print ISSN 1660–2854 | Neurodegenerative Dis 4(suppl 1) 1–364 (2007) | 4 S1 07 | online ISSN 1660–2862 | www.karger.com/ndd

ISBN 978–3–8055–8282–7

Neuro–degenerative Diseases

ABSTRACTS

Alzheimer's and Parkinson's Diseases: Progress and New Perspectives

8th International Conference AD/PD
Salzburg, Austria, March 14–18, 2007

Guest Editors
Abraham Fisher, Nes Ziona
Israel Hanin, Tucson, Ariz.
Werner Poewe, Innsbruck
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Printed in Switzerland on acid-free paper by
Kenes International, 17 rue du Cendrier
CH-1211 Geneva 1, Switzerland
ISBN 978–3–8055–8282–7

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Vol. 4, Suppl. 1, 2007

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Neuro–degenerative Diseases

Neurodegenerative Dis 2007; 4(suppl 1):1-350

Wednesday, March 14, 2007

1

Basic Research on Parkinson's Disease and the Discovery of the Nigrostriatal Dopamine Pathway

O. Hornykiewicz

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When, in 1960, the dopamine (DA) deficit in the striatum of patients with Parkinson's disease (PD) was discovered, nothing was known about whether the amine occurred in the neuronal or glial striatal elements; and in case of a neuronal localisation, whether dopamine might occur in the intrinsic striatal neurons or in striatal terminals of a pathway or pathways, originating outside the striatum. An answer to this question was crucial for deciding about whether the striatal DA deficit was specific and of functional importance for the Parkinsonian disorder. Some observations obtained in the 1960 study already hinted at the possibility of an extrinsic origin of the striatal DA. Of the extra-striatal areas, the only brain region known to be consistently affected by neuronal cell loss was the substantia nigra, especially its melanin-containing compacta neurons, and soon the research interest was focused on this brain region. Today, the existence of the nigrostriatal DA-containing pathway is a self-evident fact, being part of common textbook knowledge. In the early 1960s, however, many difficulties and prejudices had to be overcome before the existence of this pathway and its (nigrostriatal) direction were generally accepted. Today, more is known about the physiology and pathology of the nigrostriatal pathway than about any other fibre system in the mammalian brain. In the lecture, an attempt will be made at reconstructing some of the by now historic events during this exciting period of basic human brain DA research.

2

Novel Therapeutics for Alzheimer's Disease Based on Gene Discoveries

R.E. Tanzi

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Alzheimer's disease (AD) is a genetically complex disease involving both fully penetrant gene mutations and susceptibility gene polymorphisms. While >160 mutations in APP, PSEN1, and PSEN2, guarantee early-onset familial AD, the ε4 variant of APOE increases susceptibility for late-onset (>60) AD. AD-associated DNA alterations in all four of these genes harbor increase the accumulation of β-amyloid and its chief component, the Aβ peptide, in the brain. Most mutations in these three genes increase the relative levels of Aβ42, which promotes neurotoxic/synaptotoxic oligomeric species of Aβ. APOE appears plays a role in the aggregation and export of Aβ from brain. These findings have prompted therapies targeting either production or clearance of Aβ. We have found that ischemia triggers a vicious cascade of Aβ production and apoptotic cell death, beginning with the stabilization of β-secretase (BACE) owing to caspase-mediated depletion of the chaperone molecule, GGA3. In our search for novel AD genes, we have identified IDE on chromosome 10, and UBQLN1, on chromosome 9. Using cell-based and animal models, we have demonstrated a role for IDE in degrading cerebral Aβ, and a role for UBQLN1 as a gatekeeper for APP entering the secretory pathway. We are currently carrying out whole genome association (WGA) screens of >400 AD families using 500K single nucleotide polymorphism (SNP) chips to identify the remaining late-onset AD genes. Ultimately, the identification, validation, and characterization of novel AD genes should accelerate the means to better predict, diagnose, prevent and treat this devastating disease.

3

TDP-43, a New Disease Protein in Neurodegenerative Dementia and Motor Neuron Disease

V. M.Y. Lee

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Ubiquitination of misfolded proteins that aggregate in the cytoplasm and/or nucleus of nerve cells are key characteristics of neurodegenerative diseases. Misfolded disease proteins have been identified in many neurodegenerative disorders but the identity of the ubiquitinated disease protein(s) in UBIs (defined here as ubiquitinated cytoplasmic, nuclear and dystrophic neuritic inclusions) in frontotemporal lobar degeneration (FTLD-U), the most common form of frontotemporal dementias (FTDs), and amyotrophic lateral sclerosis (ALS) are unclear. FTDs are clinically, genetically and pathologically heterogeneous, and the second most common cause of dementia under age 65. FTDs present with progressive changes in social, behavioral, and/or language dysfunction and a subset of patients also develop parkinsonism or motor neuron disease (MND). Conversely, ALS, a common form of MND, is often associated with FTD and UBIs as in FTLD-U. Thus, the clinical overlap and shared ubiquitin pathologies in FTLD-U and ALS syndromes suggest that they represent different ends of a clinicopathological spectrum of the same neurodegenerative disorder. Here we showed that TDP-43 was the major disease protein in each of these disorders. Pathologic TDP-43 was hyperphosphorylated, ubiquitinated and cleaved to generate C-terminal fragments

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and was recovered only from affected central nervous systems regions including hippocampus, neocortex, and spinal cord. TDP-43 represents the common pathologic substrate linking these neurodegenerative disorders.

4

The Neuronal Sortilin-Related Receptor SORL1 is Genetically Associated With Alzheimer Disease

E Rogaeva[1], Y Meng[2], JH Lee[3], YJ Gu[1], T Kawarai[1], F Zhou[4], O Andersen[5], G Willnow[5], W Hampe[6], N Graff-Radford[4], D Dickson[4], RE Petersen[1], S Sorbi[7], A Bruni[8], S Younkin[4], L Mayeux[3], R Farrer[2], P St George-Hyslop[1]

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Background: The recycling of the amyloid precursor protein (APP) from the cell surface via the endocytic pathways plays a key role in the generation of amyloid β-peptide (Aβ) in Alzheimer's Disease (AD). Methods: We used a combination of family-based and case-control genetic association studies to explore single nucleotide polymorphisms in candidate genes potentially involved in the intracellular trafficking of amyloid precursor protein (APP). We used an initial probe set of two different FAD pedigree cohorts, a replication series of 4 additonal FAD or sporadic AD case:control cohorts, and an independent set of 4 sporadic case:control series. Results: We report here that inherited variants in the SORL1 neuronal sorting receptor are associated with late-onset AD. These variants, which occur in at least two different clusters of intronic sequences may regulate tissue-specific expression of SORL1. We also show that SORL1 directs trafficking of APP into recycling pathways, and that when SORL1 is under-expressed, APP is sorted into Aβ-generating compartments. These data suggest that inherited or acquired changes in SORL1 expression or function are mechanistically involved in causing AD.

5

The Relevance of the Amyloid Cascade Hypothesis for Sporadic Ad

B. De Strooper

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While the amyloid hypothesis finds considerable support in the rare genetic forms of the disease it is less clear how it could explain the sporadic forms of Alzheimer's disease (AD). We will discuss our recent findings on the regulation of BACE and APP expression in the brain and how this could be relevant for the understanding of sporadic AD. We will also discuss the potential of drug targets identified along the amyloid cascade pathway for the treatment of sporadic AD.

6

Molecular Analysis of Presenilin Function

S Choi[1], K Veeraraghavelu[1], G Lin[1], X Zhang[1], S Koide[2], I Ubarretxena-Belandia[3], S Sisodia[1]

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Mutations in genes encoding amyloid precursor protein (APP), or presenilins (PS1 and PS2) cause autosomal dominant, familial Alzheimer's disease (FAD). Genetic and biochemical evidence has revealed that PS interacts with nicastrin, APH1 and PEN-2 in high molecular weight complexes. I will discuss the temporal assembly of these membrane proteins, the nature of subunit interactions and the enzymatic mechanism(s) by which the complex promotes intramembranous, "g-secretase" processing of APP, Notch and other type 1 membrane proteins. Structural studies of affinity-purified g-secretase complexes are ongoing and results of these efforts will also be presented. In parallel, I will discuss the role of FAD-linked mutant PS1 in neurogenesis. We have demonstrated that expression of mutant PS1 in transgenic mice leads to marked reductions in environmental enrichment-mediated proliferation and survival of hippocampal progenitors and altered differentiation. To develop an understanding of the signaling mechanism(s) that impact on these observations, we have been examining neurosphere cultures prepared from the hippocampi and subventricular zones of these transgenic mice. Extending these studies, additional investigations have been initiated to address issues relevant to cell autonomous, versus cell non-autonomous roles of mutant PS1 in adult neurogenesis.

Supported by NIA, Ruth K. Broad Biomedical Research Foundation, Ellison Medical Foundation, Alzheimer's Association, American Health Assistance Foundation and Edward Levi Fund

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The γ/ε-Secretase-Derived APP Intracellular Domain Fragments Regulate P53

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Amyloid β-peptide (Aβ), which plays a central role in Alzheimer Disease, is generated by presenilin-dependent and presenilin-independent γ-secretase cleavages of β-amyloid precursor protein (βAPP). We report that the presenilins (PS1 and PS2) also regulate p53-associated cell death. Thus, we established that PS deficiency, catalytically inactive PS mutants, γ-secretase inhibitors and βAPP or APLP2 depletion reduced the expression and activity of p53, and lowered the transactivation of its promoter and mRNA levels. p53 expression was also reduced in the brains or βAPP-deficient mice or in brains where both PS had been invalidated by double conditional knock out. AICDC59 and AICDC50, the γ- and ε-secretase-derived C-terminal fragments of βAPP, respectively, trigger the activation of caspase-3, p53-dependent cell death, and increase p53 activity and mRNA. Finally, HEK293 cells expressing PS1 harboring familial AD (FAD) mutations or FAD-affected brains, all display enhanced p53 activity and p53 expression. Our studies demonstrate that AICDs control p53 at a transcriptional level, in vitro and in vivo and unravel a still unknown function for presenilins.

8

Regulation of Amyloid Processing and ER Stress

M. Takeda, T. Kudo, M. Okochi, T. Tanaka, K. Kamino, T. Morihara, S. Tagami, R. Hashimoto, H. Tanimukai

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Key issue of amyloid cascade hypothesis is the elucidation of biological and pathological function of presenilin complex; how the activity of gamma-secretase is regulated, what is the mechanism leading to increased level of amyloid 42 production in AD brain compared with that of amyloid 40. There should be a regulatory mechanism which modulates the activity of ganma- and ipsilon-cutting sites, which themselves show rather broad specificity of regulated intramembraous proteolysis (RIP). We have found ratio of fragments produced by several cutting sites of ganma- and ipsilon-cut is drastically modified by experimental conditions, and these findings will be utilised to develop pharmacological modification to reduce amyloid burden of AD brain without interfering proteolytic processing of other signal molecules including notch.

ER stress is another important field of our study because ER stress functions in many neurodegenerative disorders. It is speculated accumulated beta amyloid is causing ER stress and unfolded protein response (UPR) is significantly impaired in AD brain.Understanding of the molecular mechanism of neuronal degeneration in Alzheimer disease is opening the door to understand other CNS disorders.

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Presenilin (PS) Signaling, Neuronal Survival and Ad Pathology

N.K. Robakis, L. Baki, N. Arbez

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Presenilin regulates cell signaling through gamma secretase-dependent and gamma secretase-independent mechanisms. The first involves a gamma-secretase catalyzed cleavage at the epsilon site of cell surface receptors, adhesion factors and cell-cell communication proteins. The produced cytosolic peptides containing the intracellular domain (ICD) of cleaved proteins have been shown to regulate gene expression by interacting with transcriptional factors. Some ICD peptides, however, may control intracellular signaling by mechanisms other than gene expression. Thus, we obtained data showing that the ICD peptide ephrinB2/CTF2 which derives from the gamma-secretase processing of ephrinB proteins activates the Src family of tyrosine kinases by binding Src and inhibiting its association with inhibitory factor Csk.

Gamma secretase-independent mechanisms of PS signaling include regulation of the PI3K/Akt cell survival pathway. PS inhibits apoptosis and promotes neuronal survival by activating this pathway. PS-dependent activation of PI3K/Akt signaling downregulates both, GSK-3 kinase and tau phosphorylation at AD-related residues. In contrast, PS FAD mutants are unable to stimulate the PI3K/Akt signaling thus inhibiting the ability of PS1 to promote neuronal survival and to control tau overphosphorylation. Since GSK-3 activity and neuronal apoptosis have been implicated in increased Aβ production, we examined the effects of PI3K/Akt signaling on the production of Aβ peptides. Our data show that downregulation of PI3K/Akt signaling results in increased production of Aβ. Our results support the theory that PS FAD mutations promote neuronal degeneration, tau overphosphorylation and production of Aβ peptides by downregulating the PI3K/Akt cell survival pathway.

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Susceptibility Genes, Their Proteins and Treatment Targets in Sporadic Parkinson's Disease

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Background and aims: recently a number of high throughput gene-based platforms studies have attempted to define the gene expression profile of human post-mortem substantia nigra (SN) in sporadic Parkinson's disease (PD) brains. Our studies are the first to identify 20 differentially expressed genes in sporadic PD SN pars compacta (pc), partly validated by real time RT-PCR and immunohistochemistry.

Results: the major findings were the particular decreased gene and protein expression of SKP1A, a member of the SCF (E3) ligase complex (ubiquitin-proteasome) and HSC-70 chaperone and aldehyde dehydrogenase 1 (ALDH1A), specifically in the SNpc of sporadic PD. On the other hand, increased dysregulation of extracellular matrix cytoskeleton components and iron-oxygen sensor, EGLN1 (hypoxia inducible factor (HIF)-1 prolyl-4-hydroxylase) were observed. The new cluster of gene expression changes may very well represent the point of convergence or central core by which dopamine neurons degenerate in SNpc of sporadic, as well as familial PD. Therefore, we are currently developing a new model of sporadic PD based on a systematic silencing and over expression of selected genes identified in sporadic PD SNpc, which may be part of the final pathway of neurodegeneration and more representative of the animal models employed to study PD. Conclusions: the signatures unveiled by these approaches could provide crucial information on diagnosis and development of surrogate markers for PD and reliable candidate genes as predictive early biomarkers to identify individuals at risk (susceptibility genes) before disease onset.

11

Influence of Pharmacogenetic Factors on Alzheimer's Disease Therapeutics

R. Cacabelos

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Pharmacogenetic and pharmacogenomic factors account for more than 70% of efficacy and safety issues in AD therapeutics. About 1,400 human genes influence 20-95% variability in drug disposition and pharmacodynamics, and more than 200 genes are potentially involved in AD-related neurodegeneration and premature neuronal death, together with epigenetic, cerebrovascular and environmental factors. Some cholinesterase inhibitors and 30% of CNS drugs are metabolized via enzymes of the CYP gene superfamily, especially monooxigenases encoded by the CYP2D6, CYP3A4, and CYP1A2 genes. Different polymorphic variants of the CYP2D6 gene (22q13.1) are responsible for the phenotype of Extensive Metabolizers (EM)(*1/*1, *1/*10)(51.61%), Intermediate Metabolizers (IM)(*1/*3, *1/*4, *1/*5, *1/*6, *1/*7, *10/*10, *4/*10, *6/*10, *7/*10)(32.26%), Poor Metabolizers (PM)(*4/*4, *5/*5)(9.03%), and Ultrarapid Metabolizers (UM)(*1xN/*1, *1xN/*4)(7.10%) in the European population. PMs and UMs accumulate genotypes of risk associated with APOE-, presenilin-, ACE-, and PRNP-related variants. In AD patients treated with donepezil in a combination therapy for one year, the best responders are EMs and IMs, and the worst responders are PMs and UMs. *1/*10-EMs (r=+0.96) respond better than *1/*1 (r=+0.44). The best IM responders are *1/*3 (r=+0.98), *1/*6 (r=+0.93), and *1/*5 (r=+0.70), whereas *1/*4, *10/*10, and *4/*10 are poor responders. Among PMs and UMs, the poorest responders are carriers of the *4/*4 (r=-0.98) and *1xN/*1 genotypes (r=-0.97), respectively. The CYP2D6-related therapeutic outcomes are modified by the presence of the APOE-4/4 genotype which converts EMs and IMs into phenotypic PMs. Furthermore, drug liver metabolism and drug distribution can also be influenced by alterations in lipid metabolism associated with APOE variants.

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Premenopausal Estrogen Deficiency and Risk of Parkinsonism or Dementia

W.A. Rocca

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Background: There is increasing laboratory evidence for a neuroprotective effect of estrogen during aging; however, the clinical and epidemiological evidence remains limited and conflicting. We studied the association of oophorectomy performed before the onset of menopause with the risk of parkinsonism or dementia. Methods: We included all women who underwent either unilateral or bilateral oophorectomy before the onset of menopause for a non-cancer indication while residing in Olmsted County, MN, between 1950 and 1987. Each member of the oophorectomy cohort was matched by age to a referent woman in the same population who had not undergone oophorectomy. In total, we studied 1,293 women with unilateral oophorectomy, 1,097 women with bilateral oophorectomy, and 2,390 referent women. Women were followed through death or end of study using a combination of direct or proxy interviews, medical records in a records-linkage system, and death certificates. Results: Women who underwent either unilateral or bilateral oophorectomy before menopause had an increased risk of parkinsonism (HR=1.68; 95% CI, 1.06 to 2.67; P=0.03) or dementia (HR=1.43; 95% CI, 1.11 to 1.85; P=0.006) compared to referent women. The risk increased with younger age at oophorectomy (test for linear trend with decreasing age; P=0.01 for parkinsonism and P<0.0001 for dementia). These associations were similar regardless of the indication for the oophorectomy, and for women who underwent unilateral or bilateral oophorectomy considered separately. Conclusions: Both unilateral oophorectomy and bilateral oophorectomy performed before the onset of menopause are associated with an increased risk of parkinsonism or dementia. The effect is age-dependent.

13

The Economics and Pharmacoeconomics of Dementia

H. Fillit

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Considerable data now demonstrate that Alzheimer's disease and related dementias (ADRD) are among the most costly diseases to societies throughout the world. With the rapid aging of the developed and developing world, the costs of ADRD will continue to increase disproportionately to other common chronic illnesses of aging. These excess costs to society are occurring in the context of finite health care system budgets, increasing payer restraints, and greater regulatory requirements for the demonstration of cost-effectiveness of new therapies. As a result, pharmacoeconomics are an increasingly important component of drug development. Here we will review existing pharmacoeconomic data regarding current AD therapies. We will also discuss the challenges in

pharmacoeconomics for the development of disease-modifying drugs for AD.

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Pharmacoeconomics in Alzheimer's Disease

B. Winblad, A. Wimo

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The socioeconomic impact of dementia disorders worldwide is enormous. Over 25 million people are suffering from dementia and we have estimated the annual total worldwide costs from a societal perspective to over 200 billion US$. The combination of an expensive care, a highly prevalent group of disorders, a financial crisis in the public health care systems and a heavy impact of the family members combined with a great contribution of unpaid informal care indeed focus on the fundamental questions in any health economical analysis. Demands to show cost-effectiveness from those who finance care will probably be the case for any kind of treatment of dementia. However, to analyse the cost-effectiveness of treatment of dementia demands a set of prerequisites that need to be fulfilled.

Assessments of resource utilization should be done with valid and comprehensive instruments, aiming at calculating costs from a societal perspective. We have developed an instrument, the RUD (Resource Utilization in Dementia) in two versions: the basic comprehensive version and the short version, RUD Lite, where the use of resources are measured from a societal perspective.

Both costs and effects should be analysed in the same study and and there should also be a comparison between at least two alternatives. Even if these prerequisites not are controversial, there are today very few published pharmacoeconomical studies of dementia care that fulfil these simple criteria. Most studies focus only on costs (e.g cost of illness studies and most pharmacoeconomical studies of antidementia drugs). Although such studies are of interest, they can hardly be used as a basis for e.g priority discussions and reimbursement decisions by drug authorities (or similars). There is a great need for methodological improvement and more studies in this field, particularly in the light of the interest and probable future demands from drug authorities. Results from some pharmacoeconomical studies of drugs used in dementia will be presented.

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How and When Environment Affects Alzheimer's Disease Risk: The "Learn" Model (Latent Early-Life Associated Regulation) May Explain Triggering the Disease

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Background and Aims: Amyloid beta-peptide (Aβ), derived from the Aβ precursor protein (APP) is a leading candidate for immediate cause of Alzheimer's disease (AD). Both appear in healthy individuals. What triggers APP and Aβ to be overexpressed in sporadic AD? When and how does it operate? We hypothesize that environmental/dietary factors at early stages of development perturb APP gene transcriptional regulation, leading to latent overexpression and subsequent Aβ overproduction. Methods: Rat pups were pooled, randomly reassigned to litters, and divided into three groups. Control dams received unsupplemented tap water, pups given ordinary tap water after weaning. Early Pb group (Pb-E) was exposed to 0.2% lead acetate from Post-natal days (PND) 1 through 20 through the dam. Late Pb (Pb-L) group was exposed to Pb from 18 to 20 months of age. Animals sacrificed at PND 5, 50, 350, 600. Levels of APP mRNA and protein, Aβ, SP1 DNA binding, and Pb were measured. Results: developmental exposure of rats to Pb resulted in delayed (PND 600) overexpression of APP mRNA, and an elevation in levels of APP, Aβ, and SP1 DNA binding. These were unaffected in Pb-L rats. Conclusions: We suggest that developmental exposure to Pb elevates Aβ levels in old age via reprogramming synthesis of APP through disturbing DNA methylation, imposing a "somatic epitype" on the SP1 promoter and SP1-regulated genes such as APP. APP and associated genes would be subject to latent early-life associated regulation (LEARn), resulting in a pathogenic or pathologically vulnerable phenotype. Supported by NIH grants.

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Substrate Spectrum of BACE1 and Modulation of Beta-Secretase Cleavage

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The β-secretase BACE1 catalyzes the first step in Aβ-peptide generation. At present, little is known about the substrate spectrum of β-secretase and about cellular mechanisms controlling APP β-secretase cleavage. Regarding the substrate spectrum, a candidate approach was used. We tested several membrane proteins, which undergo shedding by an α-secretase, for a potential additional cleavage by BACE1. We found that transfection of BACE1 strongly increased proteolytic processing of the interleukin-1 receptor II (IL-1R2). Surprisingly, BACE1 induced IL-1R2 cleavage at the same peptide bond, where the α-secretase cleavage occurs. This suggests that a) BACE1 acts as an alternative α-secretase like protease for IL-1R2 and that b) α-secretase-like ADAM proteases could compensate for the loss of BACE1 cleavage in BACE1-deficient cells. In fact, processing of IL-1R2 was not significantly altered in BACE1-deficient cells. Potentially, BACE1 may have a more general role as alternative α-secretase and could be involved in the shedding of additional membrane proteins known to undergo ectodomain shedding. With regard to cellular control mechanisms modulating β-secretase cleavage, we used RNA interference in HEK293 cells and identified a new member of the sorting nexin (SNX) family of intracellular transport proteins, SNX33, as a novel protein limiting Aβ-peptide generation. Mechanistic analysis revealed that SNX33 modulates the cellular localization of APP and limits the availability of APP for β-secretase

cleavage and thus for Aβ generation. In summary, our work contributes to a better understanding of the substrate spectrum of BACE1 and of the cellular modulation of APP β-secretase cleavage.

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Ubiquitinated and Phosphorylated TDP-43 in Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis

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Ubiquitin-positive, tau- and alpha-synuclein- negative inclusions are the hallmark lesions of frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U). The generation of monoclonal antibodies raised against insoluble protein extracts from FTLD-U brains allowed us to identify TDP-43 as the major disease protein in the cytoplasmic, neuritic and intranuclear inclusions in sporadic and familial FTLD-U, including cases with mutations in the progranulin and valosin-containing protein gene. Moreover, pathological inclusions in sporadic amyotrophic lateral sclerosis (ALS) were also found to be TDP-43 positive, providing evidence that these diseases represent syndromic variants of a clinico-pathological spectrum of disorders that share similar pathological mechanisms which culminate in the progressive degeneration of different selectively vulnerable populations of neurons. TDP-43 is a ubiquitously, highly conserved nuclear protein, that may act in transcription regulation and as a scaffold for nuclear bodies. In FTLD-U and ALS, TDP-43 becomes hyperphosphorylated, ubiquitinated and cleaved to generate C-terminal fragments.

Thus, TDP-43 is implicated in FTDL-U and ALS pathogenesis and represents the most robust marker for the specific neuropathological diagnosis of these diseases.

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Modality-Specific Patterns of Working Memory Impairment in Alzheimer's Disease, Huntington's Disease, and Parkinson's Disease With Dementia

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A deficit in working memory (i.e., a limited capacity memory system in which information that is the immediate focus of attention can be temporarily held in mind and manipulated) is a common consequence of most neurodegenerative disorders that cause dementia. There is some evidence, however, that distinct neurodegenerative disorders differentially affect working memory for specific types of information. To assess this possibility, we administered the Recognition Span Test (RST) to elderly normal control (NC) subjects and patients with Alzheimer's disease (AD), Huntington's disease (HD), or Parkinson's disease with dementia (PDD) using spatial, verbal, and facial stimuli. In each condition, subjects had to remember an increasing number of stimuli presented, in series, across trials. Memory span length was the number of stimuli that could be correctly identified until an error was made. Results showed that AD patients were equally impaired (compared to NC performance) across modalities ($z = -1.54$, -1.33, and -1.75 for spatial, verbal, and facial, respectively), whereas patients with HD were more impaired for spatial and facial stimuli ($z = -1.85$ and -2.00, respectively) than for verbal stimuli ($z = -0.98$) ($p<.001$). PDD patients performed similarly to HD patients with greater impairment for spatial and facial stimuli ($z = -2.00$ and -1.57, respectively) than for verbal stimuli ($z = -0.97$) ($p<.001$). These results suggest that patients with mild dementia due to basal ganglia dysfunction may have disproportionately severe spatial working memory deficits due to disruption of posterior caudate connections to parietal cortex.

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Leucine-Rich Repeat Kinase-2 Stimulates Extracellular Signal-Regulated Kinases and Induces Alpha-Synuclein Expression

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The two monogenic autosomal-dominant hereditary Parkinson's disease genes encode leucine-rich repeat kinase-2 (LRRK2) and α-synuclein. Here we show that these two genes are linked in a pathway whereby LRRK2 induces α-synuclein mRNA and protein expression in transiently transfected HEK293E cells. α-Synuclein induction depended on LRRK2 kinase activity, because kinase-dead mutant [K1906N]LRRK2 failed to increase α-synuclein mRNA (measured by qRT-PCR) and protein (measured by ELISA). Both wild-type and the most common Parkinson's disease-associated mutant [G2019S]LRRK2 induced α-synuclein expression. We have raised novel polyclonal antibodies and demonstrated equal expression levels, but no evidence for destabilization, breakdown, or obvious aggregation of LRRK2 and the mutants investigated here. Since LRRK2 shows homology to mitogen-activated protein kinase (MAPK) kinase kinases, we have systematically investigated the three MAPK modules. Transient transfection of LRRK2 stimulated the activating tyrosine phosphorylation of extracellular signal-regulated kinases (ERK1 and ERK2), but not c-Jun N-terminal kinases and p38 MAPKs. The selective stimulation of the ERK pathway was further augmented by blocking tyrosine phosphatases with pervanadate. LRRK2 was found to be tyrosine phosphorylated under these conditions. We propose that the physiological role of LRRK2 involves initiation of the ERK pathway and eventually induction of α-synuclein expression as part of a pro-survival pathway, and we discuss the disease-causing effects of clinical mutations in this context.

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Role of Ubiquilin and Erasin in the Disposal of Misfolded Proteins

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Many neurodegenerative disorders are associated with an accumulation of misfolded and aggregated proteins in the brain. Thus, identifying factors that aid in the recognition and disposal of misfolded proteins in cells may provide a useful strategy to prevent and/or treat these diseases. We have identified two proteins, ubiquilin and erasin, each approximately 66-kDa in size that are involved in clearing misfolded proteins in cells.

Ubiquilin proteins contain an N-terminal ubiquitin-like domain, a central more variable domain, and a C-terminal ubiquitin-associated domain. Ubiquilin interacts with presenilins and with huntingtin (htt) proteins containing expanded polyglutamine tracts. Overexpression of ubiquilin reduced aggregation of GFP-Htt fusion proteins containing expanded polyglutamine tracts and suppressed cell death and toxicity in cell and Caenorhabditis elegans models of Huntington's disease (HD). Pulse-chase studies revealed that overexpression of ubiquilin selectively enhances the degradation of GFP-Htt fusion proteins containing 74 polyglutamine repeats compared to those containing 28 polyglutamine repeats.

Erasin is a new ER-localized protein that we found promotes ER-associated protein degradation (ERAD), a regulated pathway used by cells to eliminate misfolded proteins in the ER. We found erasin is present in a complex with other ERAD components, most notably p97/VCP, Derlin-1, and the ubiquitin ligase gp78. In addition, we found erasin binds ubiquilin, which we propose forms a functional complex to deliver misfolded proteins as they are extracted from the ER to the cytosol for degradation by the proteasome. Our findings suggest ubiquilin and erasin are novel proteins that function in the elimination of misfolded proteins in cells.

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Susceptibility Sets for Alzheimer's Disease Identified From Diverse Candidate Loci

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Background: Alzheimer's disease has been associated with gene variants found for APOE4 and diverse candidate loci. We defined susceptibility sets for AD from information on variants found for 18 genes located on chromosome 10q (32 loci) & 25 located elsewhere (34 loci) + CSF tau & Abeta42 levels.

Methods: The 938 AD patients and 397 control subjects were enrolled in Scotland & Sweden. A fuzzy latent classification approach, grade-of-membership analysis (GoM), was taken to identify risk sets. Individuals are automatically related to each set via graded membership scores.

Results: Set I: unaffected + ↓ CSF tau + ↑ CSF Abeta42 + multiple protective alleles. High intrinsic risk sets II to VI differed in onset age and relevant risk alleles: close resemblance, i.e. > 75% aggregate membership, multiplied risk of AD > 100-fold at ages 65 to 84.

Conclusions: AD likely has multiple determinants including APOE polymorphism and gene variants located on chromosome 10q and elsewhere. This information and approach can be employed to better define AD pathogenesis and risk for individuals.

Neuro–degenerative Diseases

Neurodegenerative Dis 2007; 4(suppl 1):1-350

Thursday, March 15, 2007

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The Impact of Subthalamic Nucleus Dbs on Motor and Cognitive Functions in PD

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The beneficial motor effects of STN DBS largely mimic the response to levodopa and this response remains the best predictor of benefit from DBS. Motor fluctuations and dyskinesias are particularly improved with this therapy. However, persistent parkinsonian features in the on-period gradually worsen over time and at five years bradykinesia and particularly axial symptoms (e.g. freezing, postural instability, dysarthria) are worse than before surgery. Newer DBS targets (e.g., the pedunculopontine nucleus) are being explored for levodopa- and STN DBS-resistant postural and gait abnormalities. A variety of cognitive, mood and behavioral changes may occur in patients undergoing STN DBS. The pathogenesis of these changes is multifactorial. Pre-existing cognitive status, psychiatric and psychosocial factors are critical. The procedure is relatively safe from a cognitive perspective in carefully selected patients; the most consistent adverse effects occur in the realm of verbal fluency. Behavioral changes include depression, hypomania/mania and a variety of other miscellaneous disorders. These can relate to direct effects of the surgery, the stimulation or changes in medication. Apathy is another important problem that may be aggravated by reduction in LDE but also may worsen with time as an indication of progression of the disease which seems unaltered by DBS. Finally, suicide (attempts and successful events) are approximately 12 times more frequent than in control populations in the first postoperative year and this falls to baseline by the third postoperative year. This represents an important and potentially modifiable risk for postoperative mortality.

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Etiology and Pathophysiology of Alzheimer Disease, Parkinson Disease, Lewy Body Dementia, and Frontotemporal Dementia

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Successful longitudinal kindred studies have formed the basis of recent molecular genetic discoveries. They also have led to new classifications of neurodegenerative conditions such as tauopathies (progressive supranuclear palsy, corticobasal degeneration, Alzheimer disease, frontotemporal dementia with parkinsonism linked to chromosome 17), amyloidopathies (Alzheimer disease, dementia with Lewy bodies), progranulinopathies (ubiquitin-positive frontotemporal dementia), alpha-synucleinopathies (Parkinson disease, dementia with Lewy bodies, multiple system atrophy), and polyglutaminopathies (Huntington disease, spinocerebellar ataxia). These emerging classifications are based on the predominant pathologic abnormalities seen in autopsy material. However, the pathologic findings present in brains from affected family members carrying the same genetic defect (sometimes despite uniform clinical presentation) are similar, as evident in families with LRRK2 gene mutations. More frequent are major interfamilial and intrafamilal clinical phenotypic differences observed in kindreds, with affected family members carrying the same gene defect (probably the best examples are carriers of MAPT and PGRN gene mutations). The genetic classifications based on gene discovery (for example, PARK1–PARK13) are helpful but have limited usefulness in clinical practice because the phenotypes vary substantially (as does the pathology). In addition, in a genetic locus, more than one gene can be found (as recently was discovered for the chromosome 17 wld locus: FTDP-17/MAPT and FTD-U/PGRN). It remains to be seen how all these new classifications, whether based on pathologic or genetic abnormalities, will advance clinical practice. However, there is no doubt that progress in genetic studies will improve understanding of the pathophysiology of common sporadic neurodegenerative conditions and lead eventually to curative therapies.

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The Role of Progranulin (PGRN) in Neurodegenerative Disease

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We recently demonstrated that mutations in Progranulin (PGRN) cause MAPT-negative FTDP-17. PGRN encodes a 68.5 kDa secreted mitogenic factor, thought to be involved in the regulation of multiple processes including development, wound repair and inflammation. Mutations in PGRN are associated with the formation of MAPT-negative, ubiquitin-positive neuronal intracytoplasmic and intranuclear inclusions recently shown to contain TAR DNA binding protein

(TDP43). This neuropathological phenotype distinguishes these cases from FTDP-17 patients with mutations in MAPT given that these individuals inevitably have MAPT-positive neurofibrillary lesions and do not develop consistent TDP-43 inclusion pathology. Mutations in PGRN are thought to account ~5% of all FTD cases.

All pathogenic mutations in PGRN identified to date create functional null alleles implying that they cause disease by producing a deficiency of PGRN. However the role of PGRN in neuronal survival and the explanation for why partial loss of this factor causes adult-onset neurodegenerative disease remains uncertain. What is known is that PGRN has been consistently implicated in the mechanism of wound repair. It is therefore likely significant that PGRN is upregulated in activated microglia in response to the development of multiple neurodegenerative diseases including Alzheimer's disease. In addition, the dramatic accumulation of PGRN in dystrophic neurites surrounding senile plaques suggests that PGRN is involved in the localized brain response to amyloid deposition in AD. However whether PGRN is critical to the mechanism of brain repair and whether deficiency in this process is responsible for the development of FTD in patients with PGRN mutations remains to be determined.

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Activated Microglia Affect the Nigro-Striatal Dopamine Neurons Differently in Neonatal and Aged Mice Treated With MPTP

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Background and aims: Microglia play an important role in the inflammatory process that occurs in Parkinson's disease (PD). Activated microglia produce cytokines and neurotrophins and may have neurotoxic or neurotrophic effects. Since during the neonatal period microglia are most proliferative and easily activated, we examined the effects of neonatal microglia activated with lipopolysaccharide (LPS) on the nigro-striatal dopamine neurons in mice treated with MPTP, in comparison with activated microglia from the aged mice.

Methods and Results: By MPTP administration to neonatal mice, the number of dopamine neurons in the substantia nigra (SN) was significantly decreased, whereas that in the mice treated with LPS and MPTP was recovered to normal, along with significant microglial activation. Tyrosine hydroxylase (TH) activity, the levels of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC), and the levels of pro-inflammatory cytokines IL-1β and IL-6 in the midbrain were elevated in the neonates treated with LPS and MPTP. On the contrary, although the number of dopamine neurons in the 60-week-old mice treated with MPTP was also significantly decreased, the microglial activation by LPS treatment caused a further decrease in their number.

Conclusions: These results suggest that the activated microglia in neonatal mice are different from those in aged mice, with the former having neurotrophic potential towards the dopamine neurons in the SN, in contrast to the neurotoxic effect of the latter.

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Multimodal Neuroprotective and Neurorescue Drugs for Preventing Parkinson's and Alzheimer's Diseases Pathologies

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Alzheimer's disease (AD), is initiated by cascade of neurotoxic events, that includes oxidative stress, brain iron dysregulation, glutamate excitotoxicity, inflammatory process, neurotxic processing of APP and misfolding of proteins. AD subjects are benefiting from drugs developed to act on a single molecular target. Such drugs have limited symptomatic activities and current pharmacological approaches are limited in their ability to significantly modify the course of the disease, offering incomplete and transient benefit to patients. However, the new therapeutic strategies for neurodegenerative diseases are those in which drug candidates are designed expressly to act on multiple neural and biochemical targets involved in the neurodegenerative process. Monoamine oxidase (MAO) activity, iron, and glutamate excitotoxicity increase in brains of AD. The iron deposition in microglia, amyloid plaques, neurofibrillary tangles can induce oxidative stress via interaction with hydrogen peroxide produced by MAO and other oxidative processes. The presence of 5'UTR iron responsive element on amyloid precursor protein (APP) promoter has indicated that iron increases APP translation and processing to neurotoxic Aβpeptide. We have develop molecular entities that combine two or more of cholinesterase inhibition, brain selective MAO inhibition, iron chelation, inhibitors of glutamate release and anti apoptotic-neurorescue activity. They possess range of pharmacological activities, including neuroprotective, pro-survival neurorescue effects, induction of neuronal differentiation and regulation of amyloid precursor protein (APP) and reduction Aβ levels in cell culture and in transgenic mice These properties indicate that compounds might serve as an ideal drug for neurodegenerative diseases, such as AD and PD.

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Design of AChE Inhibitors as AD Therapeutics Guided by the Functional Architecture of the Enzyme

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Structure based drug design of AChE inhibitors as AD therapeutics relies on the fine mapping of the multiple

functional domains within the active center of the enzyme. Exploration of the functional architecture of the active center has been carried out by x-ray crystallography, extensive targeted mutagenesis and through use of specific ligands that probe the various facets of the enzyme's reactivity. In particular, charged and uncharged chiral phosphonates were used to uncover the roles of the cation binding and the acyl pocket subsites in determining the stereoselectivity of HuAChE toward these inhibitors. Another active center subsite, the hydrophobic pocket, was explored for its flexibility in accommodating structurally diverse ligands, including the AD drugs tacrine and galanthamine. The hydrophobic subsite is also a major binding element contributing to the stability of HuAChE Michaelis complexes with covalent inhibitors such as carbamates, including the recently approved AD agent rivastigmine. In this context, we have recently discovered further aspects of the role of both the unique structure and the flexibility of the "hydrophobic patch" in determining the reactivity and stereoselectivity of HuAChE toward certain carbamates, including analogs of physostigmine. The different modes of HuAChE inhibition by these inhibitors (e.g. covalent vs noncovalent) are determined by the structures of their respective Michaelis complexes, and thus allow for a better understanding of the role played by these sites for further refinement of inhibitor design.

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Inhibition of Amyloid-Beta Peptide Via Actions on Its Precursor Protein as a Treatment Strategy for Alzheimer's Disease

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Alzheimer's disease (AD) is typified by a synaptic loss, cholinergic dysfunction and abnormal protein depositions in the brain. The amyloid beta-peptide (Aβ), a proteolytic fragment of amyloid precursor protein (APP), aggregates to form neuritic plaques and has a causative role in AD. Soluble forms of Aβ induce oxidative stress, perturbed calcium homeostasis, synapse dysfunction and loss, and, eventually, cell death. A present focus of AD research is to develop safe Aβ-lowering drugs. Aβ levels can be regulated at many stages, such as by lowering its rate of initial production. As APP is the precursor of Aβ and other potentially toxic carboxyl terminal fragments, we targeted its synthesis to lower Aβ. As APP levels are regulated, in part, post-transcriptionally at the level of APP mRNA translation via signals in its 5'-untranslated region [PNAS 98:7605-10, 2001; J Biol Chem. 277:45518-28, 2002; J Alz Dis. 5:81-90, 2003], we focused our medicinal chemistry towards this target. To this end, we have synthesized agents on the hexahydropyrrolo-indole backbone of the anticholinesterase, phenserine, with depleted cholinergic activity. Phenserine dose-dependently reduces APP levels but, like other anticholinesterases, is dose limited by its primary cholinergic actions [Curr Alz Res 2:482-92, 2005; JPET 318:855-62, 2006]. We have also synthesized chelating agents based on the piperazine backbone of EDTA. Compounds from both classes dose-dependently lowered Aβ levels in cell culture and animals models without apparent toxicity, and are candidates for AD clinical trials.

Supported by the Intramural Research Program, NIA1, by NIH grants2,4,6, the Alzheimer's Association and Axonyx Inc.2,4

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Cholinesterase Variants: From Molecular Mechanisms to Neurodegenerative Diseases

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Two human genes encode for aetylcholine hydrolysing enzymes, acetyl- and butyrylcholinesterase (ACHE, BCHE), and both are actively involved in neurodegeneration. The acetylcholinesterase (AChE) protein product is not one, but a combinatorial series of proteins having variant N- and C-termini due to alternate promoter usage and 3'-alternative splicing. Neuronal AChE variants show indistinguishable enzymatic activity yet differ in their expression, multimeric assembly and membrane association patterns. Moreover, they show distinct non-hydrolytic properties and interact with different protein partners. Transcriptional and post-transcriptional regulation of AChE pre-mRNA appears to serve as a neuroprotection strategy but may entail long-term damages. The composition, neuronal localization and functional roles of the AChE protein variants suggest causal involvement of these variants in neuronal development, functioning and pathological states. Thus, AChE-S acts as a detrimental accentuator of various neuropathologies, including amyloid fibrils formation, whereas both AChE-R and BChE operate as neuroprotective agents. The recurrent involvement of cholinesterases in a variety of different pathophysiological states including neuropathologies and stress-related syndromes places them as the main target for drug development and therapy of such disorders. The uniqueness of each variant is accentuated by its expression timing, i.e. during health or disease, during normal or stressful situations; its expression compartment, i.e. in neuritic processes, on synaptic membranes, etc. and its known and yet unknown interaction partners. These three factors together determine an additional dimension in the intricate control over the variant cholinesterase proteins. Effective characterization of the molecular pathways involved may hold promise and hope for treating cholinesterase-related neuropathologies.

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Glutamatergic Approches to Treatment of Cognitive and Behavioural Symptoms of AD

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Understanding of the neurochemical changes that occur in Alzheimer's disease in relation to particular symptoms has greatly helped in devising rationale therapeutic strategies. Knowledge of changes to the cholinergic system and relation to both attentional aspects of memory and non-cognitive behavioural changes such as apathy and psychosis has underpinned findings of the range of therapeutic benefits in clinical trials afforded by cholinesterase inhibitors. Our recent studies indicate an early loss of glutamatergic synapses in AD (Kirvell et al, 2006). The glutamatergic system has long been recognised for its role in learning and memory and efforts to produce drugs which address changes in the glutamatergic system in AD are well advanced (eg memantine and drugs in development such as APMAkines). Much less is know about the possible role of glutamate in non-cognitive behavioural changes, however recent data from clinical trials suggest that memantine reduces agitation and aggressive behaviour in AD patients. In this context it is important to investigate underlying mechanisms to understand better the full range of possible benefits of existing drugs but also to help identify new treatment approaches to replace the use of antipsychotics in this vulnerable population.

Kirvell SL, Esiri M, Francis PT. Down-regulation of vesicular glutamate transporters precedes cell loss and pathology in Alzheimer's disease. J Neurochem. 2006; 98(3):939-50.

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Future of Cholinergic Therapy in Dementia

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Twenty years after the first report of Summers in 1986, several million Alzheimer Disease (AD) patients, have been treated with cholinesterase inhibitors (ChEI) with no evidence of severe side effects .There is no evidence that treatment is not cost effective. The time limit in long-term clinical effects is at least 3 yrs. The recent discovery of the role of butyrylcholinesterase (BuChE) in brain points to this enzyme as a new target for AD treatment in advanced AD cases. Selective BuChEI should be tested in more severe cases. Based on the functional role of the cholinergic system, indication for ChEI treatment should be extended to those diseases or syndromes showing a cholinergic deficit such as Lewy Body Disease, Vascular Dementia, Parkinson Dementia, Delirium ,Brain injury ,attention deficits and HIV dementia..Bifunctional ChEI are being developed to add non-cholinergic to cholinergic effects such as APP synthesis inhibition ,anti-oxidation, MAO inhibition , A-beta anti-aggregation, 5HT uptake inhibition.ChEI will continue to play an important role in AD therapy for many years to come.The possibility of rescuing forebrain cholinergic cells from degeneration is being attempted with NGF therapy directly , through gene therapy or through genetically modified stem cells This approach may double the effect-length of ChEI.

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Longitudinal Effects on Cognition in Alzheimer Patients Measured by CDR

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Background: MMSE and ADAS-Cog are commonly used tools for measuring cognitive function in Alzheimer's disease (AD) patients. More recently developed tests are the AQT and computerized tests such as the CDR. Information is scarce regarding how these various tests relate to each other and their sensitivity to detect disease progression.

Objectives: To determine the sensitivity of ADAS-Cog, MMSE, AQT and CDR to detect disease progression and how these measures relates to each other over time.

Method: Patients had to fulfill the DSM-IV diagnosis of AD of mild to moderate severity (MMSE>15) and to be on continuous treatment with acetylcholine esterase inhibitors. Within 4 weeks from the first visit the cognitive tests were performed. The tests were repeated after 6 months.

Results: A significant change in cognition over time was detected with the MMSE, CDR and AQT (tendency) whereas no such change was evident with the ADAS-Cog. When compared to normative CDR data the levels of pre-existing cognitive impairment were lower in this study than what could be expected in patients with a similar range of MMSE scores and the impairment was less pronounced than what has previously been reported on AD patients treated with placebo. The test batteries were highly correlated when measured at one time point. However, the correlation between test batteries with regard to change over time was almost absent.

Conclusion: This indicates that the different test batteries though measuring the same underlying cognitive functions at one time point, measure different aspects of cognitive decline over time.

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Longitudinal Effects on Biomarkers in Alzheimer Disease

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Biomarker levels in cerebrospinal fluid (CSF) may serve as surrogate markers for treatment efficacy in clinical trials of disease-modifying drugs against Alzheimer's disease (AD). A prerequisite, however, is that the marker is sufficiently stable

over time in individual patients. Here, we tested the stability of the three established CSF biomarkers for AD, total tau (T-tau), phospho-tau181 (P-tau) and the 42 amino acid fragment of beta-amyloid (Abeta42), over 6 months in a cohort of AD patients on standard symptomatic treatment. Forty-seven patients completed the study (19 women, 28 men, mean age ± standard deviation [SD] 75 ± 8.0 years). Mean levels (± SD) of T-tau, P-tau and Abeta42 at baseline were 690 ± 290, 90 ± 35 and 450 ± 230 ng/L, respectively. These levels did not change significantly during the 6-month study period. On the contrary, intra-individual biomarker concentrations on the two sampling occasions were highly correlated with Pearson r-values above 0.95, $p<0.0001$, for all three markers. We conclude that T-tau, P-tau and Abeta42 concentrations in CSF are remarkably stable over time in individual AD patients. If these biomarkers indeed reflect the disease process in AD, the results bode well for their usefulness as surrogate markers for treatment efficacy in disease-modifying anti-AD drug trials.

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Relationship Between ADAS-Cog and the CDR System, Plus Recent Data From Clinical Trials in PDD, DLB & AD

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While the ADAS is widely accepted as the gold standard in registration trials for treatments for Alzheimer's disease, there is no regulatory mandate for this. Its strengths are its history of use and ability to assess important aspects of cognition such as memory. Its weaknesses are that it not automated, and therefore subject to bias and misinterpretation, and also that it does not assess attention and executive function. Working groups have long recommended replacing the ADAS if suitable automated alternatives can be found (eg Ferris et al, Alzheimer Disease and Associated Disorders 1997, 11: 34-38). One candidate is the Cognitive Drug Research (CDR) computerised assessment system which has been widely used in dementia work for 20 years. Research has shown good correlations between the memory scores from the CDR and the ADAS, while the CDR system also assesses attention, articulatory and spatial working memory, information processing and the speed of retrieval of memory. The utility of the system in evaluating and differentiating the major dementias will be described, as well as its ability to track deterioration over time. Its validation as a core measure of cognitive dysfunction in the dementias will be described, as will work showing that various CDR measures relate closely to activities of daily living. The sensitivity of the CDR system to anticholinesterases will be demonstrated in AD, Dementia with Lewy bodies and Parkinson's dementia. Finally, the CDR system has a large normative database which allows treatment effects in dementia to be put into an unambiguous clinical perspective.

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The Early Development of TC-1734 (AZD3480) in Volunteers and Older Subjects Indicating a Beneficial Effect on Cognition

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Background: TC-1734 (AZD3480) is a highly selective partial agonist at the α4β2 neuronal nicotinic (acetylcholine) receptor (NNR) that has demonstrated pro-cognitive and neuroprotective properties in multiple preclinical models. It has minimal effect at muscle and ganglion NNRs.

Phase 1 Development: In studies involving young and older healthy volunteers (n=78), the compound demonstrated an acceleration profile on pharmaco EEG, after both single and multiple dosing. Cognitive performance was measured using a computer test battery (CDR). No effect was seen in a single dose study involving young volunteers but in a multiple dose trial lasting 10 days, a beneficial effect was seen on cognition, in particular attention and memory (both immediate and delayed word recall). In an elderly PK/PD study, cognition was enhanced for up to 48 hours following a single dose of medication (elimination half life =5-7 hours).

Phase 2a Development: A three week proof of concept study (n=76) was undertaken in older subjects with subjective and objectively defined age associated memory impairment (AAMI). A number of doses were evaluated and a dose of 50 mg TC-1734 (AZD3480) was found to have the broadest enhancement. At that dose results on 4 of the 5 CDR factors (PoA, CoA, QoEM and SoM) favored the compound.

Phase 2b Study in AAMI: In this trial (n=193) the results on all three co-primary outcome measures (PoA, QoEM factors from the CDR and a subject rated global impression scale) tested in a hierarchical fashion, favored 50 mg TC-1734 (AZD3480).

Results from the studies will be presented.

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A Immunotherapy: Use of Monoclonal Antibodies for Potential Treatment and Amelioration of AD Neuropathology

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The field of Aβ Immunotherapy was ushered in for potential treatment of Alzheimer's disease (AD) in 1999 with the observation that immunization of Aβ1-42 in APP transgenic mice resulted in slowing and even reversal of AD-like neuropathology. It was also observed that administration of monoclonal antibodies directed to Aβ, termed passive Aβ Immunotherapy, could also elicit a similar effect in these models, demonstrating that the effect was antibody mediated. This resulted in a number of clinical trials for AN 1792 (a

synthetic form of Aβ1-42 plus the adjuvant QS-21) which ultimately ended in an interrupted phase 2 trial that demonstrated positive clinical signals for a number of endpoints and biomarkers but had to be terminated due to a 6% incidence of encephalitis in some patients. Since this time, extensive basic research and clinical effort has resulted in numerous Aβ Immunotherapy clinical candidates that are currently undergoing testing worldwide, exploring both active and passive approaches for treatment of AD. In particular, it has been shown by our laboratories and others, that passive immunotherapy can result in reversal of Aβ plaque pathology, synaptic reduction and cognitive deficits in APP transgenic models. This work has been extended into the clinic with the ongoing development of bapineuzumab which has completed phase 1 testing and is currently in phase 2 clinical development, and as such is at the most advanced stage of clinical development of all of the various Aβ immunotherapeutic approaches. Based upon available and ongoing mouse and human testing data, it is anticipated that Aβ immunotherapy has and will likely ultimately provide a key test of the Amyloid Hypothesis for treatment of AD.

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Alpha-Synuclein Clearance and Vaccination Strategies in the Development of New Treatments Parkinson's Disease

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Background and Aims. It has been proposed that the neurodegenerative alterations in Parkinson's Disease(PD) and Alzheimer's Disease(AD) conditions might be related to abnormal accumulation of Abeta and α-synuclein aggregates at the synaptic sites, leading to lysosomal leakage and signaling abnormalities. Development of therapies aimed at protecting synapses by regulating signaling pathways or preventing α-synuclein aggregation holds significant promise. Development of anti-parkinsonian treatments includes anti-aggregation compounds, neuroprotective compounds, neurotrophic agents, vaccination, and stem cell and fetal cell grafting. Special interest has emerged in developing treatments to block abnormal compartmentalization and aggregation of α-synuclein.

Methods. We have focused or the use of monoclonal antibodies with passive and active vaccination The hypothesis is that antibodies against α-synuclein might cross the BBB and block the formation of toxic α-synuclein aggregates. For this purpose, α-synuclein transgenic(tg) mice were vaccinated with recombinant full-length or fragments of α-synuclein or received injections of monoclonal antibodies against α-synuclein.

Results. In mice that produced high-affinity antibodies there was decreased accumulation of aggregated α-synuclein in neuronal cell bodies and synapses that was associated with reduced neurodegeneration. Furthermore, antibodies produced by immunized mice recognized abnormal α-synuclein associated with the neuronal membrane and promoted the degradation of α-synuclein aggregates probably via lysosomal pathways.

Conclusions. These results suggest that vaccination is effective in reducing the neuronal accumulation of α-synuclein aggregates and that further development of this approach might have a role in the treatment of PD. Such an anti-amyloidogenic property might also provide a novel strategy for the treatment of other neurodegenerative disorders.

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Potential Mechanisms of Abeta Plaque Clearance in Patients Immunized With Abeta

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Animal models of Aβ immunotherapy have provided evidence of at least three different mechanisms by which Aβ can be removed from the brain: binding of antibody to plaque Aβ resulting in Aβ solubilisation; opsonisation of plaque Aβ by antibody prompting phagocytosis by microglia; and plasma antibody altering the equilibrium of Aβ across the blood-brain-barrier. Within the limitations of the human studies here we explore to what extent we can confirm these mechanisms occur in humans. Neuropathological evidence from patients with Alzheimer's disease (AD) immunized with Aβ in the initial Elan Pharmaceuticals trials has confirmed that plaque removal occurs. Plaque removal is associated with (i) the presence of Aβ in microglia, confirming that phagocytosis occurs (ii) increased vascular Aβ, including capillary angiopathy, likely to reflect solubilised Aβ leaving the brain along perivascular drainage pathways and across the blood-brain barrier. There is a substantial increase in the amount of Aβ42 associated with the vasculature, likely to be derived from plaques. The active phase of plaque removal may therefore be associated with processes that, at least transiently, may not be helpful, namely increased microglial activation, increased soluble Aβ, and increased vascular Aβ. It would seem logical to intervene as early in the Alzheimer process as possible.

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Neurogenetics: Preclinical and Clinical Aspects of AD, PD and FTD

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To date 12 forms of familial PD have been mapped to certain loci on the chromosomes and 7 disease genes have been identified. PARK1 is an autosomal dominant form cause by mutations of the alpha-synuclein gene. The missense mutations are very rare, however, recently, duplication and triplication of the entire coding region and exons of adjacent gene have been reported more frequently. The triplication of the gene cause parkinsonism and dementia, i.e., clinical phenotypes are either PDD or DLB. Recently, we had a chance to exam five families with duplication of the alpha-synuclein gene. Clinical phenotypes were either PD without dementia or PDD. One of the autopsied patients had PDD clinically and autopsy showed transitional type of Lewy body disease. Generally live patients with duplications showed relatively mild parkinsonism responding well to levodopa. In any autosomal dominant families, possibility of duplication

should be considered. PARK2 is an autosomal recessive form caused by parkin gene mutations. One of the hot topics on PARK2 is whether or not single heterozygotic mutation of the parkin can cause PD or serves as a risk factor for sporadic PD. To answer this question, we reviewed our cases and cases reported in the literature. According to our hand, 28 cases of parkin heterozygotes were identified among 1035 families studied. There was no clear autosomal dominant inheritance in heterozygotes. Parents of affected heterozygotes were usually not affected. These findings suggest that single heterozygotes are not suffice to cause PD; it appears more likely that the second mutations are difficult to find with the current technique. Also many heterozygotes are known in PARK6. This will be also discussed in this talk. PARK8 is another autoromal dominant familial PD caused by mutations of LRRK2 and clinical features are very similar to those of sporadic PD.

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Genes Associated With Familial Parkinson's Disease Share Pathological Actions That Converge on the Mitochondria

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Mutations in α-synuclein (AS), parkin, DJ-1, Pink1 and LRRK2 are associated with familial Parkinson's disease (PD). Our studies in cell culture and in vivo point to three general biochemical systems that are regulated by these genes: protein degradation (mediated by the proteasome and autophagy), neuroprotection (mediated by the Akt and stress kinase cascades) and mitochondrial function. In vitro studies suggest that AS modulates proteasomal function and autophagy. AS also exhibits a high tendency to aggregate. AS aggregation is readily detectable in vivo, but symptomatic transgenic mice or C. elegans over-expressing AS do not show much evidence of proteasomal inhibition, which suggests that other mechanisms of toxicity might play a more important role in PD. Parkin, DJ-1 and Pink1 have all been shown to modulate cellular pathways associated with neuroprotection, such as the Akt pathway. Our studies indicate that LRRK2 also modulates the Akt and stress kinase cascades, which highlight the potential role of neuroprotection in the mechanism of action of familial PD genes. Using C. elegans we compared the actions of each of these genes in vivo. C. elegans lines are selectively vulnerable to mitochondrial inhibition if they express AS or LRRK2, or lack parkin, DJ-1 or Pink1. These results suggest that mitochondria are a point of convergence for the action of many genes implicated in PD. Based on these data, we hypothesize that the neurodegenerative response associated with PD derives from stresses that are associated with mitochondrial dysfunction.

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Gene Profiling Within Cholinergic Basal Forebrain Neurons During the Progression Alzheimer's Disease

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The molecular mechanisms underlying the selectively vulnerable of cholinergic basal forebrain (CBF) neurons within the nucleus basalis (NB) during the preclinical stage of Alzheimer's disease (AD) remain unknown. CBF expression was assessed using single cell gene profiling applied to tissue harvested from cases with a premortem clinical diagnosis of no cognitive impairment (NCI), mild cognitive impairment (MCI), and AD. CBF neurons displayed a significant down regulation of trkA-C receptors with an intermediate reduction in MCI and the greatest decrement in AD. In contrast, we found a lack of regulation of p75NTR expression. Profiling indicated no significant differences in expression levels for the six- tau isoforms across groups. However, a significant decrease in the 3Rtau/4Rtau expression ratio was found in MCI and AD relative to NCI. CBF single cell analysis revealed a lack of differential regulation of calcium/calmodulin-dependent protein kinase II, alpha, cyclin-dependent kinase 2, cyclin-dependent kinase 5 subunit p35, or mitogen activated protein kinase p38 across clinical groups. There was a significant down regulation of protein kinase B in AD and an intermediate reduction in MCI. Down regulation of p44 extracellular signal-regulated kinase 1 and p42 extracellular signal-regulated kinase 2 occurs in AD and MCI compared to NCI. A significant up regulation of cyclin-dependent kinase 5 occurs in AD compared to MCI and NCI, which may be relevant for tau phosphorylation events in CBF neurons. These results suggest that within CBF neurons alterations occur in several classes of signal transduction and tau genes during the progression of AD.

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Clinical and Pre-Clinical Aspects of Familial Parkinson's Disease

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Considerable progress has been achieved in the understanding of the molecular pathogenesis of Parkinson's disease (PD) through the mapping and cloning of genes for monogenically inherited forms. In a small number of families with autosomal dominant inheritance and typical Lewy body pathology, mutations have been identified in the gene for a-synuclein. Aggregation of this protein in Lewy bodies may be a crucial step in the molecular pathogenesis of familial and sporadic PD. Importantly, not only point mutations, that may physically alter the aggregation properties of the protein, but also gene multiplications (duplications and triplications) have been found to cause PD, indicating that a mere protein overload is sufficient to damage dopaminergic neurons. A

second and much more common autosomal-dominant form of PD has recently been attributed to mutations in the gene for leucine-rich repeat kinase 2 (LRRK2). Patients with these mutations have typical a-synuclein pathology in most, but not in all cases. The clinical picture is very similar to idiopathic PD, and depending on the population studied, a surprisingly high percentage of apparently sporadic PD patients are found to carry LRRK2-mutatations, and increasing evidence is emerging that relatively common variants in the a-synuclein and LRRK2-gene also play a role in the etiology of sporadic PD.

These findings now allow to study the natural course and spectrum of clinical manifestations in PD patients with defined genetic contributions to the disease process.

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In Vivo and Post Mortem Clinicoanatomical Correlations in FTDP-17

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Apoe4 and Paraxonase 1 Genetic Variants Affect Both Risk Levels and Cholinergic System Integrity in AD

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The discovery that the apolipoprotein E4 (apoE4) allele is strongly linked to sporadic late onset Alzheimer's disease (AD) raises the possibility that a dysfunction of the lipid transport system could seriously affect lipid homeostasis in AD subjects and could compromised lipid-dependent neurotransmitter systems such as the cholinergic system. We have shown a little while ago that phospholipids transported by apoE4-HDL particles are significantly more oxidized than those transported by apoE3-HDL complexes. This suggests a possible interplay between apoE4 and the lipoprotein-bound antioxidants vitamin E and Paraxonase 1 (PON1). These observations prompted us to examine the role of PON1 in the human brain as a function of cholinergic integrity in AD and to map the effect of known polymorphisms in the PON1 gene on risk levels and cholinomimetic drug responses in living patients. Results obtained so far clearly establish a highly significant association between PON1 genetic variants and AD risk and a marked genotype-dependent reduction of choline acetyltransferase activity and m1 receptor density in the hippocampal and cortical areas in AD. Ongoing studies using AD subjects treated with cholinomimetic agents will be put to contribution to replicate a recent report suggesting a genotype-dependent effect of PON1 polymorphisms on Aricept and Exelon efficacies in mild-to-moderate AD. Supported by grants from CIHR and the Alcan corporation. J.P. is a Canadian Institute on Aging senior scientist.

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Apolipoprotein E and Neurodegenerative Diseases

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Background: In accordance with the reductionistic paradigm, various molecules (DNA, proteins, lipids, sugars, and free radicals) are implicated in neurodegenerative diseases. The discovery of genetic mutations for familial forms of Alzheimer's disease (AD) and Parkinson's disease (PD) has given rise to heightened expectations for better understanding of the etiology of such diseases and, ultimately, effective therapies. An important assumption underlying this approach is that molecular mechanisms operating in familial forms of disease are also primarily involved in sporadic cases. An alternative possibility is that familial and sporadic forms share common end-points but arrive there via different etiologies. If so, it is important to identify factors that confer a disproportionate risk for sporadic forms of disease and elucidate the mechanisms that confer such risk. Apolipoprotein E (apoE) genotype is a highly dominant genetic risk factor for AD and is implicated in PD and other neurodegenerative diseases. However, the precise role played by apoE, especially apoE4, in these disorders remains unknown and largely unexplored.

Methods and Results: We have been pursuing the hypothesis that proteolytic fragments of apoE contribute, in an isoform-specific manner, to neuronal degeneration. Recent findings suggest that full-length apoE protects against toxicity of apoE-related peptides and that proteolytic generation of apoE fragments may involve aspartic proteases such as cathepsin D. The latter reaction is inhibited by oligomeric or fibrillar Aß42.

Conclusions: ApoE may play a direct role in neurodegeneration. We hope to identify mechanisms that may explain the profound contribution of apoE genotype to the risk of neurodegenerative disease.

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The Fatal Attraction Between Amyloid Beta and Apolipoprotein E4 in Alzheimer's Disease

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A growing body of evidence suggests that the pathological effects of amyloid beta (Abeta) are mediated by soluble Abeta oligomers. ApoE4, the most prevalent genetic risk factor of Alzheimer's disease is associated with increased Abeta deposition and with impairments in neuronal plasticity. We presently examined the hypothesis that these seemingly different pathological effects of apoE4 are both mediated by soluble Abeta oligomers, whose levels and neurotoxicity are specifically elevated by apoE4.

ApoE4 and apoE3 transgenic mice were subjected to activation of the amyloid cascade by inhibition of the Abeta degrading enzyme neprilysin. Biochemical measurements revealed that this triggered the formation of intracellular 55kD Abeta oligomers specifically in apoE4 mice. Immunohistochemical studies revealed specific accumulation of Abeta in CA1 neurons of the apoE4 mice which was associated with apoptotic loss of CA1 neurons and with learning and memory impairments.

Environmental stimulation triggered neurogenesis and synaptogenesis in the hippocampus of apoE3 mice, whereas it triggered apoptosis in the hippocampus of apoE4 mice. Furthermore, environmental stimulation prompted the formation of 55 kD Abeta oligomers, specifically in the apoE4 mice . The levels of oligomerized Abeta were particularly high in hippocampal dentate gyrus neurons of the apoE4 mice , which are the neurons most markedly affected by apoE4 following environmental stimulation.

These findings suggest that the amyloid cascade and neuronal plasticity-related pathological effects of apoE4 are both mediated via distinct Abeta oligomers. The mechanisms underlying the effects of apoE4 on the oligomerization of Abeta and the associated neuropathology , will be discussed

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Apoe Alleles in Parkinson's Disease: Relation to Cognitive Decline and Hallucinations. A Longitudinal Study

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Background: Apolipoprotein e (Apo e) is a polymorphic proteinwith three major alleles: 2, 3, and 4, yielding six possible genotypes and translating into three major isoforms of the protein: ApoE2, ApoE3, and ApoE4. The 2 allele is considered protective in Alzheimer disease (AD). The 4 allele is associated with an increased risk of developing AD and a lower age at disease onset. Alzhemer-type changes contribute to dementia in Parkinson's disease (PD), but evidence for the role of APO E in PD has been inconclusive. Some studies have shown associations of the 4 allele with PD and development of dementia or hallucinations in PD, but conflicting results have been reported.

Objective: To explore the role of Apo e alleles in the progression of cognitive decline and hallucinations in PD.

Methods: Patients were recruited from an ongoing population based study in Rogaland, Norway. 245 Patients were followed prospectively for up to 12 years, and assessed with MMSE, Neuropsychiatric Inventory and UPDRS. A subgroup of the patients has come to autopsy and showed brain chances compatible with PD. Blood from 96 patients were screened and the Apo e allele-type characterised and correlated with the longitudinal clinical data.

Results: Neither e2-alleles nor e4 alleles were associated with age at onset of PD nor the longitudinal course of cognition, hallucinations or parkinsonism.

Conclusion: We cannot confirm an association of Apo e-alleles with cognitive decline or hallucinations in PD.

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Transgenic Mouse : Amyloid and Tau Pathology Are Linked by GSK-3b

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We generated single and bigenic transgenic mice that develop all pathological hallmarks of AD. Our APP-V717I transgenic mice present amyloid pathology characterized by intracellular amyloid, diffuse and senile plaques, vascular deposits, progressively with ageing. This late pathology is preceded by early defective cognition and LTP, not rescued by inactivation of Presenilin1 (Moechars et al,1999; Van Dorpe et al, 2000; Dewachter et al, 2002). Our Tau-P301L mice display morbid tauopathy with intracellular tau-filaments and mortality before age 1 year (Terwel et al, 2005) but, surprisingly, preceded by improved cognition (Boekhoorn et al, 2006). Ageing APP-V717I x tau-P301L bigenic mice (13-18 months) have combined AD-like pathology in hippocampus and cortex ("plaques and tangles"), with more extensive amyloid than the parent APP-V717 mice, and dramatic enhanced forebrain tauopathy, particularly in hippocampus CA1. Remarkably, tau-P301L mice die before age 1 year, the APP-V717I x tau-P301L bigenic mice survive longer, which is tightly correlated to alleviation of brainstem tauopathy that is much less prominent than in the parental tau-P301L mice. Even more remarkable, also tau-P301L x GSK-3b bigenic mice have normalized lifespan relative to tau-P301L mice, again correlating with strongly reduced brainstem tauopathy, despite dramatic forebrain tauopathy with "tangles in almost every neuron". The combined data corroborate that (i) neither amyloid not neurofibrillary tangles are toxic per se, (ii) GSK-3b is a missing link between amyloid and tau-pathology, (iii) with important brain-regional differences in its action.

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GSK3 Inhibitors for Alzheimer´S Disease

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The development of dementia highly correlates with the formation of intracellular neurofibrillary tangles (NFT), and brain atrophy. Abnormal hyperphosphorylation of the microtubule associated protein tau results in cytoskeletal abnormalities and its subsequent post-translational modification result in the formation of paired helical filament (PHF), the major constituents of the NFT. GSK3 a serine / threonine kinase has been implicated as a major kinase in the aberrant hyperphosphorylation of tau leading to NFTs in AD.

GSK3 phosphorylates the majority of PHF phosphorylation sites in AD brain. Transgenic mice overexpressing GSK3β exhibit extensive pre-tangle pathology such as tau hyperphosphorylation at PHF sites, neurodegeneration, gliosis and cognitive deficits, thereby providing a strong link between increased GSK3 activity and tau pathology in AD.. Accordingly, inhibition of pre-tangle pathology via GSK3 inhibition would be expected to slow down the progression of NFT formation and neurodegeneration in AD.

We report here the discovery of a series of GSK3 inhibitors. The pyrazine chemical series were found to be selective and potent inhibitors of GSK3 in vitro and in vivo. We provide evidence for cellular and in vivo reduction of tau phosphorylation and microtubule deficits in brains of mice overexpressing the tau mutation P301L. In vivo exposure studies showed that these compounds are brain permeable. The pyrazines may thus have important implications in the treatment of AD and realed dementias

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Down-Regulation of Brain-Derived Neurotrophic Factor by Oligomeric Amyloid as a Mechanism for Alzheimer-Associated Dysfunction

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The mechanism by which amyloid-beta (Aβ) might cause progressive synaptic dysfunction and degeneration in selective areas of the Alzheimer's disease (AD) brain is unclear. Down-regulation of brain-derived neurotrophic factor (BDNF) is one such mechanism. BDNF maintains survival and function of specific neurons and their connections that are compromised in AD and regulates neuronal excitability, dendritic growth, neurogenesis, synaptic transmission and plasticity and learning and memory. BDNF mRNA and protein are dramatically decreased in brain areas that are compromised in AD, and this occurs early in the disease. Transgenic mice with similarly decreased BDNF levels exhibit deficits in LTP and learning and memory tests. In human subjects, decreased cortical BDNF correlates with decreased cognitive scores.

We used in vitro and in vivo models to define the mechanism of BDNF down-regulation in AD. Seven different transcripts of the BDNF gene are expressed in human cortex, but only one major and two minor transcripts are specifically down-regulated in AD. In human neuroblastoma cells, oligomeric but not fibrillar Aβ down-regulates the major BDNF transcript. Transgenic mice expressing high levels of Aβ, but not those over-expressing APP, exhibit decreased BDNF mRNA. The degree of BDNF down-regulation correlates with the molecular form of Aβ in different transgenic mouse strains. Thus, oligomeric Aβ down-regulates BDNF in vitro and in vivo, suggesting that decreased BDNF plays a role in synaptic and neuronal dysfunction underlying cognitive impairment in AD.

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Alzheimer Disease-Like Cortical Imbalance of Neurotrophin Factors in Tau Transgenic Mice With Memory Deficits and Neurofibrillary Degeneration

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Tau transgenic mice are valuable models to investigate the role of tau protein in Alzheimer's disease (AD) and other tauopathies. However, motor dysfunction and dystonic posture interfering with behavioral testing are the most common undesirable effects of tau transgenic mice. Therefore, we have generated a novel mouse model (THY-Tau22) that expresses human 4-repeat tau mutated at sites G272V and P301S under a Thy1.2-promotor, displaying tau pathology in the absence of any motor dysfunction. THY-Tau22 shows hyperphosphorylation of tau on several AD-relevant tau epitopes, neurofibrillary tangle-like inclusions and PHF-like filaments. These mice also display impaired behaviour characterized by increased anxiety, delayed learning and reduced spatial memory. There are no signs of motor deficits or changes in motor activity at any age investigated. Decreased levels of brain-derived-neurotrophic-factor (BDNF) and increased levels of nerve-growth-factor (NGF) in the hippocampus and cortex very similar to what is reported from AD post mortem brain were also observed. BDNF mRNA and protein levels are reduced by 6 months in tau transgenic animals. Interestingly, the loss of BDNF correlates negatively with tau abnormally phosphorylated at AT100. In the entorhinal cortex and hippocampus, neurons accumulate NGF-immunoreactivity. Interestingly, some of these neurons colocalize accumulated NGF and AT100, but not AT8 indicating a crucial role of abnormal phosphorylated tau in this process. This mouse model therefore displays several of the pathophysiological disturbances observed during neurofibrillary degeneration. It is also the first mouse model showing an AD-like imbalance of BDNF and NGF in cortex and hippocampus.

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Seeding Neuritic Plaques From the Distance: A Possible Role for Brainstem Neurons in the Development of Alzheimer's Disease Pathology

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Our goal is to understand the pathogenesis of neuritic plaques in the Alzheimer's disease (AD) brain. It is thought that the amyloid-β (Aβ) deposits within the cerebral cortex and hippocampus are initiated by a "bad seed" of oligomeric Aβ. The origin of this seed is unknown. We established a cell culture system, where brainstem-derived, catecholaminergic, neuronal cells (CAD) accumulate within their processes large amounts of Aβ peptide, similar to what is believed to occur in brain neurons, in the initial phases of AD. This accumulation of Aβ begins within neurites, prior to any detectable sign of neurodegeneration. Neuritic accumulation of Aβ is restricted to a small population of cells with normal levels of amyloid-β precursor protein (APP), but showing redistribution of β-secretase into the processes, where it co-localizes with Aβ and markers of late endosomes and autophagic vacuoles. Unlike cultured cortical and hippocampal neurons, the brainstem-derived CAD cells accumulate oligomeric Aβ primarily at the terminals of their processes. Since brainstem neurons innervate many brain regions, including the cerebral cortex and the hippocampus, they could provide the "bad seed" that nucleates plaques. These results suggest that Aβ accumulation is initiated in a small number of brainstem neurons by altered APP metabolism. We propose that plaque formation in the cerebral cortex and hippocampus is seeded by oligomeric Aβ accumulated at the terminals of projections of brainstem neurons. CAD cells appear to recapitulate the biochemical

processes leading to Aβ deposition, thus providing an in vitro experimental system for studying the pathobiology of AD.

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Elevated Cortical Zinc in Alzheimer's Disease

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Background: Recent studies suggest a possible role of biochemical interaction between amyloid b-peptide (Ab) and biometals in the pathogenesis of Alzheimer's disease (AD). Material and Methods: Here we studied the association between metal (zinc, copper, iron, cobalt, manganese and aluminum) and Ab levels in post-mortem brain neocortical tissue from patients with AD (n=10), schizophrenia (n=26), schizophrenia with AD pathology (n=8) and normal age-matched controls (n=14). Severity of cognitive impairment was assessed with the Clinical Dementia Rating Scale.

Results: There was a significant, more than twofold, increase of tissue zinc in the AD-affected cortex compared to the other groups. Zinc levels positively correlated with tissue Ab42 levels. Zinc levels were significantly ($p< 0.001$) elevated in the most severely demented cases (CDR 4-5) and in cases that had an amyloid burden greater than 8 plaques/mm2. Copper levels significantly ($p<0.001$) declined with age in non-AD subjects. Levels of other metals did not differ between groups.

Conclusions: These data indicate that brain copper levels decline with age and that brain zinc accumulation is a prominent feature of advanced AD.

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Amyloid Beta and Neuromelanin Toxic or Protective Molecules? The Cellular Context Makes the Difference

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Alzheimer's disease (AD) and Parkinson's disease (PD) share several pathological mechanisms. The parallels pathways and roles of amyloid beta (Aβ) in AD and alpha-synuclein in PD have been discussed in several reports. However, studies of the last few years show that Aβ also shares several important characteristics with neuromelanin (NM), whose role in PD is emerging. First, both molecules accumulate with aging, the greatest risk factor for AD and PD. Second, in spite of their different structures, Aβ and NM have similar characteristics that could also lead to neuroprotection. Metals are required to catalyze their formation and they can bind large amounts of these metals, generating stable complexes and thus playing a protective role against metal toxicity. Moreover, they may be able to remove toxic species such as oligopeptides and excess cytosolic dopamine. Third, both Aβ and NM have been implicated in some of the same aspects of the neuronal death that underlie AD and PD, respectively. For example, both molecules can activate microglia, inducing release of toxic factors such as tumor necrosis factor-alpha, interleukin 6 and nitric oxide. A careful analysis of these parallel effects of Aβ and NM, including their seemingly paradoxical ability to participate in both cell death and protection, may lead to an improved understanding of the roles of these molecules in neurodegeneration and also provide insights into possible parallels in the pathological mechanisms underlying AD and PD.

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The Rho GTPases Play a Role in Actin Polymerization Stimulated by the Abeta(1-42) Amyloid Peptide in Hippocampal Neurons

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Alzheimer´s disease is a multifactorial brain disorder affecting the elderly, that has become on the major public health problems with more than 24 millions affected throughout the world. Several cognitive and neurodegenerative disorders, like Alzheimer´s disease, involve alterations in both microtubules and actin filaments cytoskeleton. We have found that the Rho GTPases Cdc42 and Rac1, essential for actin dynamics regulation, increase their activities, as related with actin polymerization in Abeta-treated hippocampal cells. Interestingly, Rac1 up-regulation, as a result of Abeta treatment, involves the participation of Tiam1, a Rac guanine-nucleotide exchange factor, through a mechanism highlighted by Tiam1 activation by changes in calcium transients. These studies suggest that Rho GTPases constitute a target in Abeta-induced neuronal degeneration in Alzheimer´s pathology, through the remodeling of the actin cytoskeleton. A set of neuronal proteins are also involved in the signaling pathway regulated by the Rho GTPases. These results contribute to elucidating the signaling mechanism around the pathogenesis of Alzheimer´s disease. (Financed by the International Centre for Biomedicine (ICC) and grant 1050198 from Fondecyt).

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Herpes Simplex Virus Type 1 (HSV1) in Brain is a Cause of the Neuropathological Features of Alzheimer's Disease (AD)

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Background: The causes of AD have still to be elucidated, despite many studies on the main neuropathological features of the disease – amyloid plaques and neurofibrillary tangles. Our research has implicated the common virus, HSV1, as a major factor in the condition when it is present in brain of people carrying the type 4 allele of the apolipoprotein E gene (APOE). (The role of HSV1 and APOE in AD is strongly if indirectly supported by our discovery that APOE determines outcome of infection by four different viruses.)

Methods: In situ PCR, immunocytochemistry, immunohistochemistry.

Results: Our recent findings link HSV1 directly to the abnormal brain features of AD: firstly, HSV1 infection of several different cell types in culture leads to an intracellular accumulation of β-amyloid – the main component of plaques; secondly, HSV1 infection of mice results in β-amyloid deposition in the brain; thirdly, HSV1 infection of cultured cells causes abnormal, AD-like, tau phosphorylation, the main component of tangles.

Our current findings have explored further the association between HSV1 and AD neuropathological features: we determined the proximity of the HSV1 DNA to amyloid plaques in AD brain. Remarkably, we found that the viral DNA is almost wholly localised within the plaques.

Conclusions: These and our previous findings strongly implicate HSV1 in plaque formation and in the development of AD. In addition, they support our proposal that antiviral agents be used to prevent the deterioration of AD patients and possibly in future, vaccination against HSV1 to prevent the occurrence of the disease.

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Fulfillment of Hypothesis: A Direct, or Surrogate, Not Necessarily Unique, Role for Helicobacter Infection in the Pathogenesis of Idiopathic Parkinsonism

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The conventional concept for an environmental cause of idiopathic parkinsonism (IP) is of an insult, temporally remote from diagnosis. To the contrary, we described the fit of Helicobacter pylori (Hp) (Medical Hypotheses 2000;55:93-98). Following Helicobacter eradication, 'malignant' IP appears converted to 'benign', whilst marked deterioration accompanied failure (Helicobacter 2005;10:267-297). Although a minority of probands are urea-breath-test positive, the serum immunoblot Hp antibody-profile predicts risk, presence, severity and deterioration. Continuing low-density infection, dectable in biopsies only by molecular methods, seems to be important, and past infection may be seminal to a cascade of current events.

We report follow-up, to a mean of 468 days after deblinding (scheduled 1 year post-treatment), of the randomized, double-blind, placebo-controlled efficacy study of Hp eradication on the time-course of measures of IP, in 30 recruits, taking no, or stable long-t½, anti-parkinsonian medication. (Levodopa use was an exclusion.) Those still infected following placebo received open-active. Protocol analysis on the principal outcome (time-trend in mean stride-length, at free-walking-speed) showed a clinically-relevant effect (7.3 (95% C.I. 1.4,13.2) cm/year, P<0.01), in favour of biopsy-proven successful, blinded-active eradication over placebo (which had no effect), irrespective of presence/absence of anti-parkinsonian medication. The improvement was echoed in those receiving open-active subsequent to placebo (9.5 (1.2, 20.1) cm/year, P=0.04). Improvement following blinded-active did not fall-off after deblinding, and, similarly, that after open-active had plateaued after 1 year.

It remains to be seen whether follow-up to 5 years will show any deterioration predicted from serology, and whether that could be pinned-down to another remediable cause.

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Beta-Secretase as a Therapeutic Target

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Background: Finding inhibitors of Abeta generation is a major goal of Alzheimer's disease drug development. Two target protease activities, beta-and gamma-secretase, were operationally defined in the early 90s, but progress in this area was slow, because the actual enzymes were not understood at the molecular level.

Results and Conclusions: Some years ago we have identified a novel membrane bound aspartic protease, BACE1, as beta-secretase. This finding has been confirmed and BACE1 and its homolog BACE2 have been characterized in detail by many groups. Major progress has been made in two areas: First, the x-ray crystal structure, which is critical for rational inhibitor design, has been solved and shown to be similar to that of other pepsin family members. Second, knockout studies show that BACE1 is critical for Abeta generation, but knockout mice show no gross pathology, raising the possibility that therapeutic BACE1 inhibition could be accomplished without major mechanism based toxicity. However, as more BACE1 substrates are identified, new mechanism based liabilities may emerge. While various peptidic beta-secretase inhibitors have been published, the key challenge now is the generation of more drug-like compounds that could be developed for therapeutic purposes. Other current areas of investigation, including identification of additional BACE1 substrates, the potential role of BACE1 overexpression in AD and the phenotype of BACE2 knockout mice will be discussed.

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Stimulation of the Non-Amyloidogenic Alpha-Secretase Pathway by the Neuropeptide PACAP in Vitro and in Vivo

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Increase of alpha-secretase activity by overexpression of the alpha-secretase ADAM10 in mice transgenic for human APP[V717I] enhanced the production of neuroprotective sAPP-alpha, reduced the formation of soluble A-beta peptides, and prevented their deposition in plaques. Functionally, impaired cognitive deficits were alleviated. Therefore, alpha-secretase stimulation in the brain provides an attractive strategy for the treatment of Alzheimer's disease (AD).

Analysis of cortical gene expression in several transgenic AD mouse models and in human AD temporal cortex showed a downregulation of the neuropeptide pituitary adenylate cyclase-activating polypeptide PACAP, which has neurotrophic, neuroprotective and anti-apoptotic properties and is involved in learning and in memory processes. The PAC1-receptor, a G protein-coupled receptor of type B, is specific for PACAP and is localized in brain areas affected by AD. We examined the effect of PAC1-receptor activation on alpha secretase cleavage of APP in vitro and in vivo. Stimulation of endogenously expressed PAC1 receptors with PACAP in human neuroblastoma cells increased sAPP-alpha secretion which was completely inhibited by a PAC1 receptor-specific antagonist. The increase of alpha-secretase activity by PACAP was abolished by hydroxamate-based metalloproteinase inhibitors including an ADAM 10 inhibitor. By using several specific protein kinase inhibitors we show that the MAP-kinase pathway and PI3-kinase mediate the PACAP-induced alpha-secretase activation. Intranasal application of PACAP-38 in transgenic APP[V717I] mice for three months significantly increased the generation of neuroprotective sAPPalpha and reduced the production of soluble A-beta-40 and A-beta-42 peptides. Our results suggest that PAC1-receptor agonists might be of therapeutic value for the treatment of AD.

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in Vitro and in Vivo Modulation of the Gamma Secretase Enzyme Complex With Orally Bioavailable Small Molecules

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A large body of scientific evidence from genetic, biochemical, biophysical, pharmacological, pathological and epidemiological studies has revealed that the Aß42 peptide is one of the "prime suspects" in the pathogenesis of Alzheimer's disease. Hence, numerous drug discovery strategies have centered on lowering the physiological levels of this particular Aß peptide, by either reducing its production or by enhancing its degradation/clearance.

We recently discovered and optimized a series of small molecules (Series591) that modulate the membrane associated gamma secretase enzymatic complex without affecting its overall catalytic activity. A number of these compounds are able to substantially lower the levels of Aß42 and, to a lesser degree, Aß40 while causing a concomitant increase in the formation of shorter, less amyloidogenic Aß38 and Aß37 peptides in both in vitro and in vivo paradigms. In vitro studies have shown that compounds within Series591 show no detectable inhibition of other known gamma-secretase substrates such as Notch or E-cadherin at concentrations at least 1000-fold over the IC50 necessary for Aß42 inhibition. In addition, Series591 compounds are effective at lowering Aß42 levels in the medium of primary neuronal cultures derived from embryonic mouse brain. Finally, in vivo studies have demonstrated that certain compounds from this series are effective at sustaining significant reductions of Aß42 levels in mouse brain and plasma for at least two weeks following daily oral administration and without significant toxic side effects.

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Gamma-Secretase Modulation: Drug Discovery and Development

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Notwithstanding the tremendous progress in the understanding of Alzheimer's Disease (AD), there remains the challenge to develop bona-fide disease modifying therapies. The central etiologic agents in AD are believed to be the Abeta peptides, more specifically, the Abeta42 isoform, the principal component of the prefibrillar oligomeric aggregates and senile plaques. Much work has been focused on defining the mechanisms of Abeta production/degradation and to develop appropriate therapeutic strategies; i.e., inhibiting production or enhancing degradation of Abeta. Some of the most substantial advances have been made with respect to inhibitors of the gamma-secretase (GS) activity. Although shown to reduce total Abeta in various model systems, the GS is also involved in various, vital physiological pathways, e.g. NOTCH signaling; thus limiting its therapeutic usefulness. However, directing production of Abeta away from the 42 amino acid isoform while leaving the GS activity intact may prove to be more preferable. This GS modulation (GSM), although not fully resolved on the molecular level most likely involves the direct interaction with components of the GS complex and/or its substrates. Therefore, we will present our current efforts at the identification and characterization of highly potent, small molecule GSMs. Additionally, we will review important aspects of Abeta measurement in non-transgenic animals; which may be more appropriate for preclinical characterization of Abeta pharmacological agents.

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Catabolic Clearance of Abeta Following Treatment With Pai-1 Inhibitors

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Alzheimer's Disease (AD) is a progressive neurodegenerative disease and the leading cause of dementia. According to the amyloid cascade hypothesis the amyloid β-peptide (Aβ) assembles into a number of aggregated species including oligomers, fibrils and amyloid plaques, leading to the pathogenesis of AD. Here we describe a novel therapeutic strategy involving the activation of a physiologically relevant proteolytic pathway to enhance the clearance of Aβ.

Tissue plasminogen activator (tPA) cleaves plasminogen to generate plasmin, a serine protease previously demonstrated to cleave Aβ species in vitro. tPA activity is inhibited in vivo by plasminogen activator inhibitor-1 (PAI-1), a serine protease inhibitor whose expression levels are induced in response to certain inflammatory conditions. Since tPA, plasminogen and PAI-1 are expressed in brain, we tested the hypothesis that treatment with small molecule inhibitors of PAI-1 is efficacious for enhancing the proteolytic clearance of Aβ in AD transgenic mouse models expressing human APP. Our data demonstrate that orally administered PAI-1 inhibitors penetrate the blood-brain-barrier and lead to a lowering of plasma and brain Aβ levels, and an improvement of cognitive deficits associated with the expression of soluble Aβ species in pre-plaque and plaque-bearing transgenic mice. In vitro reconstitution leading to the cleavage of Aβ and the in vivo activation of hippocampal tPA following treatment with a PAI-1 inhibitor are supportive of our model.

The therapeutic implications of inhibiting PAI-1, restoring tPA and plasmin activities, and potentially restoring Aβ homeostasis with this novel strategy will be presented.

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Structural Polymorphisms in Amyloid Oligomers

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Amyloidogenic sequences of many different proteins assemble into morphologically distinct hydrogen bonded beta structures that include prefibrillar oligomers, pore-like annular protofibrils and amyloid fibrils. Immunization of mice and rabbits with stable and homogeneous preparations of prefibrillar oligomers, annular protofibrils and fibrils results in the production of three distinct conformation-dependent antibodies that recognize unique generic epitopes on distinct types of amyloid aggregates, indicating that the assembly states are conformationally distinct. These antibodies specifically recognize these assembly states from many different types of amyloids, indicating that the assembly states share a generic structural motif. Analysis of in vitro assembled Aß oligomers by western blotting indicates that the antibodies specifically and mutually exclusively stain homogeneous preparations of prefibrillar oligomers, annular protofibrils and fibrils, but the sizes of bands recognized by the different antibodies are broadly overlapping. These results indicate that size is not a reliable indicator of oligomer conformation. All three antibodies detect naturally occurring amyloid oligomers in human AD brain, but the soluble fibrillar oligomers detected by the anti-fibril antibody correlate best with disease. Taken together, these results indicate that there are 3 general classes of soluble amyloid oligomers (prefibrillar oligomers, annular protofibrils and fibrillar oligomers) and that there are polymorphisms within a class. These results indicate that the problem of identifying and quantifying soluble amyloid oligomers is considerably more complex that previously imagined. This work was supported by NIH NS 38298, AG00538 and a grant from the Larry L. Hillblom foundation.

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The Importance of Steady State Blood Dopa Levels in Reducing Motor Fluctuations

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In the late nineteen seventies Dr Shoulson and colleagues demonstrated that patients with off periods during the waking day on optimal oral drug therapy could be improved in the short term with intravenous l-dopa infusions provided the patient remained at rest and lying down. Standing up or immersion of a hand in cold water, however led to an immediate switch off. Further studies in the early nineteen eighties with steady state intravenous or intraduodenal l-dopa infusions confirmed that the striatal dopamine receptors remained responsive to dopaminergic receptors even during refractory off periods and that the number of waking hours "On" could be markedly improved. The lack of solubility of l-dopa with the need for large and cumbersome pump technology and either duodenostomy or Portacath intravenous lines into the subclavian vein limited this therapeutic approach to all but a few dedicated research centres. More recently continuous subcutaneous apomorphine steady state infusions have been successfully used to improve refractory motor fluctuations in some countries and avoid the need for consideration of deep brain subthalamic nucleus stimulatiion which carries a higher mortality and morbidity.

More recently a new more soluble formulation of l-dopa (Duodpa has been introduced for enteral therapy in patients with severe on-off effects which cannot be controlled by oral medication and several hundred patients have now been treated in Europe. The mechanisms underlying the benefits of steady state delivery of l-dopa to the brain in late stage PD will be discussed including the importance of tolerance and sensitisation.

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Strategies to Get An Optimal Continuous Dopaminergic Stimulation

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In the course of Parkinson´s disease (PD), with progressive loss of dopaminergic neurons, the levodopa-induced therapeutical effect will decrease, and patients will be more dependent on the plasma levodopa levels. As a consequence, due to more discontinuous and pulsatile stimulation of the postsynaptic dopamine receptors, neuroplastic changes of the striatal output neurons will occur with therapeutical response fluctuations.

Treatment options then include both high-frequency deep brain stimulation (DBS) of the subthalamic nucleus (STN) and constant non-pulsatile administration of dopaminomimetic drugs (e.g.transdermal, subcutaneous, intratestinal and/or intravenous).

Through functional inhibition, DBS-STN has been established to induce long lasting reductions of motor parkinsonism up to 60-70%. Apart from surgical and hardware-related complications, DBS related complications comprise dysarthria, eyelid apraxia and behavioral changes such as cognitive deterioration (40%), depression (8%), hypomania (4%), anxiety (2%)1. The latter changes might be multifactorial, and related to pre-existant psychiatric vulnerability, disease progression and/or the reduced need for levodopa. In the selection of patients for DBS-STN a psychiatric screening program is mandatory. Up to approximately 80% of the PD patients were found to have a lifetime psychiatric diagnosis, making them not very suitable for DBS.2 Continuous dopaminergic stimulation (CDS) might offer a safer way to treat both dopamine deficiency-related and therapeutical fluctuations-related motor and non-motor symptoms in PD. Continuous intestinal levodopa delivery appears to be the most promising option in terms of both efficacy and safety. Further experience with this and other CDS strategies are necessary.

1. Temel Y et al. Behavioural changes after bilateral subthalamic stimulation in advanced Parkinson's disease: A systematic review. Parkinsonism Relat Disord 2006;12:265-272.

2. Voon V et al. Psychiatric symptoms in patients with Parkinson's disease presenting for deep brain stimulation surgery. J Neurosurg 2005;103:246-251.

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Duodenal Levodopa Infusion for Advanced PD: A 12-Months Treatment Outcome

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Motor fluctuations and dyskinesia in Parkinson's disease (PD) patients cause severe disability and may not be adequately controlled by oral treatment. A novel gel form of levodopa has enabled its infusion through percutaneous endoscopic gastrostomy directly into the duodenum.

We assessd clinical and quality of life changes after 12 months continuous levodopa duodenal infusion in 9 patients with advanced PD (Hoehn and Yahr Stage ?3) with severe motor fluctuations and dyskinesia who started continuous daily levodopa duodenal infusion and were evaluated at baseline, and at 6 and 12 months.

Seven patients completed the follow-up period. Number and duration of "off" periods and time with disabling dyskinesia was significantly reduced ($p<0.01$). Total daily dose of levodopa infused did not differ from baseline equivalents. There were significant improvements in UPDRS-II (activities of daily living) and -IV (motor complications) in the "on" condition ($p<0.02$), and in four PDQ-39 domains (mobility, activities of daily living, stigma, bodily discomfort; $p<0.05$). Two patients withdrew for adverse events.

Levodopa duodenal infusion improved motor fluctuations and reduced disabling dyskinesia, resulting in significant benefit in quality of life. Our results demonstrate that a satisfactory therapeutic window can be achieved and maintained for several months in advanced PD patients.

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Clinical Characterization of Familial Frontotemporal Dementia and/or Parkinsonism Associated With Mutations in Progranulin

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Background & Aims: We recently identified mutations in the gene encoding progranulin (PGRN) among families with frontotemporal dementia and parkinsonism (FTDP). We report the clinical characterization of 8 kindreds, each with different mutations in PGRN.

Methods: All clinical information was analyzed on affected relatives in eight kindreds in which the probands have been followed in the Mayo Clinic Alzheimer's Disease Research Centers.

Results: There have been 44 affected individuals (18 male), with an age of onset from 49 to 88 years (mean 64.1 years) and disease duration from 1 to 14 years (mean 6.5 years). Inheritance has followed an autosomal dominant pattern. One mutation carrier is asymptomatic at age 73. Clinical diagnoses have included dementia not otherwise specified (NOS, n=16), FTDP (n=3), dementia with parkinsonism NOS (n=4), amnestic mild cognitive impairment (n=1), Alzheimer's disease (AD, n=4), AD with parkinsonism (n=1), frontotemporal dementia (FTD, n=5), parkinsonism NOS (n=2), corticobasal syndrome (n=1), primary progressive

aphasia (PPA, n=6), or mixed FTD/PPA (n=1). None exhibited features of motor neuron disease (MND).

Conclusions: These data indicate wide ranges of onset age and disease duration are associated with PGRN mutations, with some individuals exhibiting initial symptoms well beyond age 70. Penetrance appears high, but may not be 100%. An array of cognitive, behavioral, and motor features can be manifested, although MND appears to be uncommon.

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The Neuropathology of Frontotemporal Lobar Degeneration Caused by Mutations in the Progranulin Gene

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The most common pathology in frontotemporal dementia (FTD) is tau-negative, ubiquitin-immunoreactive (ub-ir) neuronal inclusions (FTLD-U). Recently, we identified mutations in the Progranulin gene (PGRN) as the cause of autosomal dominant FTLD-U linked to chromosome 17. Here, we describe the neuropathology in 13 patients from six different families, each with FTD caused by a different PGRN mutation. The most consistent feature was the presence of ub-ir lentiform neuronal intranuclear inclusions (NII) in the neocortex and striatum. In addition, the neocortex showed moderate-to-severe superficial laminar spongiosis, chronic degenerative changes, ub-ir neurites and well-defined ub-ir neuronal cytoplasmic inclusions (NCI). In the striatum, there were numerous ub-ir neurites. NCI in the hippocampus usually had a granular appearance.

All ub-ir inclusions were also immunoreactive for TDP-43 but showed no staining with a panel of antibodies that recognize the entire PGRN protein. In contrast, familial FTLD-U cases without PGRN mutations had no NII, less severe neocortical and striatal pathology and hippocampal NCI that were more often solid. Eight cases in which genetic analysis was not available also had NII and an overall pathology similar to those with proven mutations. None of our cases of FTLD-U without NII showed the same pattern of pathology as those with mutations. These findings suggest that FTD caused by PGRN mutations has a recognizable pathology with the most characteristic feature being ub-ir NII.

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Analysis of Progranulin in the Manchester FTLD Cohort

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Objective: To assess the contribution of Progranulin to the aetiology of Frontotemporal lobar degeneration (FTLD) in the Manchester cohort.

Background: FTLD is the second most common form of primary degenerative dementia after Alzheimer's disease and is thought to represent up to 20% of presenile dementia. 35-50% of patients have a family history of dementia suggesting a large genetic contribution to the aetiology of this disease. It has been established that mutations in the Progranulin gene on chromosome 17q21 is a cause of FTLD. However, the exact contribution of this gene to the aetiology of FTLD is unknown

Methods: Full sequencing of progranulin was performed in the Manchester cohort (n=270). Furthermore, SNPs at a density of 1 SNP per 1kb covering the progranulin gene were genotyped using the Sequenom MassArray system.

Results: Stop or frameshift mutations were identified in 4% of cases. Some of these were found in apparent sporadic cases. Mutations were only found in 38% of autopsies with intranuclear inclusions. Linkage disequilibrium mapping identified one haplotype associated with disease when restricted to cases with a family history.

Conclusions: Progranulin mutations, in a variety of clinical FTLD syndromes, were found at a similar frequency to that of tau in the Manchester cohort. While intranuclear inclusions were found in all mutation carriers that had undergone autopsy examination not all autopsy cases with intranclear inclusions had progranulin mutations. There are likely other genetic causes for this particular pathological feature.

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Founder Effect of LRRK2 Arg1441Cys in Belgian Parkinson's Disease Patients

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Background and aims: Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene were shown to account for at least 5% of familial Parkinson's disease (PD) patients. Also, in approximately 2% of sporadic patients mutations in this gene were observed. Previous studies showed that carriers of the most prevalent mutations shared the same disease haplotype suggestive of a common ancestor. We performed a mutation analysis of the Roc domain in LRRK2 and four other exons in which mutations have been reported. Methods: Mutation analysis was performed in 270 Belgian PD patients and 270 control individuals by direct sequencing. Results: There was no replication of known mutations; except for the Arg1441Cys mutation – which was identified in five unrelated patients. All other novel mutations were also located in the Roc domain, but were present in only one patient each. Next, available relatives of the probands with LRRK2 mutations were genotyped for 18 intragenic and flanking STR markers spanning a region of 16 Mb. Haplotype and/or allele sharing analysis showed that the Belgian Arg1441Cys haplotype consisted of 11 consecutive markers spanning a 5,8 Mb region.

Analysis in 180 control individuals showed that the shared haplotype was not present in the control population. In addition, segregation analyses of the other mutations in the Roc domain indicated that LRRK2 mutations were not completely penetrant. Conclusion: These data suggested that the Arg1441Cys mutation in Belgium was transmitted from a single common founder. Furthermore, our data shows that family analyses should be approached very carefully.

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Implications From Systematic Meta-Analyses of Genetic Association Studies of AD and PD

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Background and Aims: Alzheimer's disease (AD) and Parkinson's disease (PD) are genetically complex and heterogeneous disorders. To date, a small number of genes have been identified for both diseases causing predominantly early-onset forms with Mendelian inheritance. The majority of AD and PD cases, however, show no obvious familial aggregation and are likely governed by a variety of genetic and non-genetic factors that define an individual's risk. In the past decade, literally hundreds of reports have been published claiming or refuting genetic association between putative AD/PD genes and disease risk or other phenotypic variables, but only a handful of these potential risk factors have shown consistent effects over time.

Methods: We have created two online databases that serve as unbiased, continously updated and publicly available collections of all AD or PD genetic association studies. Data is collected following systematic searches of scientific literature databases, as well as the table of contents of speciality journals. For all polymorphisms with genotype data available in at least three independent case-control samples, we routinely calculate meta-analyses based on allelic crude odds ratios from each study.

Results and Conclusions: All data and results can be retrieved online ("AlzGene": www.alzgene.org, and "PDGene": www.pdgene.org). For both diseases, a number of genetic variants have emerged to show significant risk effects in the meta-analyses. This presentation will highlight the most significant findings from both databases, with a particular focus on genes/variants that concurrently modulate the risk for both disorders, potentially indicating common denominators for neurodegeneration in AD and PD.

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Findings From An Extended Functional Genome Scan on Late-Onset Alzheimer's Disease

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Background and aims: Late onset Alzheimer's disease (LOAD) is the major form of progressive dementia in the elderly. Susceptibility to LOAD is influenced by genetic factors such as APOE4 and others that remain to be identified/validated. We have recently reported novel susceptibility genes for LOAD through genotyping ~20,000 single nucleotide polymorphisms (SNPs) in multiple large case-control series from the UK and US (2000 cases x 2000 controls) (Grupe et al., the 10th International Conference on Alzheimer's Disease and Related Disorders). To increase the coverage of interrogated genes and variants, we have continued to test another large set of putative functional SNPs, totaling ~4,700. Methods: These markers target mostly missense or predicted transcription factor binding site variants (~80%), are distributed across all chromosomes, and cover a wide range of allele frequencies. We have used a multi-stage approach to identify markers that show the strongest association. In the first stage, lead candidate variants were identified after testing ~4,700 markers in one sample set followed by the second stage, where the significant markers were tested in a second sample set. Replicated markers were then moved to stage three for further testing in two other independently collected case-control sample sets. Results: This allowed us to identify SNPs in APOC1 near the APOE locus (full sample P=3.8x10-17), POMT1 (full sample P=0.0006) and several other genes showing association with LOAD. Conclusions: Novel genetic variants are associated with LOAD.

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Characterizing the Pro-Survival Function of Parkin: Relevance to Mitochondrial Integrity

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Autosomal recessive mutations within the ubiquitin E3 ligase, parkin, are responsible for one of the most common forms of inherited Parkinson's disease. These mutations are presumed loss-of-function, but the mechanism by which parkin prevents degeneration in the substantia nigra and locus coeruleus is not known. Knockout of the Drosophila homolog of parkin results in spontaneous apoptosis and degeneration of flight muscle cells and abnormal mitochondrial morphology in the fly.

Mitochondria can play a significant role in the regulation of apoptosis and, consistent with a potential role for parkin in mitochondrial health, we and several groups have reported a pro-survival effect of parkin over-expression. Therefore, we sought to expand our work to include specific parkin-dependent changes in mitochondrial function and behavior.

To determine whether parkin expression simply down-regulates pro-apoptotic proteins (i.e. via increased ubiquitin-dependent turnover), we analyzed several BH3 family members, including Bax and Bim, and found that parkin expression did not grossly alter the levels of these proteins. We did find, however, that over-expression of parkin decreased the apoptotic response of isolated mitochondria. This effect was independent of the cell line tested (MES, SY5Y, HeLa) or the mechanism of parkin expression (i.e. plasmid-based or viral-based). These data provide a novel link between the fly genetics and the pro-survival effects of parkin reported by many groups in mammalian models. This work may lead to critical insights into the role of the parkin protein in cell survival and in Parkinson's disease.

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Novel Regulation of Parkin as a Mechanism of PD Progression

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Mutations in parkin, an E3 ubiquitin ligase, are the most common cause of autosomal recessive Parkinson's disease. Whether parkin plays a role in sporadic forms of Parkinson's disease remains unclear. Here we show that parkin interacts with and is phosphorylated on tyrosine 143 by the stress signaling non-receptor tyrosine kinase, c-Abl both in vitro and in vivo. Under oxidative and dopaminergic stress, both in vitro and in vivo and in the striatum of patients with Parkinson's disease, c-Abl is activated and tyrosine-phosphorylates parkin, causing loss of parkin activity and solubility and the accumulation of parkin substrates, AIMP2 (p38/JTV-1) and FBP-1. Tyrosine phosphorylation of parkin by c-Abl also leads to loss of parkin's cytoprotective function as parkin is unable to rescue cells from AIMP2-induced toxicity. STI-571, a specific c-

Abl inhibitor, prevents tyrosine phosphorylation of parkin and restores its E3 ligase activity and cytoprotective function. Our results show that tyrosine phosphorylation of parkin by c-Abl is a major post-translational modification that leads to loss of parkin function, and describe a novel and compelling role for the stress signaling kinase c-Abl, in the pathophysiology of Parkinson's disease.

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Dementia With Lewy Bodies: A Comparison of Clinical Diagnosis, Datscan Imaging and Neuropathological Diagnosis

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Background: Dementia with Lewy bodies (DLB) is a common form of dementia. The presence of Alzheimer's disease (AD) pathology modifies the clinical features of DLB, making it harder to distinguish DLB from AD clinically during life. Our aim was to determine, in a series of patients with dementia in whom autopsy confirmation of diagnosis is available, whether functional imaging of the nigrostriatal pathway improves the accuracy of diagnosis compared to diagnosis by means of clinical criteria alone.

Methods: A SPECT scan was carried out with a dopaminergic pre-synaptic ligand [123I]-2beta-carbometoxy-3beta- (4-iodophenyl)-N- (3-fluoropropyl) nortropane (FP-CIT) on a group of patients with a clinical diagnosis of DLB or other dementia. An abnormal scan was defined as one in which right and left posterior putamen binding, measured semi-quantitatively, was more than 2 standard deviations below the mean of the controls.

Results: Over a ten year period it has been possible to collect twenty patients who have been followed from the time of first assessment and time of scan through to death and subsequent detailed neuropathological autopsy. Eight patients fulfilled neuropathological diagnostic criteria for DLB. Nine patients had AD, mostly with co-existing cerebrovascular disease. Three patients had other diagnoses. The sensitivity of the FP-CIT scan for the diagnosis

Conclusions: FP-CIT SPET scans substantially enhanced the accuracy of diagnosis of DLB by comparison with clinical criteria alone.

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Results of a Multi-Centre Study of Datscan in Dementia With Lewy Bodies

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Clinically based diagnostic criteria for DLB have limited accuracy. The availability of a biomarker to assist with diagnosis would be a major advance. Severe nigro-striatal degeneration and dopamine loss occurs in DLB but not in most other dementia subtypes offering a potential system for a biological marker.

In the PDT-301 study, 326 patients with dementia with clinical diagnoses of probable or possible DLB, or non-DLB dementia established by a Consensus panel, had a FP-CIT SPECT brain scan labelling the dopamine transporter (DAT) reuptake site in the striatum. Three readers, blinded to clinical diagnosis, classified the images as normal or abnormal by visual inspection. This study which was conducted across 40 European sites, confirms the high correlation between abnormal (low uptake) DAT activity measured using FP-CIT SPECT and a clinical diagnosis of probable DLB.

The diagnostic accuracy is sufficiently high for this to be clinically useful in distinguishing DLB from AD.

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The Impact Datscan Can Have on Dementia Patients: Case Studies

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Although good epidemiological data do not yet exist, Dementia with Lewy bodies (DLB) is increasingly recognized as one of the most common causes of dementia after Alzheimer's disease (AD). The identification of DLB has important implications in terms of prognosis and patient management. These patients frequently develop motor, psychiatric, and sleep-related disturbances in addition to the dementia syndrome, and information and re-assuring of patients and caregivers are important. In addition, drug treatment of patients with DLB is different from that of AD patients. DLB patients may respond better to cholinergic and dopaminergic agents, but are more likely to develop severe side-effects when treated with anti-psychotic agents, including the atypical ones.

However, diagnosing DLB may be difficult. Several studies have demonstrated a low diagnostic sensitivity compared to AD when using international consensus criteria for a clinical diagnosis of DLB. Thus diagnostic markers are needed to improve diagnostic accuracy. Although emerging data indicate that neuro-imaging techniques such as structural MRI and perfusion SPECT may differentiate AD and DLB at group level, there is too much overlap for these techniques to be useful in the diagnosis of individual patients. Accordingly, the finding that Dat scan can reliably distinguish patients with DLB from those with AD, even DLB patients without parkinsonism, can improve patient management. The most important situation is a patient fulfilling DLB criteria but without parkinsonism, where Dat scan ascertain involvement of the nigrostriatal system typical for DLB. Another potentially important situation is a patient with dementia and parkinsonis and psychosis treated with antipsychotic, where it is unclear whether parkinsonism is secondary to the antipsychotic drug treatment or part of the dementia syndrome. Cases illustrating these clinical dilemmas will be presented and discussed.

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Cellular Prion Protein Regulates Amyloidogenic Processing of the Alzheimer's Amyloid Precursor Protein

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Alzheimer's disease (AD) is characterized by the presence of extracellular senile plaques and intracellular neurofibrillary tangles within the afflicted brain. The major constituents of senile plaques are amyloid beta (Aβ)-peptides which are derived from the amyloid precursor protein (APP) through sequential proteolytic cleavage by β- and γ-secretases. Transmissible spongiform encephalopathies are caused by a conformational conversion of normal cellular prion protein (PrPC) to an infectious form, PrPSc. The normal function of PrPC remains unclear. Here we show that PrPC expression in human neuroblastoma (SH-SY5Y) cells dramatically down-regulates β-secretase cleavage of APP and subsequent Aβ-peptide formation. Conversely, siRNA reduction of endogenous PrPC levels in murine neuroblastoma (N2a) cells lead to an increase in Aβ-peptide formation. In addition, a number of prion mutations were used to demonstrate that the N-terminal positively charged amino acid sequence, KKRP, known to participate in glycosaminoglycan binding is essential for decreased amyloidogenic processing of APP. Collectively these data suggest that PrPC may serve to protect the brain from excessive Aβ-peptide production.

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Gamma Secretase Expression During Neurodevelopment

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Background and aims: Amyloid precursor protein (APP) and Presenilins (PSs) appear to play important roles in the etiology and pathogenesis of Alzheimer's disease (AD). Amyloid beta protein is generated by the APP cleavage by two complexes, beta- and gamma-secretase. In particular, gamma-secretase complex requires the presenilin 1 and 2 heterodimers (PSEN1/PSEN2) which build up its active site, beyond three cofactors: Anterior pharynx defective-1 (Aph-1), Presenilin enhancer-2 (Pen-2), and Nicastrin (Ncstn). During ontogenesis, PSs play crucial roles in the maintenance of neuronal progenitor cell proliferation and the temporal control of neuronal differentiation. The aim of the study was to monitor the levels of expression of gamma-secretase components and to discriminate the sexual dimorphism of their expression in different cerebral areas (cortex, hippocampus, striatum and cerebellum).

Methods: We carried out a quantitative analysis of the gamma secretase expression by Real Time PCR.

Results: Preliminary results on cerebral rat tissues show that PSEN1 is expressed throughout postnatal development, although mRNA levels in males appear to be lower than in females in all the brain districts, suggesting a different transcriptional regulation of these genes. We also observe a stronger PSEN1 and PSEN2 gene expression, in both sexes, at the birth (day 0) relative to the other postnatal day (7,14, 21 p.n.d and adult).

Conclusions: Our data seem to confirm the existence of a modulation in the expression of gamma-secretase and show for the first time dramatic differences, in PSEN1 expression, between males and females during brain ontogenesis.

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Presenilin 1 Gene Mutations Increase the Expression and the Activity of BACE1

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Background and aim: Presenilin 1 gene (PSEN1) mutations, the major cause of familial early-onset Alzheimer's disease (FAD), alter the gamma-secretase cleavage on the beta-amyloid precursor protein (APP), leading to overproduction of beta-amyloid (42) species. We show that PSEN1 mutations also alter the activity of the beta-secretase (BACE1), the limiting step of Abeta production.

Results: Three PSEN1 mutations (M146V, S170F, L392V), expressed in HEK-293 cells, determined an increase of BACE1 mRNA, protein levels, and activity. The elevation of all BACE1 parameters was much higher when PSEN1 mutations were expressed in PS1 deficient cells. The activation of BACE1 operated by the PSEN1 mutations required the presence of APP 695, as shown in APP deficient cells, as well as silencing APP 695 with RNA interference. The increase of BACE1 expression was significantly correlated with the levels of secreted Abeta 42, but neither with those of Abeta 40 nor with the amount of the APP intracellular domain (AICD). In brain tissue of 12 FAD cases with different PSEN1 mutations the expression and the activity of BACE1 was significantly higher than in normal controls.

Conclusions: The activation of BACE1 induced by PSEN1 mutations implies the physiological presence of a positive feedback loop from the gamma- to beta-secretase cleavages of APP (Tamagno et al., submitted). The increased activity of BACE1 can influence the N-terminal heterogeneity of Abeta species, thus modifying the composition of the soluble aggregates of Abeta and their properties of aggregation and toxicity.

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Effects of Presenilin 1 Mutations on the Gamma Secretase Cutting of P75NTR in Vitro

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The cholinergic neurons of the basal forebrain are amongst the first to degenerate in Alzheimer's disease. The cholinergic neurons are unique in the brain, in that they express the tyrosine kinase receptor, TrkA and the common neurotrophin receptor p75NTR; both of which bind nerve growth factor. Binding of nerve growth factor to the TrkA receptor is important in the maintenance of cell viability, whereas the p75NTR receptor has been implicated in apoptosis. p75NTR undergoes regulated intramembrane proteolysis, by gamma secretase, which involves the multi-transmembrane aspartyl protease presenilin. Mutations in presenilin 1 are the major cause of familial Alzheimer's disease.

The changes in regulated intramembrane proteolysis of p75NTR elucidated in these studies may have an effect on the balance between TrkA and p75NTR receptors and their respective signalling pathways. This balance is vital in the maintenance of healthy cholinergic neurons. Thus alterations in cleavage of p75NTR may influence this equilibrium in disease states such as Alzheimer's disease.

PC12 cells were stably transfected with familial mutations M146V, A246E and deltaE9 and wild type presenilin 1. These were examined for their effect on regulated intramembrane proteolysis of p75NTR.

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The Patient With De Novo S170F Presenilin 1 Gene Mutation. Case Report

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PSEN 1 mutations are most prominent cause of familial, early onset Alzheimer's disease (AD). There are more than 150 described mutations, although de novo mutation cases are really rare.

We present the case of 31 years old woman, with very fast progressing Alzheimer's disease, with previously described mutation . Age of onset was 29 years old, after giving a birth to second child.

Three months after delivery patient was diagnosed with puerperal depression and treated with anti – depressants.

She was later admitted to hospital, due to cognitive disturbances (especially short-term memory), mioclonic jerks and bradykinesia. MRI showed symmetric, moderate cortical atrophy of the brain hemispheres and cerebellum, without focal changes.

Patient was tested for Creutzfeld – Jacob disease – cerebrospinal fluid was checked for the presence of 14-3-3 protein, tonsil biopsy was also conducted. All results were negative. Also test for neuroboreliosis, HIV infection, Wilson's and Huntington diseases, SSPE, metabolic diseases were negative. Neuropsychological assessment after 16 months of the disease showed severe impairment of short-term memory, language abilities and orientation. MMSE score was 11 points. Parkinsonian symptoms soon progressed and epileptic sizeures appeared. Patient was diagnosed as possible early onset Alzheimer's . We have conducted sequencing of PS1 gene, finding S170F mutation. Sequencing of parents' DNA didn't show the presence of mutation.

We confirmed de novo mutation, using AmpfSTR Identifiler kit, from Applied Biosystems.

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Apolipoprotein E and Neuropsychological Presentation in Alzheimer's Disease: A Preliminary Study

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Background and objectives : specific apolipoprotein E allele is known to be related to the age of onset in Alzheimer's disease (AD). Moreover, there have been some reports showed that the presence of apolipoprotein E epsilon 4 allele might affect the neuropsychological complaints of patients with AD. Detailed analyses of the neuropsychological pattern in AD based on apolipoprotein E allele have not been studied. This study is to explore whether there are differences in the age of onset and neuropsychological manifestations in AD by the presence of apolipoprotein E epsilon 4 allele.

Methods : We studied 59 patients with probable AD underwent apolipoprotein E genotyping. All of them were newly diagnosed and underwent detailed neuropsychological evaluation at the initial visit. We analyzed demographic features and patterns of cognitive function according to apolipoprotein E genotyping.

Results : AD with apolipoprotein E epsilon 4 allele didn't differ in age, age of onset or duration of education from AD without apolipoprotein E epsilon 4 allele. In the analyses of subgroups divided by age of onset (based on 65 years), early and late-onset, their cognitive impairment of each domain such as memory, language, visuospatial function and word fluency were same regardless of having apolipoprotein E epsilon 4 allele within each group.

Conclusion

Our study shows the different apolipoprotein E alleles do not affect the age of onset and neuropsychological manifestation in AD. Within the group of similar age of onset, we couldn't find significant role of apolipoprotein E epsilon 4 allele in cognitive function

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Brain Glutamate Metabolism in Alzheimer's Disease

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We proposed to employ systems approach to glutamate metabolism in human brain in health and mental pathology (Boksha, 2004). Relative amounts of glutamate metabolizing enzymes, such as glutamate dehydrogenase (GDH - three isoenzymes, Burbaeva et al., 2002), glutamine synthetase (GS), GS-like protein (GSLP, Boksha et al. 2000), and phosphate-activated glutaminase (PAG) were evaluated in prefrontal cortex of control subjects and patients with Alzheimer's disease (AD). The target proteins were quantified by ECL-Western immunoblotting in extracts from brain tissue prepared by two different techniques separating enzymes preferentially associated with cytoplasm (GDH I and II isoenzymes, GS, and partially GSLP) and membrane (GDH III, PAG, and partially GSLP) fractions. Amounts of all listed enzymes were found significantly increased in the patient group compared with controls. Some links between the measured values of the enzymes were observed in the control. No links were found in the group of patients with AD. The results may suggest for the pathological interruption of regulatory relations between distinct enzymes of glutamate metabolism in brain of patients with AD.

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Audit of Dementia and Non-Insulin Dependent Diabetes - Are We Jumping the Gun?

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Background-Alzheimer's disease (AD) and Vascular Dementia (VD) lead to cognitive decline via different aetiologies. Studies show that Non-Insulin Dependent Diabetes Mellitus (NIDDM) patients are at higher risk of AD than controls. NIDDM patients are also prone to cardiovascular co-morbidities, predisposing them to VD.

Methods- We investigated the relationship between NIDDM, AD and VD by analysis of registry data.

Results-Between 2000-2006, 16,238 patients were admitted with NIDDM (Mean age 66.2 years); 677 with AD (83.5 years); 445 with VD (83.1 years). Of 16,238 NIDDM patients, 40 had AD and 39 had VD. However, 7% of AD patients and 12% of VD patients also had NIDDM. NIDDM patients had a significantly higher prevalence of cardiovascular co-morbidities compared to AD and VD patients, including angina (9.8% vs 4.1% AD; 3.9% VD), MI (9.1% vs 0.9% AD; 1.1% VD), hypertension (34% vs 13.7% AD; 20.4% VD); hyperlipidaemia (9.1% vs 0.6% AD; 1.6% VD). NIDDM patients with AD had fewer co-morbidities than NIDDM patients without AD.

Conclusions-Patients are unlikely to develop AD or VD as a direct result of NIDDM although it is risk factor in a significant proportion of AD and VD patients. The higher prevalence of cardiovascular co-morbidities conferring higher mortality in NIDDM patients and the higher mean age of AD and VD patients, suggests that NIDDM patients may be dead before they develop AD or VD. AD may be a complication of NIDDM but is only likely to manifest in rare groups of elderly patients lacking cardiovascular co-morbidities.

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Dietary Preference and Diabetes Among Alzhiemer

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Some previous studies have revealed a tendency towards sweety foods. A previously published work of us revealed a relation between diabetes and Alzheimer's patients. The aim of this study was to concentrate on the diet of Alzheimer's patients and the occurrence of diabetes.

Material: Fifty patients with probable (mild to moderate) Alzheimer's disease were included. A twenty-control of matching age were included.

Results: There was a greater preference than normal controls for sweet beverages and food. High complex carbohydrates were second in preferences. Patients with moderate Alzheimer's disease who were not previously known to be diabetic started to show non-insulin dependent diabetes mellitus. Once diabetes was revealed, the course of the disease was rapidly progressive.

Discussion and Conclusion: Results provide a possible relation and link between the craving for sweet food and the occurrence of later diabetes. This might reflect a neuronal metabolic disturbance with the need of more energy than already supplied, resulting in the need for a higher fast acting energy supply, which might be in the form of sweets.

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Specific Neuroautonomic Changes by Heart Rate Variability Analysis in Parkinson's Disease, Multiple System Atrophy, and Lewy Bodies Dementia

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Background and aims:recently, a lot of interest is gained by three diseases sharing some neurologic aspects and in which some neuroautonomic involvement has been documented or suspected: Parkinson's Disease (PD), Multiple System Atrophy (MSA), and Lewy Bodies Dementia (LBD).Heart Rate Variability (HRV) analysis is a reliable technique to evaluate the autonomic cardiac control

Increased mortality and sudden death were described in these patients. It has been well estabilished that reduced HRV is associated with increased sudden cardiac death

Methods: we evaluated 26 subjects, 9 affected by PD, 5 by MSA, 5 by LBD, and 7 as control subjects (C)

They underwent an accurate clinical and laboratoristic assessment, cognitive and motorial tests, 24 hours ambulatory blood pressure monitoring, 24 hours electrocardiographic monitoring to allow HRV analysis by time domain and frequency domain analysis (power spectral analysis)

Results:we found a significant reduction of SDNN, rMSSD, HRV index, LFp, HFp in MSA vs C (p=0.01, 0.01, 0.04, 0.002, 0.002 respectively), of SDNN, rMSSD, HFp in LBD vs C (p=0.02, 0.02, 0.03 respectively) and of HRV index in Parkinson vs C (p= 0.04)

A significant relationship was found between the degree of HRV parameters changes and the length of the disease (LF r=-0.301, HRV index r=-0.355)

Conclusion:our findings show specific impairment of HRV parameters, reflecting parasympathetic modulation in patient with LBD and a global one (sympathetic and parasympathetic) in patients with MSA.

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Pulmonary Function Tests in Idiopathic Parkinson's Disease

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Background: Pulmonary dysfunctions are common in idiopathic Parkinson's disease(IPD), and contribute as a limiting factor for activities of daily living. The aims of our study were to estimate the severity of pulmonary dysfunction in IPD patients with pulmonary function test(PFT) and to determine the correlation between parameters of PFT and severity of IPD. Methods: PFT has been performed in 28 patients (20 non-smoker and 8 smoker; 12 tremor-dominant and 8 postural instability and gait disturbance patients). The clinical disability was indicated by Unified Parkinson disease rating scale(UPDRS) part II & III. The parameters of PFT were not different between IPD and control group, and between non-smoker IPD and smoker IPD group. We use Speraman correlation test for statistic method. Results: There is no significant difference in the parameters of PFT between tremor dominant and postural instability and gait disturbance group. UPDRS part II score was significant positive correlation with the maximal flow rate at 75% of forced vital capacity(FEF75%)($p<0.05$). UPDRS part III score was significant positive correlation with FEF75%($p<0.05$), and significant negative correlation with forced vital capacity($p<0.05$). Conclusions: We conclude that less coordinated and less explosive muscle force contributes to the change of FEF75%, and suggest parameters of PFT may be used as indicators of severity of IPD. Therefore PFT may be used in IPD for preventing pulmonary complications. Also, PFT may be used as indicators of severity of IPD.

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Prevalence of Neuropsychiatric Manifestations and Sleep Disturbances in Parkinson's Disease

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Introduction: Neuropsychiatric, perceptual and cognitive deficits are increasingly recognized as non-motor manifestations of Parkinson's disease (PD). Many patients with Parkinson's disease (PD) experience sleep-related symptoms.

Aim: The aim of the study was to determine the prevalence of neuropsychiatric symptoms and sleep disturbances in PD patients attending a Movement Disorder Clinic (MDC).

Methods: Data was collected from MDC computerised database. All patients with PD (diagnosed using UK Brain bank criteria) having neuropsychiatric symptoms and sleep disturbances were included. Information was collected by retrospective notes review using performa's.

Results: 43 of 379 patients (11%) with PD had neuropsychiatric symptoms. Mean age was 77yrs (range 64-86). 86% had advanced PD (Hoehn & Yahr stage ^{3} 3). Average duration of PD was 7.6 years, MMSE 23. 98% of patients were on L-Dopa. Visual Hallucinations were present in 88%, (mean duration of PD-5.2 years), followed by depression (54%; duration 4.6 yrs), Confusion (44%; duration 5.6yrs), Delusion (23%; Duration 7.1 yrs). Agitation, anxiety, irritability and apathy were also noted. 70% had sleep disturbances (mean duration of PD 4.8 years).

Conclusions: Visual hallucinations, sleep disturbances, depression and confusion are the most commonly occurring symptoms in this elderly cohort. Average onset of symptoms is about 5 years after diagnosis and appears to be related to the severity of PD. Awareness of these non-motor symptoms and effective management can improve the quality of life for patients with PD.

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Neuropsychological Assessment of Moderate Stage Parkinson's Disease Dementia (Pdd): Comparison With Moderate Stage of Alzheimer's Disease(AD) -A Preliminary Study-

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Background & Objective : The aim of study is to compare and assess the degree of cognitive impairment, psychiatric problem and acitivity of daily living between moderate stage PDD and AD. Methods : The neuropsychological battery test results (attention, memory, visuospatial function, frontal executive function, language, neuropsychiatirc inventory, K-IADL test) of three subject groups(20 PDD patients, 20 AD and 10 Normal) were reviewed and analyzed. They were all matched for age and education period. PDD and AD patients diagnosed as moderate dementia($12 \leq$K-MMSE≤ 18) were selected Result : Compared with the normal control group, PDD and AD patients showed cognitive decline with significant attentional deficits, frontal executive and visuospatial dysfunctions, memory dysfunctions and impairment on language, psychiatric impairment and IADL impairment ($p<0.05$). Neuropsychological tests involved in the cognitive domain revealed no statistically significant differences between PDD and AD. However, PDD patients were worse in the neuropsychiatirc inventory score and IADL score than AD patients ($p< 0.05$). PDD patients showed the worse on the depression, anxiety, hallucination and sleep disturbance rather than AD. Conclusion : Patients with moderate Parkinson disease dementia showed worse impairment of psychiatric symptom and activity daily living compared with moderate AD, in spite of similar degree of cognitive impairment. These findings support the importance of psychiatric problem on activity of daily living in the PDD compared with AD, beyond the motor symptom.

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Amnestic Mild Cognitive Impairment - Two Subgroups According to Spatial Navigation Defficit

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Background: Spatial navigation is impaired early in Alzheimer's disease (AD) and even in Mild cognitive impairment (MCI). Not retrieval but memory consolidation refers to hippocampus. Methods: 50 patients were classified based on Petersen's criteria to non-amnestic MCI (naMCI)(n-7) and amnestic MCI (aMCI)(n-43) subdivided to single (aMCIs)(n-13) and multiple domain (aMCIm)(n-30). Further aMCI group was divided in memory consolidation (hippocampal –HaMCI (n-13)) and retrieval (mainly frontal – NHaMCI (n-30)) impairment. Results were compared to control group (n-29) and AD group (n-22). All subjects underwent precise neuropsychological testing, MRI and ApoE sampling. Spatial navigation was tested in an enclosed arena 2.9m in diameter with orientation cues on the wall and in a computer version with a map view of the arena. The subjects should locate a hidden goal inside of the arena in 4 subtests using the start position and/or cues on the wall for navigation to focus on either allocentric or egocentric navigation.

Results: HaMCIm group was significantly impaired in all subtests ($p<0.01$); HaMCIs group in subtests focused on allocentric navigation after 30 minutes delayed ($p<0.01$); NHaMCIm group in subtests focused on allocentric navigation ($p<0.05$); NHaMCIs and naMCI groups weren't impaired in any of the subtest. AD group was significantly impaired in all subtests ($p<0.01$) and was similar to HaMCIm group ($p>0.48$).

Conclusions: Spatial navigation deficit in HaMCI is more profound than in NHaMCI and naMCI. Spatial navigation testing can be profitable for identification of individuals at higher risk of AD among the heterogeneous MCI population.

Supported by grant GACR 309/05/0693

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Activities of Daily Living – Outcome During Two Years in Galantamine Treated Alzheimer Patients

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Objectives: To analyse and present the outcome of longitudinal change and possible subgroups with respect to ADL function measured by different scales (IADL, PSMS and FAST) in patients treated with galantamine for two years (N=122).

Methods: The Swedish Alzheimer Treatment Study (SATS) is an open, 3-year, multicentre study in a routine clinical setting. The patients were assessed with several rating scales including Activities of Daily Living at baseline and every 6 months for a total period of two years. A twostep cluster analysis was performed to reveal any natural groupings (clusters) of the patients based on the ADL scores at baseline.

Results: After two years of galantamine treatment the total mean change in IADL score was 4,3 ± 4,9 points, PSMS 1,0 ± 2,4 and in FAST 1,0 ± 1,7 points. The different ADL scales were linearly correlated ($p<0,001$) with each other and with cognition measured by MMSE and ADAS-cog. Age at onset and baseline showed significant correlations ($p<0,05$) with IADL score at baseline. A cluster analysis showed two subgroups that significantly differ in age ($p<0,05$), cognition ($p<0,001$) at baseline, proportion of apoE4-bearers ($p<0,05$) and the long-term outcome of PSMS score ($0,01<p<0,05$).

Conclusions: The instrumental ADL scale shows a faster decline of function. A strong linear correlation between the three ADL scales and cognition was observed. Cluster analysis shows two subgroups based on ADL scores at baseline, one older, more cognitive impaired group with lower proportion of apoE4-bearers and faster decline measured by PSMS in contrast to the other group.

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Acute Parkinsonism With Bilateral Basal Ganglia Lesions in Uremia

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Background and aims: The involuntary movements seen in uremic encephalopathy typically consist of asterixis and myoclous, which reflect cortical involvement. Other types of abnormal movement disorder have only been very rarely reported as a consequence of uremia. We report a case of acute parkinsonism with bilateral basal ganglia lesions in uremia.

Methods: We performed brain MRI in a uremic patient with acute parkinsonism.

Results: A 47-year man who had hypertension, diabetes mellitus for 7 years. The diabetes had been regularly treated with oral hypoglycemic agents. Uremia was noted in January 2005, and he had been on dialysis since March 2005. In June 2005, he suddenly developed bradykinesia, generalized limb muscular rigidity, dysarthria, and dysphasia. The BUN level was 56 mg/dl and serum creatinine level was 6.1 mg/dl. Glucose level was 136 mg/dl and electrolyte levels were with normal limits. T2 weighted MRI images showed extensive hyperintensity over the basal ganglia bilaterally, extending to the adjacent periventricular white matter. On T1 weighted images the lesions was hypointense. Supportive management and hemodialysis were given. His symptoms improved gradually and he recovered after 4 weeks of treatment.

Conclusions: Exacerbation of glucose utilization failure or vasogenic edema is suggested as the etiology of basal ganglia lesions, but the exact underlying pathophysiology is unknown.

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Outcomes of Patients With Delirium Admitted to Medical Wards of the Hutt Hospital

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Objective: To determine Cognitive and Functional status of patients with delirium and matched controls, who were identified during the Delirium audit (2001).

Methods: In this Cohort study, 53 patients with Delirium and 108 matched controls that were identified during the delirium audit (2001), admitted to medical wards of the Hutt hospital were followed up in 2004 and 2005. Their vital status, place of residence, activities of daily living & cognitive performance was determined, by visiting living patients and by telephone interview of carers of the deceased patients. Functional assessment was done using Barthel Index or Modified Rankin's Score in the deceased group. Cognition was assessed using Folsteins Mini Mental State Examination (MMSE).

Results: Only presence of Delirium was predictor of development of Dementia (p 0.015). MMSE mean range of score in delirium group was 19.4, that in control group was 25.7 (p 0.005). Mean Rankin's score in Delirium group was 5 that in control group was 4 (P 0.004). More patients in control group ceased driving (p 0.2687). Significant patients in delirium group were in residential care (p 0.0013). There was no difference in the mortality between the groups.

Conclusions: Delirium is associated with worse functional status and increased need for residential care. Delirium may also identify patients at risk for further cognitive decline. Diagnosis of delirium in acute setting may be important in organising appropriate follow up plans for frail elderly patients.

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What Linguistic Variables Influence Naming in Alzheimer's Disease? Longitudinal Study Based on a Single Stimulus and Single Patient Approach

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In a longitudinal investigation, two Alzheimer's patients were tested in single-case study of picture naming, aimed at analysing the effect of different psycholinguistic factors on naming success. The variables considered in this experiment were lexical frequency, age of acquisition, stimulus familiarity, word length, and imageability. The difficulty of this type of inquiry, scarcely represented in the literature, depends on two facts: (i) the same stimulus may give rise to inconsistent responses, even on a close repetition of the task; (ii) the explanatory variables are highly inter-correlated. Therefore, a special methodology is necessary for addressing this class of studies. In this survey we used a recently introduced statistical approach (Capitani and Laiacona, 2004) that takes into account the inconsistency of the response to each stimulus at a given clinical stage. The method is based on the multiple presentation of the same naming battery and on the use of logistic regression analysis, where the response given to each stimulus is weighted in proportion to the different types of response observed in distinct trials at each clinical stage.

Two middle-severity Alzheimer's patients were studied with a 80-picture battery, and the examination was repeated after two years. For one patient the progressive decline affected lately acquired words, whereas for the other it affected the names corresponding to low-familiarity items. The findings of this study are discussed with reference to the quality of errors and to current cognitive and neuro-linguistic models of naming.

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Melatonin Receptor Alterations in Alzheimer's Disease

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Melatonin is an endogenous regulator of circadian and seasonal rhythms in vertebrates. In addition, melatonin has various physiological effects including vasoactive, visual and neuroprotective properties. A major production site of melatonin is pineal gland. With age and in patients with Alzheimer's disease (AD), parallel to diminishing melatonin levels, amplitude and timing of the circadian system change. This results in disturbances in sleep, arousal and behavioural symptoms affecting patients' autonomy and providing a big burden for their caregivers. Two different subtypes of G-protein coupled receptors, MT1 and MT2, transmit some of melatonin's effects in mammals. We have studied melatonin receptor alterations in different brain areas of AD patients using immunohistochemistry. Pyramidal neurons express both receptors in the human hippocampus. Whereas MT1 was decreased, the expression of MT2 was increased in AD hippocampus. Ganglion, amacrine, and photoreceptor cell of the retina expressed MT1 and MT2. The expression of both receptor was decreased in AD retina. MT1 and MT2 were also localized in the pineal gland and occipital cortex. In the pineal gland both MT1 and MT2 were localized to pinealocytes, whereas in the cortex both receptors were expressed in some pyramidal and non-pyramidal cells. In patients with AD, parallel to degenerative tissue changes, there was an overall decrease in the intensity of receptors in both brain regions. The results indicate that both melatonin receptors may be involved in mediating the effects of melatonin in different brain areas and their expression is heavily impaired in AD.

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The Spectrum of Neuropsychological Deficits Associated With Mutations in Progranulin

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Background & Aims: Mutations in PGRN are associated with frontotemporal dementia +/-parkinsonism. We report on the variability in neuropsychological testing among patients who were proven to have mutations in progranulin (PGRN) associated with neurodegenerative disease.

Methods: Ten affected members in six kindreds with mutations in PGRN underwent neuropsychological evaluation on one or more occasions, which included standard assessments of learning and memory, language functioning, attention/executive functioning, and visuospatial skills. Mayo Older American Normative Studies (MOANS) norms were used to determine scaled scores for these tests, and results at or below 1.5 standard deviations from the mean were considered abnormal.

Results: Mean age at first testing was 62.6 years, and mean education was 13.2 years. Two had a profile of significantly impaired language functioning with relative sparing of other domains. Two individuals initially manifested significant amnestic difficulties without deficits on attention/executive tasks. Three individuals exhibited early prominent visuospatial deficits to a degree not typical of frontotemporal dementia. Although all patients ultimately developed executive dysfunction, this was not a marked feature in the initial testing of four subjects. Deficits in four members of one large kindred varied widely, including one with predominant

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Familial Parkinsonism and/or Dementia Associated With the C.910_911insTG Mutation in Progranulin

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Background & Aims: We recently identified mutations in progranulin (PGRN) associated with familial frontotemporal dementia and parkinsonism (FTDP). We now report a family with parkinsonism-predominant features associated with a mutation in (PGRN).

Design & Methods: We analyzed all available clinical, radiologic, genetic, and pathologic data in all affected individuals in the F147 kindred evaluated and followed by the authors.

Results: We previously evaluated a male executive who at age 60 began experiencing frontotemporal dementia and postural tremor. Neuropsychological testing demonstrated impaired learning, memory, and executive functions. MRI revealed left greater than right anterior temporal and bifrontal atrophy as well as diffuse white matter hyperintensities. SPECT showed left greater than right anterior temporal and right greater than left frontal hypoperfusion. Autopsy revealed frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U), leukoencephalopathy, pallidonigral pigment-spheroid degeneration, and frontopontine fiber degeneration. The proband's sister presented with levodopa-responsive parkinsonism and later developed dementia, with FTLD-U findings also found at autopsy. Another sister had FTD, a brother had dementia (not otherwise specified), and the proband's father had parkinsonism. PGRN analysis in the proband and one sibling revealed a 2 base pair insertion in exon 8 (c.910_911insTG) that results in a frameshift (Trp304LeufsX58) and creation of a premature termination codon, and likely creates a null allele through nonsense mediated decay.

Conclusions: The features and findings in the kindred expand the clinical features associated with mutations in PGRN, with a parkinsonism-predominant phenotype in many affected members, including one individual with initial levodopa-responsiveness.

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MRI Findings in Familial Frontotemporal Dementia and/or Parkinsonism Associated With Mutations in Progranulin

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Background & Aims: To report MRI findings in affected members of eight kindreds having mutations in progranulin (PGRN) associated with frontotemporal dementia and parkinsonism (FTDP).

Methods: We reviewed all available MR images among affected members of eight kindreds followed in the Mayo Alzheimer's Disease Research Center.

Results: MRI scans were available for review in 15 patients. Atrophy was present in all, with frontotemporal predominance in 13. Parietal cortical atrophy was present in 4. Subcortical white matter changes with frontotemporal predominance were identified in 6 patients. In 3 subjects, striking subcortical signal changes were present on FLAIR, most prominently in regions of maximal cortical atrophy. Six patients had longitudinal MRI scans spanning 2 or more years, and all showed progression of atrophy and subcortical white matter signal changes. In most kindreds, topography of cortical atrophy was highly variable. In one kindred with frontotemporal dementia and corticobasal syndrome features (exon 9 c.1145delC mutation), the right cerebral hemisphere was maximally affected in all three symptomatic siblings. The three affected siblings of family PPA1 (exon 9 c.998delG mutation) had familial primary progressive aphasia, and the left cerebral hemisphere was maximally affected on MRI.

Conclusions: A variety of neuroimaging findings can be seen in patients with FTDP associated with mutations in PGRN. In addition to frontotemporal atrophy, other regions of atrophy as well as prominent subcortical white matter changes may be present. It is unclear whether the lateralization of MRI findings in two families is coincidental or represents some biologically relevant process inherent to progranulin pathophysiology.

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Clinical Heterogeneity in Familial Dementia With or Without Parkinsonism Associated With the C.154delA Mutation in Progranulin

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Background/Aims: Mutations in progranulin (PGRN) are associated with familial frontotemporal dementia and parkinsonism. We report a family of Danish descent with variable clinical features affecting eight individuals over two generations.

Design/Methods: We analyzed all available clinical, radiologic, genetic, and pathologic data in individuals of the F142 kindred evaluated at our center.

Results: Eight individuals in two generations became symptomatic. Clinical material was available in four in generation 1 (G1) and three in generation 2 (G2). Mean age of onset in G1 was 69 years (range 69-80), and 61 years in G2 (range 51-66). Duration of symptoms was 4.3 years (range 2-8) in G1 and 4.0 years (range 1-7) in G2, with those in G2 still living. Clinical diagnoses included mild cognitive impairment, Alzheimer's disease, frontotemporal dementia, primary progressive aphasia, and corticobasal syndrome; some diagnoses changed during the clinical course, and some carried overlapping diagnoses. Autopsies in four individuals revealed frontotemporal lobar degeneration with ubiquitin-positive intracytoplasmic inclusions and neuronal intranuclear inclusions (FTLD-U NII), with one also having mixed AD/vascular pathology. PGRN sequence analysis in all subjects revealed a single base pair deletion in exon 2 (c.154delA). This mutation caused a frameshift

(Thr52HisfsX2), creating a premature termination codon, and likely created a null allele through nonsense mediated decay.

Conclusions: A wide array of cognitive, behavioral, and parkinsonian features are associated with the c.154delA mutation in PGRN. The earlier age of onset in G2 and lack of overlap in ranges of age of onset between generations raise the possibility of anticipation-like effect.

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Familial Frontotemporal Dementia and/or Corticobasal Syndrome Associated With the C.1145delC Mutation of Progranulin

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Background/Aims: We extend the previously reported kindred (F129) in which the proband and his father exhibited the corticobasal syndrome (CBS) and his brother exhibited frontotemporal dementia (FTD).

Design/Methods: We analyzed all available clinical, radiologic, genetic, and pathologic data in affected individuals of F129.

Results: The proband had neuropathological findings initially consistent with dementia lacking distinctive histopathology. His brother later developed hemiparkinsonism and has since expired, and ubiquitin-positive inclusions were identified (i.e., frontotemporal lobar degeneration with ubiquitin-positive intracytoplasmic inclusions plus neuronal intranuclear inclusions: FTLD-U NII), and the same were found upon re-examination of the proband using newer staining techniques. Their sister developed FTD, then mild hemiparkinsonism, and then classic CBS features. All four affected individuals have had motor features maximal in the left-sided limbs, and MRIs in three have shown temporal more than frontal and parietal cortical atrophy, right more than left. PGRN sequence analysis in the brother and sister revealed a single base pair deletion in exon 9 (c.1145delC). The effect of this mutation is to cause a frameshift (Thr382SerfsX30), creation of a premature termination codon, likely creating a null allele through nonsense mediated decay.

Conclusions: These findings support the observations that 1) the corticobasal syndrome is not specific for corticobasal degeneration, 2) variable FTD/CBS features can occur in individuals with presumably the same neurodegenerative disorder, 3) familial FTD/CBS with underlying FTLD-U NII pathology can be associated with mutations in PGRN. Why the same cerebral hemisphere is maximally affected in all symptomatic individuals in this kindred is not known.

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Identification of New Cys606Arg Mutation of NOTCH3 Gene in Family With Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy

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Background; Cerebral autosomal dominant arteriopathy and subcortical infarcts and leukoencephalopathy (CADASIL) is a rare genetic disorder inherited in an autosomal dominant manner. The phenotype and genotype of CADASIL in Caucasians have been characterized, but CADASIL is less recognized and reported in Asian populations. Case; Here we investigated the first known Korean family affected by CADASIL and identified an uncommon NOTCH3 mutation. The affected family members had recurrent strokes, early-onset dementia, depression, and migraine. Genetic analysis was conducted in four of seven family members, two sisters were dead and one refused. A Cys606Arg mutation at exon 11 of NOTCH3 was found in three out of four who were analyzed. A sister who was asymptomatic didn't have mutation. Neuropsychological evaluation showed vascular dementia in one of three affected family members. Brain MRI showed multiple infarcts in bilateral basal ganglia, thalami, and periventricular white matter in all affected but not in unaffected sister. A skin biopsy in an affected family member did not show characteristic pathological findings of CADASIL on electron microscopy except increased collagen deposits. Comments; To our knowledge, the Cys606Arg Mutation of NOTCH3 Gene at exon 11, which was identified in the studied family, has not been reported yet. Our findings emphasize the importance of genetic analysis of NOTCH3 for the patient and family with a phenotype typical of CADASIL.

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Clinical Characteristics of Familial Parkinson's Disease Patients With and Without PINK1 Mutations

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Background: PTEN induced putative kinase 1 (PINK1) is one of several genes implicated in autosomal recessive forms of Parkinson's disease (PD). PINK1 encodes a serine/threonine protein kinase that localizes to mitochondria, and is thought to protect cells from stress-induced mitochondrial dysfunction. A family-based genetic linkage

study has been carried out as a collaboration between the pharmaceutical company GlaxoSmithKline, investigators from Tunisia, and investigators from Mayo Clinic, Florida to investigate familial PD genetics. Methods: Eighty multiplex PD families and 11 families with one PD patient were recruited from the Institut National de Neurologie in Tunis, which provides a specialized neurological service to the entire country. All PD patients and available family members received neurological examinations and completed standardized questionnaires with demographic and clinical information. PD clinical characteristics included age at onset, dominant symptom, Hoehn and Yahr score, Schwab and England ratings, Epworth Sleepiness score, and dopamine medication. Results: After a significant linkage peak was identified on chromosome 1p36 in the PINK1 region (MERLIN LOD= 6), all eight exons of PINK1 were sequenced in probands. Sequencing has identified two known mutations P196L (rs35802484) and Q456X, and several novel mutations, which are undergoing segregation analysis and will be screened in an unrelated Tunisian control population. A comparison of the clinical characteristics between individuals with and without specific PINK1 mutations will be reported.

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Familial Alzheimer Disease With Parkinsonism Associated With a Novel Presenilin 2 Mutation

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Background and aims: autosomal dominant Alzheimer Disease is a heterogeneous condition that has been associated with mutations in three different genes: the Amyloid Precursor Protein (APP), Presenilin1 (PSEN1) and Presenilin2 (PSEN2). The aim of the present study was to describe the clinical features of an atypical dementia with Parkinsonism, associated with a novel Presenilin2 mutation. Methods:we reconstructed a large familial pedegree from North Sardinia, identifying twelve affected members on the first, second and third generation and we personally evaluated eight family members by clinical examination and genetic analysis.

Results: The disease onset ranged from 54 to 76 years. The clinical history varied within family members including, in addition to dementia, psychiatric symptoms such as obsessive-compulsive behaviour, visual hallucinations, delusions and extrapyramidal movement disorder such as lead-pipe rigidity, bradykinesia and arms tremor. Four siblings presented clinical features similar to Parkinsonian disorder, four siblings developed progressive dementia fulfilling the clinical diagnosis of Alzheimer's Disease criteria, while three siblings Lewy Body criteria and finally one patient presented severe psychosis.

Conclusions: This large Sardinian pedegree shows a peculiar and heterogeneous phenotype. Sometimes patients show a predominant picture of dementia or parkinsonism or psychosis, sometimes they show different clinical entities overlapping each other in a specific way. Only the association of the different clinical pictures and molecular analysis are able to allow a correct diagnosis, unifying different symptoms, in a possible new syndrome.

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Common Genetic Variants of the Low Density Lipoprotein Receptor-Related Protein 6 Are Associated With Late-Onset Alzheimer´S Disease

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Background and aims: Genome-wide linkage studies have defined a broad susceptibility region for late-onset Alzheimer disease (AD) on chromosome 12, which contains the Low Density Lipoprotein Receptor (LDLR)-Related Protein 6 (LRP6) gene. We and others proposed that altered function of Wnt signaling components may be involved in AD, leading us to examine whether the LRP6 gene, which encodes a co-receptor for Wnt signaling, is associated with this disease.

Methods: LRP6 variants were examined for association with late-onset Alzheimer's disease in a multi-centre case-control series as well as in a family-based series ascertained by the NIMH-NIA Genetics Initiative.

Results: Haplotype tagging single nucleotide polymorphisms (SNPs) with a set of 7 allelic variants of LRP6 identified a putative risk haplotype, which includes a highly conserved coding sequence SNP in LRP6. The association was mainly dependent on Apolipoprotein E-e4 (APOE-e4) carrier status. While we observed that LRP6 is normally expressed in the adult human hippocampus, functional assays revealed that LRP6 alleles display differential activity on Wnt signaling.

Conclusions: Our study unveils a novel genetic relationship between LRP6 and APOE and supports the hypothesis that altered Wnt signaling may be central in the onset of AD.

Funding: RTM and GVD: HHMI and TLL Temple Award from the Alzheimer's Association; FW-V, AJM and JH: NIA/NIH Intermural Program, USA; AP: Swiss National Science Foundation, Helmut Horten Stiftung, and EU APOPIS program.

GVD also wish to thank support from the PEW Latin American Program in the Biomedical Sciences, FONDECYT 1050097 and PBCT ADI-04.

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Study on the Association Between DHCR24 Polymorphisms and Alzheimer's Disease Risk

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DHCR24 gene in chromosome 1 encodes Seladin 1, a cholesterol synthesizing enzyme. Seladin 1 protects neurons from AB42 mediated toxicity and participates in regulation of AB42 formation by organizing the placement of APP cleaving B-secretase in cholesterol-rich detergent-resistant membrane domains (DRMs). In Alzheimer's disease (AD) the level of Seladin 1 in affected neurons is reduced, DRMs are disorganized and AB42 formation is increased. To examine genetic association of the DHCR24 with AD, we genotyped four single nucleotide polymorphism (SNP) sites in 414 Finnish AD cases and 459 controls using TaqMan® SNP Genotyping Assays and ABI7000 fluorescence sequence detection system and calculated the allelic and genotypic distribution of both cases and controls. The single locus association analysis indicated that men carrying certain SNP genotypes had an increased risk of AD. We also estimated haplotypes between cases and controls and found overall distribution of haplotypes highly significant in both men and women. Our findings indicate that DHCR24 gene may be associated with AD risk.

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A Novel Deletion in Progranulin Gene is Associated With FTDP-17 and Cbd in Two Italian Pedigrees

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Background and aims: Frontotemporal dementia (FTD) is the second most common form of early-onset dementia. In different clinical FTD series, 20 to 50% of cases have a family history of dementia with an autosomal dominant pattern of inheritance, indicating a significant genetic contribution to the etiology of this disease. Aim of the present study was to provide an extensive clinical and genetic characterization of two Italian pedigrees presenting with familial FTD

Methods: Microsatellites analysis on chromosome 3, 9 and 17 was performed in two families (FAM047 and FAM071). FastSLink was used to calculate the power to detect linkage. Two point and multipoint linkage analyses were performed using FastLink and Simwalk respectively. Mutation analysis in the genes MAPT, PGRN, APP, PSEN1, PSEN2 and PRNP was performed by sequencing.

Results: Genetic analysis showed a conclusive linkage (LOD score: 4.173) to chromosome 17 and defined a candidate region containing MAPT and PGRN genes. In affected subjects belonging to both families, we identified a novel deletion mutation of PGRN gene associated with a variable clinical presentation ranging from FTDP-17 to corticobasal degeneration.

Conclusions: Both mutations in MAPT and PGRN genes are associated with highly variable clinical phenotypes. Despite the profound differences in the biological functions of the encoded proteins, it is not possible to define a clinical phenotype distinguishing the disease caused by mutations in MAPT and PGRN genes.

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Low Prevalence of APP Duplications in Swedish Alzheimers Disease Patients

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Background and aims: It is well known that most patients with Down's syndrome (trisomy 21) develop Alzheimer's disease (AD) in early middle age due to the extra copy of the APP gene, located on chromosome 21. Recently, two groups reported that duplications of APP, in the absence of trisomy 21, can cause familial early onset Alzheimer's disease with cerebral amyloid angiopathy (EOAD/CAA) [Rovelet-Lecrux et al. 2006; Sleegers et al. 2006]. In this study, we have screened for APP duplications in affected subjects from Swedish families and cases with EOAD.

Methods: Approximately 50 families with features of EOAD and some 35 individuals with EOAD without known familial inheritance patterns have been screened for APP gene load by quantitative PCR using the SYBR green chemistry. The number of APP copies was determined relative to β-globin and normalized to control DNA using the 2-ΔΔCt method. Two samples from patients with trisomy 21 were included as positive controls.

Results: Preliminary results show few or no cases of APP gene duplication in these Swedish families and cases. However, more regions in and near APP will be screened to verify these results.

Conclusion: Duplications of the APP gene does not seem to be a common cause of EOAD in the Swedish population, at least not in our collection of Swedish families and cases.

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HTRA2 Mutations in Parkinson's Disease

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Background and aims: Mitochondrial dysfunction is considered a key player in Parkinson's disease (PD) pathogenesis. High temperature requirement protein A2 (HTRA2, PARK13) is a nuclear-encoded mitochondrial protein, carrying an inhibitor of apoptosis protein motif, a serine protease domain and a PDZ domain that regulates its protease activity. HTRA2 has been proposed to be a proapoptotic protein. In non-apoptotic conditions it is involved in maintaining mitochondrial homeostasis. HTRA2 knockout

mice were reported to have a parkinsonian phenotype and its protease activity was suggested to be physiologically most critical. The recent identification of a G399S mutation within the PDZ domain in sporadic PD patients prompted us to perform a detailed HTRA2 mutation analysis in Belgian PD patients.

Methods: Extensive mutation analysis of 266 Belgian PD patients by direct sequencing (exons, exon-intron boundaries and regulatory regions). Association study with polymorphisms genotyped in the PD patient/control samples.

Results: We detected a novel missense mutation within the PDZ domain predicted to freeze HTRA2 into an inactive form. Sixty-seven percent of the remaining sequence variants absent in control individuals were located in the HTRA2 promoter region and occurred within consensus sequences for transcription factors binding sites. We did not identify common variants or haplotypes associated with PD risk.

Conclusions: Our results argue against a major role of common HTRA2 variants in PD, but confirmed the occurrence of HTRA2 mutations in PD patients. Potentially pathogenic mutations were mainly situated throughout the HTRA2 promoter, emphasizing a previously unrecognized role for altered HTRA2 expression in PD pathogenesis.

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Genetic Analyses of Progranulin (Pgrn) and Tar Dna Binding Protein (TDP43) in Frontotemporal Dementia (FTD) and Related Neurodegenerative Diseases

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Aim: To identify causal mutations and risk variants in progranulin (PGRN) and TAR DNA binding protein (TDP43) in patients with frontotemporal dementia (FTD) and related disorders.

Background: We recently demonstrated that mutations in PGRN cause chromosome 17-linked FTD with tau-negative, ubiquitin-positive inclusions (FTLD-U). TDP43 accumulates in these inclusions and in ubiquitinated inclusions in amyotrophic lateral sclerosis (ALS) patients.

Methods: PGRN was sequenced in 400 FTD/FTD-ALS patients. PGRN association analyses were performed in large FTD and Alzheimer's disease (AD) patient-control series using a high-density tagging SNP panel developed by sequencing the complete PGRN genomic region. TDP43 sequencing was performed in 200 FTD/FTD-ALS patients. Association analysis with the two major TDP43 haplotypes was performed using rs3765896.

Results: PGRN mutations were identified in 10% of FTD/FTD-ALS patients, 23% of patients with a positive family history and 25% of patients with FTLD-U. Mutation carriers showed age-related disease penetrance of 50% at 60 and >90% at 70 years, with language dysfunction as common presenting symptom. PGRN association analyses in FTD/FTD-ALS populations are ongoing, however, no association was identified in a Mayo Clinic community-based AD patient-control population.

We did not identify causal mutations in TDP43 nor did we identify association of TDP43 in our extended FTD/FTD-ALS population. Association studies of TDP43 in sporadic ALS and AD patient-control populations are currently performed.

Conclusions: Mutations in PGRN are a major cause of FTD, while mutations in TDP43 are rare or non-existent in FTD and/or ALS. Initial association studies did not identify genetic risk variants in PGRN or TDP43.

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Mutation Analysis of the Progranulin Gene in a Scandinavian Frontotemporal Dementia Population

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Frontotemporal dementia (FTD) is a neurodegenerative disorder clinically characterized by behavioral and personality changes, together with a progressive change in language. Neuropathologically, approximately 40 % of the patients display ubiquitin-positive intranuclear and cytoplasmic inclusions. Recently, mutations in the progranulin (PGRN) gene were found to be the cause of disease in a subset of these cases. All mutations identified so far are null mutations and lead to a loss of functional PGRN. In the present study, we performed a systematic screening of all 12 coding exons and the non-coding exon 0 of the PGRN gene in 50 FTD patients from a Scandinavian population. We also investigated PGRN copy-number alterations using quantitative PCR-based assays of exons 1 and 12. Preliminary results indicate that mutations in the PGRN gene are not a common cause for frontotemporal dementia in the Scandinavian population. A novel mutation, Leu53Pro in exon 2, was identified in a sporadic case with FTD, but not in 160 healthy controls. However, the significance of this mutation for the disease needs to be further evaluated.

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Progranulin Modifies Onset Age and Survival in Amyotrophic Lateral Sclerosis

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Background and aims: Null mutations in progranulin (PGRN) cause ubiquitin-positive frontotemporal dementia (FTD) linked to chromosome 17q21 (FTDU-17). Here we examined PGRN genetic variability in amyotrophic lateral sclerosis (ALS), a neurodegenerative motor neuron disease that bears resemblance to FTD at a clinical, pathological and epidemiological level. Methods: We sequenced all exons, exon-intron boundaries and 5' and 3' regulatory regions of PGRN in a Belgian sample of 230 ALS patients. The frequency of observed genetic variants was determined in 436 healthy control individuals. The contribution of 8 frequent polymorphisms to ALS risk, onset age and survival was assessed in an association study. Results: In ALS patients we identified 11 mutations, 5 of which were predicted to affect PGRN protein sequence or levels (4 missense mutations and one 5' regulatory variant). Moreover, one common variant (IVS2+21G>A; rs9897526) was significantly associated with a 7.7 (95% CI 3.1-12.3) years reduction in age at onset, and a reduced survival after onset of ALS (Hazard ratio 1.58 (95% CI 1.03-2.40)). Conclusions: PGRN acts as a modifier of the course of disease in patients with ALS, through earlier onset and shorter survival. This finding, in the long run, may have therapeutic implications for this fatal disease.

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Association Between Nitric Oxide Synthase Gene (NOS-3) Polymorphism and the Risk of Alzheimer's Disease

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Background: The main function of endothelial isoform of nitric oxide synthase (NOS-3) is regulation of vascular tone and blood pressure. NOS-3 is also involved in processes of learning and memory by evoking NMDA receptor-independent form of LTP. NO may also affect many other physiological processes, including oxidative stress, inflammation and apoptosis. The formation of NO by NOS-3 may be influenced by functionally important, common polymorphism NOS3, 894 G/T (Glu298Asp). Molecular-epidemiological studies have presented contradictory results concerning a potential role of this polymorphism. The objective of the study was to compare frequencies of NOS3 G/T polymorphism in AD patients and healthy Polish population. Methods: We analysed a common polymorphism of NOS3 gene in two groups of people: AD patients (n=154, F-102, M-52, mean age of onset = 71.5 ± 4.67) and the non-demented control group (n=176, F-120, M-56, mean age = 72.7 ± 6.23; MMSE score> 27). In all AD patients the disease was diagnosed as probable according to the NINCDS-ADRDA criteria. Genotyping was done using PCR-RFLP method. Results: Frequencies of NOS3 genotypes G/G, G/T and T/T in AD group were: 0.69: 0.25: 0.06. In the control group those frequencies were: 0.56: 0.35: 0.09 respectively. Genotype G/G was significantly more frequent in AD group than in the control group (X2 test, p=0.024), and the "G" allele also significantly overrepresented in AD group (X2 test, p=0.025). Conclusion: The results of the study indicate significant association between NOS3 common polymorphism and the risk of Alzheimer's disease in the Polish population. Acknowledgement: The study was supported by grant PBZ-MIN-001/P05/16

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Genetic Variation in Pgrn in a Belgian Parkinson's Disease Population

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Background and aims: Null mutations were identified in the progranulin gene (PGRN) in frontal temporal dementia patients with ubiquitin positive inclusions (FTDU), and FTDU families linked to chromosome 17q21. We know little of the protein's function in CNS but so far data have indicated that progranulin might be a neuronal survival protein, implicating that it could also play a role in other neurodegenerative brain disorders (NBD). Furthermore, FTD patients are diagnosed with additional features of motor symptoms (parkinsonism). Therefore we performed a mutation analysis of PGRN in a Belgian Parkinson's disease (PD) population. Methods: All coding and conserved regulatory regions were screened by direct sequencing in 270 Belgian PD patients. Results: Interestingly, we identified the splice donor site mutation in intron 0 (IVS0+5G>C) in one patient suffering from PD with dementia. We previously identified this mutation in an extended Belgian founder FTDU-17 family, DR8, and showed that the unspliced mutant allele was most likely degraded in the nucleus leading to haplo-insufficiency . In at least 1 branch of this founder family patients presented, apart from FTD, also with symptoms of parkinsonism. We also identified 3 missense and 2 silent mutations, however in silico predictions showed that they had no significant effect on protein function. Further, we observed 18 polymorphisms also present in control individuals. Conclusion: We conclude that mutations in PGRN are not a major cause of PD. However, clinical heterogeneity among NBD patients might warrant screening of PD patients for PGRN mutations particularly when PD is associated with dementia.

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Novel CHMP2B Mutations in Frontotemporal Dementia and Corticobasal Degeneration

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Background and Aims: The charged multivesicular body protein 2B gene (CHMP2B) has recently been identified as the gene responsible for frontotemporal dementia (FTD) linked to the pericentromeric region of chromosome 3. To date, linkage to this region is unique to a large Danish FTD family in which the original CHMP2B splice site mutation was identified, and very few other mutations were detected since. The aim of this study is to further determine the impact of CHMP2B on FTD and potentially other neurodegenerative diseases.

Methods: We performed an extensive mutation analysis CHMP2B in a Belgian patient series of 103 unrelated FTD patients, seven patients with corticobasal degeneration (CBD) and five progressive supranuclear palsy patients (PSP).

Results: Mutation analysis of CHMP2B revealed two novel mutations. A missense mutation in a CBD patient, and a nonsense mutation in a FTD patient. Both patients had a positive family history of dementia. Mutations were excluded in 918 control chromosomes.

Conclusion: In 103 FTD patients we identified one novel CHMP2B mutation giving a frequency of 0.97% (1/103) of all FTD patients, and 2.33% (1/43) of patients with a positive family history of FTD. In addition, a second novel CHMP2B mutation was identified in one CBD patient. Our results provide confirmation of the pathogenicity of CHMP2B mutations in FTD. Moreover, CHMP2B appears to be involved in other neurodegenerative diseases, including CBD.

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Progranulin Missense and Promoter Mutations in Frontotemporal Dementia

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Background and Aims: Null mutations in PGRN have recently been identified in frontotemporal dementia (FTD) with ubiquitin-positive brain pathology linked to chromosome 17 (FTDU-17). The aim of this study was to investigate if mutations other than null mutations contributed to the complex genetic etiology of FTD.

Methods: We performed an extensive mutation analysis of PGRN in a large FTD patient series from Belgium and France, comprising a total of 299 patients. The pathogenic nature of identified mutations was investigated using in silico conservation and structural analyses, assessing their effect on the biological function of PGRN and PGRN levels.

Results: We identified three novel missense and three novel promoter mutations that were not present in 1200 control chromosomes.

Conclusion: Our data support that PGRN missense and promoter mutations contribute to the genetic etiology of FTDU-17. When considering the total genetic variability of PGRN, including null mutations, missense mutations, and promoter mutations, observed in our patient series, PGRN mutations account for 9,36 % of FTD in general, 22.22 % of familial FTD, 53.33% of pathologically confirmed FTD, and 66.67% of FTDU.

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Knock-in Mutations in the Intracellular Domain of LRP1 Result in Reduced Levels of Secreted Abeta in MEF Cell Lines

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Introduction: Abeta deposition in the brain is a major hallmark in Alzheimer's disease. An increasing number of studies indicate that LRP1 (Low Density Lipoprotein Receptor-related Protein 1), especially via its intracellular domain, has a crucial dual role in production, deposition, degradation and clearance of Abeta in the brain. After binding of Abeta to ligands of LRP1, Abeta can be endocytosed and degraded in the cell. Via interactions with APP, the precursor for Abeta, however, LRP1 can also stimulate the production of Abeta.

Objectives: Different LRP1 knock-in mice carrying inactivating mutations in motifs of the LRP1-ICD were generated by RMCE (Roebroek et al. MCB, 2006). These were used to derive MEF cell lines to investigate the effect of LRP1 mutations on APP processing and degradation of Abeta.

Results: MEF cell lines derived from an NPXY(2)XXL and an ICD-truncation mutant, show significantly lower Abeta levels in the medium upon transfection with an APP751Swe construct compared to WT MEFs. This can be due to increased Abeta clearance, reduced Abeta production or a combination of both. Preliminary endocytosis experiments with PEA, heparanase or alpha2M, indicate that the basal endocytosis function of LRP1 is not affected by these mutations. So the phenotype is probably due to changes in Abeta production. A BACE1 activity assay indicates that both mutations do not affect BACE1 activity. Presently it is under investigation whether a prolonged cell surface residence of APP, might be responsible for increased alpha-cleavage resulting in less Abeta production.

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DJ-1 is Sumoylated by SUMO2/3

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Mutations in the PARK7/DJ-1 gene were found to be associated with autosomal recessive early-onset Parkinson's Disease (PD).

DJ-1 has been recently reported to be SUMOylated by SUMO1 at lysine-130 (Shinbo et al., Cell Death Differ 13(1):96-108, 2006) and to be involved in global protein SUMOylation (Zhong et al., J Biol Chem 281(30):20940-8, 2006).

To dissect the molecular events related to DJ-1 function, we have isolated a group of DJ-1 interactors from a human foetal library. Among others, Small Ubiquitin-like MOdifier 1 (SUMO1), SUMO-activating enzyme (Uba2), and SUMO-conjugating enzyme (Ubc9) were identified as DJ-1 interactors.

Here we show that DJ-1 is modified by SUMO 2/3 in vitro and in vivo. Focusing on SUMO3 modification, we report that DJ-1 is modified on a different Lys than SUMO-1. Furthermore, the PD-linked mutant L166P is ipersumoylated. We are currently investigating whether oxidative stress may affect DJ-1 sumoylation by SUMO 2/3.

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Il-1 Alpha and Il-1beta Genetic Polymorphism Have Influence on Age at Onset of Sporadic Parkinson's Disease in Taiwan

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It has been reported that IL-1α-899 C/T genetic polymorphism increased the risk of MSA and AD, but which is not confirmed in PD. IL-1β -311 C/T promoter polymorphism has been reported associated with AD, PD, MSA and SCA6. We investigate whether the genetic variability in IL-1α and IL-1β are associated with susceptibility to PD amongst Taiwanese.

Methods: A group of 493 patients with sporadic PD and and 388 unrelated healthy adult volunteers were recruited. DNA was extracted and biallelic SNAP was performed using the PCR-RFLP method.

Result: PD and control groups were stratified in the following subgroups: >50, >60, >70 years. Significant difference in genotype frequency distribution for 1α-889 C/T between PD patients and controls was observed (P =0.034) in the > 70 years subgroup. Genotype frequencies of 1α -889 C/T were notably lower in the PD patients than the controls (11.7% vs 22.9%, P = 0.021) with an odds ratios of 0.44 (95% CI: 0.22-0.88) as compared to -889 C/C genotype. There was no difference for the IL-1β-511 C/T genetype between the PD patients and controls. We also failed to detect any difference in disease onset between each genotype of the IL-1 B polymorphisms in PD.

The 1α -889 C/T IL-1β-511 C/T (8.8% vs 20.0%, P = 0.019) showed a significant difference between the PD patients and controls with an odds ratio of 0.31 (95% CI: 0.12-0.73; P =0.009).

Conclusion: IL-1α(-899) and IL-1β(-511) genetic polymorphism have influence on age- at- onset of sporadic PD in Taiwan.

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Association of Dopamine D1, D2 Receptor and Dopamine Transporter Gene With Late Onset Alzheimer Disease in Taiwan Chinese

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Purpose: The dopamine receptor and transporter genes have been proposed as candidate genes for several psychiatric and neurologic disorders. There was evidence that the presence of psychotic symptoms and aggressive behavior in AD patients and the DRD1 polymorphism. The DRD2 Taq1 genetic variation has an influence on cognitive performance in the memory impaired elders. The dopamine transporter gene (DAT1) is associated with Parkinson disease in some populations. We aimed to evaluate the relationship between the DRD1, DRD2 Taq1, DAT1 genes and late onset Alzheimer disease in Taiwan Chinese.

Patients and Methods: This study included 65 patients with late onset Alzheimer disease (AD) (onset age >65 years old) and 103 age matched, healthy controls. Polymerase chain reaction (PCR) based analysis was used to resolve the DRD1, DRD2 Taq1 and DAT1 polymorphism.

Results: There were significant differences in the distribution of the DRD2 Taq1 polymorphism between the late onset Alzheimer patients and the control subjects ($p<0.05$). The allele T frequency of the DRD Taq1 was less frequently found in the AD patients than in the healthy patients (OR=2, CI=1.23-3.32, P=0.003). However there is no association between the DRD1 gene or DAT1 gene and the late onset Alzheimer patients in Taiwan Chinese.

Conclusions: The frequency of the Allele T of the DRD2 Taq1 polymorphism was relative low in late onset AD patients. Therefore the Allele T might be a protecting factor to predicate the genetic causes of late onset Alzheimer disease patients among Chinese in Taiwan.

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An Association Study of the iNOS 22 Gene Polymorphism and Parkinson's Disease in a Greek Population

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Parkinson's disease (PD) is a common neurodegenerative disorder, characterized by the progressive loss of dopamine neurons. This degeneration could be associated with nitric oxide synthase (NOS), a biological messenger involved in nitric oxide (NO) production, which can be neurotoxic when overproduced. NOS exists in three known isoforms. The neuronal and inducible isoforms have been implicated in PD pathogenesis. Genetic variations in the NOS gene have been significantly associated with PD. It is therefore a candidate gene for PD pathogenesis. In this report, we examine the association between polymorphism iNOS 22 resulting from a GŭA substitution in position 37498, in exon 22 of the inducible NOS (iNOS) gene and PD, in an independent cohort of Greek patients and control individuals.

Our study group was composed of 72 Greek patients diagnosed with late onset PD, in Papanikolaou Hospital, Thessaloniki, Greece . The control group consisted of 90 healthy individuals and was matched for age and ethnic origin. PCR and RFLP methods were used for the genotyping of the iNOS 22 polymorphism. Comparison of genotype frequency distributions in patients and controls was done with the $\chi 2$ test of independence.

The distribution of genotype frequencies was GG= 47.23%, GA=44.45%, AA=8.33% for the patient group, and GG= 37.78%, GA=50.00%, AA=12.22% for the control group (P=0.429) (OR=1.474 95% CI=0.79-2.76).

We detected a non-statistically significant overrepresentation of the GG genotype among PD patients, compared to the control group. A larger study needs to be carried out in order to establish whether this difference can attain statistical significance.

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The G2019S LRRK2 Mutation is Uncommon in a Greek Cohort of Parkinson's Disease Patients

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Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting approximately 2% of the population over 65 years of age. Although, the etiology of PD is still unknown, the genetic background of the disease has been documented. Recently, a mutation in the LRRK2 gene, G2019S, was associated with 3%–6% and 1%–2% of familial and sporadic PD, respectively suggesting a pivotal role of LRRK2 in PD. In this report we examine the association of the G2019S mutation with sporadic late-onset PD, in an independent cohort of Greek patients and controls.

Our study group was composed of 180 Greek patients diagnosed with late onset PD, in Papanikolaou Hospital, Thessaloniki, Greece. The control group consisted of 160 healthy individuals over 65 years of age and was matched for sex and ethnic origin. The relevant portion of the LRRK2 gene was PCR-amplified and the genotyping for the G2019S mutation was accomplished with the RFLP method.

We found no subject carrying the G2019S mutation in our Greek cohort. So, our results indicate that the G2019S mutation identified to date both in familial and sporadic PD in many ethnicities is not at all common in the population of Northern Greece. Possibly, the mutational frequency of the LRRK2 G2019S shows ethnic and geographical variability. Further studies in multiple ethnicities need to be undertaken in order to establish or reassess the role of the LRRK2 gene in the pathogenesis of PD.

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Unique Pattern of Hotspots in PARK2 With Reduced Parkin Levels in Indian Parkinson`s Disease Population

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Parkin gene mutations have been implicated in early-onset Parkinsonism characterized by degeneration of dopaminergic neurons in midbrain with the prevalence rate of 19 per 100,000 in India. We report significantly altered parkin levels in patients of Parkinson's disease (PD) corresponding to the mutations in the PARK2. The non- familial PD patients grouped into young-onset PD (age <40 years) and sporadic PD (above > 40 years). Parkin gene mutation analysis by PCR, SSCP and direct sequencing and corresponding protein expression was detected by flowcytometry. We report that the occurrence of exonic deletion as well as point mutations detected by PCR, SSCP and direct sequencing, which correlates positively with altered parkin protein blood levels in both young onset and sporadic Parkinson's disease (PD) patients. This observation proves that PARK2 mutations significantly modify parkin protein expression among north Indian PD patients (P=0.03). Such loss of parkin function could directly induce rapid degeneration of nigral neurons. In addition, we have found milder phenotype among PD patients with mutations as compared to those without mutations.

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Atypical Phenotype in a Patient With Progranulin Gene Mutation

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Aim: Clinical and pathological study of an atypical patient with progranulin (PGRN) mutation.

Background: Frontotemporal lobar degeneration with ubiquitin positive inclusions (FTLD-U) has long been recognized as a tau mutation negative familial FTD. Recently, FTLD-U was linked to mutations in PGRN. Patients typically present with a frontotemporal dementia phenotype, displaying prominent behavioral changes and aphasia. Methods: The patient underwent several clinical evaluations. After death, the brain was examined by neuropathologic methods, and with tau, α-synuclein and ubiquitin immunostaining. DNA was extracted from brain tissue. Results: She was 62 when she developed stuttering, along with personality changes, slowness and gait instability, with no relevant family history. She subsequently developed marked stuttering dysarthria, reduced spontaneous language, impersistence, choreic movements of the cheek, mouth and tongue, and mild parkinsonism. She became mute over 3 years, with endstage dementia. She died at age 65. At autopsy, the brain was macroscopically normal, including the frontal and temporal lobes, hippocampus, substantia nigra and basal ganglia. Histologic exam showed only mild striatal gliosis. Immunostaining revealed sparse ubiquitin-positive, tau- and α-synuclein-negative cytoplasmic and intranuclear inclusions in cortex and basal ganglia, but none in hippocampus. Genetic analysis showed c.26C>A mutation in exon 1 of PGRN, predicted to induce an Ala9Asp change in the protein. Conclusions: This case FLTD-U with a PGRN mutation adds to the clinical and pathological diversity of the disease, with prominent dysarthria and abnormal orobuccal movements, and with nearly normal pathological examination. It again raises nosologic questions regarding the clinical, pathological and genetic definition of FTLD-U.

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Genetic Variability in Progranulin is Associated With Alzheimer's Disease

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Background and Aims: Null mutations in the progranulin gene (PGRN) were identified in frontotemporal dementia (FTD) with ubiquitin-immunoreactive neuronal inclusions (FTDU) linked to chromosome 17q21 (FTDU-17). Here, we assessed whether genetic variability in PGRN contributed to Alzheimer's disease (AD) in a large Belgian AD patient group (N = 779). Methods: Using direct sequencing we performed an extensive mutation analysis of all exons and 5' regulatory region of PGRN. Results: We identified ten missense mutations, seven of which were absent from control individuals. In silico predictions based on conservation and protein modeling provided evidence in favor of a pathogenic nature for at least two mutations. We also identified two PGRN null mutations in three patients (IVS0+5G>C and p.Arg535X). The two AD patients carrying the IVS0+5G>C mutation shared alleles at 17q21 with FTDU-17 patients of the Belgian FTDU-17 founder family (DR8), suggesting the same PGRN mutation might present with a high degree of clinical heterogeneity. Further, using SNP haplotypes we showed that PGRN is associated with increased risk for AD. Conclusion: We provided evidence that PGRN missense mutations as well as null mutations contribute to AD, with missense mutations found more frequently in patients than in control individuals (2.2% versus 0.7%). At the level of more common variation, we evidenced that two specific SNP haplotypes are associated with AD risk.

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Getenic Analysis of LRRK2 in Japanese Patients With Parkinson's Disease

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The mutations of Leucine-rich repeat kinase (LRRK2) gene cause familial Parkinson's disease (PARK8). In Europe about 1% of sporadic Parkinson's disease (PD) and about 6% of familial PD were reported to have G2019S mutation of LRRK2 gene. LRRK2 is a relatively large gene and has 51 exons and this protein consists of 2,527 amino acids. The peripheral lymphocytes express the mRNA of LRRK2. We analyzed mutations of LRRK2 gene in some Japanese patients of familial PD with RT-PCR-sequencing method using lymphocytes of peripheral blood from patients. And one of them had the R1320S heterozygous mutation. None of them had G2019S mutation. We planned to examine the frequency of these two mutations in Japanese patients with PD and healthy controls.

Subjects: We analyzed 136 sporadic PD, 10 familial PD and 113 control subjects. We used PCR-RFLP fragment analysis method for screening the R1320S and G2019S mutations.

Results: The frequency of R1320S mutation was 6.8% in sporadic PD, 10% in familial PD and 9.0% in healthy controls. None of these subjects had G2019S mutation.

Discussion: Codon 1320 is cited at the intron-exon border and this mutation might change the expression and splicing of LRRK2 mRNA. But our results showed no significant difference of the R1320S frequency between PD and control. We thought this mutation might not be the risk factor for PD. And our results showed G2019S mutation was rare in Japanese PD.

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Clinical Heterogeneity in Familial Dementia +/- Parkinsonism Associated With the c.154delA Mutation in Progranulin

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We recently identified mutations in progranulin (PGRN) that are associated with familial frontotemporal dementia and parkinsonism.

We analyzed all available data in affected individuals in the F142 kindred followed at our center.

Eight individuals became symptomatic; data was available for 4 in generation 1 (G1) and 3 in generation 2 (G2). The mean age of onset in G1 was 69 years (range 69-80), and in G2 was 61 years (51-66). The duration of symptoms was 4.3 years (2-8) in G1 and 4.0 (1-7) in G2, with all 3 in G2 still living. The clinical diagnoses among these 7 included mild cognitive impairment, Alzheimer's disease, frontotemporal dementia, primary progressive aphasia, and corticobasal syndrome; some diagnoses changed during the course of their illnesses, and some carried overlapping diagnoses. Autopsies in 4 individuals from G1 revealed frontotemporal lobar degeneration with ubiquitin-positive intracytoplasmic inclusions and neuronal intranuclear inclusions (FTLD-U NII), with one also having mixed AD/vascular disease pathology with severe frontotemporal atrophy. PGRN sequence analysis in all subjects revealed a single base pair deletion in exon 2 (c.154delA). The effect of this mutation is to cause a frameshift (Thr52HisfsX2), creation of a premature termination codon, and, likely, create a null allele through nonsense mediated decay.

The findings in this family suggest that a wide array of cognitive, behavioral, and parkinsonian features are associated with the c.154delA mutation in PGRN. The earlier age of onset in G2 and lack of overlap in ranges of age on onset between generations raise the possibility of anticipation-like effect.

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Somatostatin Genetic Variants Modify the Risk for Alzheimer`S Disease Among Finnish Patients

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Background: The levels of SOMATOSTATIN (SST) are consistently decreased in the brain and cerebrospinal fluid of Alzheimer`s disease (AD) patients. We have previously shown that the microsatellite marker D3S2436 on chromosome 3q28 was associated with late-onset AD (LOAD) in patients originating from Finland (Hiltunen et al. 2001; Neurology 57(9):1663-8). Interestingly, the SST gene is located in close proximity (~ 500 kb) to this site. Method: To assess the genetic association of SST with AD, we genotyped three single nucleotide polymorphisms (SNPs) within this gene among Finnish AD patients (n=424) and non-demented controls (n=466). AD patients were compared with non-demented control subjects using single-locus and haplotype approaches. Results: According to single locus analyses SST is a possible risk gene for AD, especially among the APOE ε4(+)-allele patients. When AD patients and controls were analyzed together in a pair-wise LD analysis (1780 alleles), a strong LD was observed between all three SNPs analyzed (D'=1.0 in all pairs, $p<0.01$) and these SNPs originated from the same haplotype block. The haplotype data reinforces the data observed from the single locus analyses. No other genes than SST are known to exist within this haplotype block. Conclusion: This first genetic association study between SST and AD indicates that genetic variations in the SST gene may modify the risk for AD among Finnish AD subjects.

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Association of Prostaglandin-Endoperoxide Synthase 2 (PTGS2) Polymorphisms and Alzheimer's Disease in Chinese

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Alzheimer's disease (AD) is the most common form of dementia among the elderly. Cyclooxygenase-2 (COX-2) is a key enzyme in the conversion of arachidonic acid to prostaglandins, encoded by prostaglandin-endoperoxide synthase 2 (PTGS2) gene. The prostaglandins produced by COX-2 are involved in inflammation and pain response in different tissues in the body. Enhanced COX-2 expression had been shown in regions of brains from patients with AD. We therefore investigated the association of polymorphisms on PTGS2 gene and the risk of AD in the Chinese population.

257 AD patients and 244 age-matched healthy Chinese subjects were recruited in this case-control study. Among the 38 SNPs from the HapMap database and data from SNP500Cancer database, four tagging SNPs were chosen to comprehensively cover the genetic variations in the PTGS2 gene. We found that PTGS2 Ex10+837T>C was associated with the risk of AD ($p=0.03$). Carriers of T allele had a 1.5 fold increase in the risk of AD. On the other hand, the haplotype G-A-T-C showed a significant reduction in the risk of AD ($p= 0.041$, 95% CI: 0.45-1.00). This study suggested that PTGS2 gene was a predisposition gene and arachidonic acid metabolism might be involved in the pathogenesis of AD.

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Pure Progressive Amnesia as a Variant of Genetically Proven Alzheimer's Disease

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In October 2000, a 50 year old, right handed, French speaking patient complained of memory deficit since 5 years. His medical history was unremarkable. His mother, grand mother and aunt were institutionalized for "memory and personality problems". Neurological examination was normal and his Minimal Mental State Examination score was 28/30. An extensive neuropsychological examination showed isolated deficit in verbal learning. His electroencephalogram, cerebral MRI and blood sample were normal. The visual analysis of his cerebral FDG-PET showed slight cortical hypometabolism on temporal. Repeated neuropsychological evaluations remained similar during the next two years.

In view of the positive familial history, DNA diagnostic testing was performed by direct sequencing of exons 16 and 17 of the amyloid precursor protein gene (APP), exons 3 to 12 of the presenilin 1 gene (PSEN1), and exons 9 to 13 of the microtubule associated protein tau gene (MAPT). The sequencing of exons 16 and 17 of APP showed the London APP717 mutation. The evolution of this patient is remarkable for the striking stability of the purely amnesic nature of his symptoms over 10 years. This case report shows that sequencing of known AD genes might be worthwhile, even in patients with a pure amnesia and probably also in patients with any other form of focal dementia when a positive familial history, an early onset of the disease, or both are observed.

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Sigma Receptor Type-1 Gene Variation in a Group of Polish Patients With Alzheimer's Disease

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Alzheimer's disease (AD) pathogenesis is dependant upon complex interactions between genetic, physiological and environmental factors. Apart from the APOE4 allele variation of several other genes is postulated to contribute to the risk of LOAD. In the sigma 1 receptor gene (SIGMAR1), which protein is implicated in learning, memory, motor control, neuritogenesis, and other functions related to AD pathogenesis, three common variants were identified: G-241T/C-240T and A61C. In the Japanese population TT/C haplotype was suggested to be a protective factor for AD.

We investigated a putative link between the SIGMAR1 variants and AD in a group of 219 Polish LOAD patients and in a control group of 308 healthy individuals. No significant deviations from Hardy-Weinberg equilibrium were observed in each group and in all groups together. Contrary to the previous reports, no statistically significant differences were observed for G-241T/C-240T and A61C genotype distributions in the LOAD group, compared with the controls (74% GC/GC, 23% GC/TT, 3% TT/TT vs 72% GC/GC, 26% GC/TT, 2% TT/TT, p=0.66 and 73% AA, 23% AC, 4% CC vs 71% AA, 27% AC, 2% CC, p=0.57, respectively). There were no differences in haplotype distributions between both groups (LOAD: 84.5% GC/A, 14.6% TT/C, 0.7% GC/C, 0.2% TT/A; Controls: 84.1% GC/A, 15.3% TT/C, 0.6% GC/C; p=0.69). The polymorphisms were in a very strong but not complete disequilibrium.

We postulate no association of the TT/C genotype with a decreased risk for LOAD in the Polish population. Further studies are required to fully elucidate the effect of TT/C polymorphism on AD.

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VEGF Promoter Polymorphism and Increased Plasma Levels Are Associated With AD Progression and MCI Conversion to AD

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Background:The expression of VEGF represents one potential pathological mechanism of AD. We investigated whether AD cases carried a functional promoter gene variant for VEGF and showed elevated plasma levels of Vegf. In addition we investigated whether patterns of association were found for mild cognitive impairment (MCI) and conversion from MCI to AD. Methods:317 AD cases and 320 control subjects,113 MCI patients and 130 control subjects were genotyped by PCR.Plasma VEGF was measured with chemiluminesc.ence Findings: VEGF AA genotype was associated with an increased risk of AD (OR = 1.616). This genotype was also associated with an accelerated cognitive decline in APOE epsilon4+ AD patients (O.R = 6.5). The VEGF AA genotype was a risk factor for MCI (O.R.= 2.5) and MCI conversion to AD in APOE epsilon4+ (O.R.= 6.5).Vegf plasma levels were higher in AD patients than controls (230pg/ml vs 42 pg/ml), being even higher in those patients with a fast cognitive decline and the APOE epsilon4 allele.Interpretation: Modulation of VEGF expression is a potential mechanism associated with the risk of developing AD and it's clinical deterioration. These data have important therapeutic implications and suggest that several drugs with positive regulatory effect on brain circulation, neurogenesis and glial activation might be used to delay the manifestation of the age-related cognitive impairment and dementia.

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A Yeast Two-Hybrid Approach to Study the Role of DJ-1 in Parkinson's Disease

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Mutations in the PARK7/DJ-1 gene were found to be associated with autosomal recessive early-onset Parkinson's Disease (PD). Genetic data suggest that a lack of functional DJ-1 leads to neurodegeneration of dopaminergic neurons of the midbrain and, ultimately, to PD. We hypothesize that an important aspect of the neurodegenerative process may involve the altered pattern of protein interactions for DJ-1 partners. Therefore, the identification of DJ-1-binding proteins may lead to the discovery of pathways fundamental for neurodegeneration.

To dissect the molecular events related to DJ-1 function, we have isolated a group of DJ-1 interactors from a human foetal library. We are currently characterizing these interactors to dissect their potential role in PD.

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Genetic Study Between SIRT1 Polymorphisms and Alzheimer Disease

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SIRT1 is the mammalian version of members of the family, known as sirtuins, which have been found in nearly all organisms studied. Sirtuins are assumed to play a key role in an organism's response to stress conditions such as heat or starvation and to be responsible for the lifespan-extending effects of calorie restriction. Our goal was to study the genetic association of the SIRT1 gene and Alzheimer's disease (AD) using the Finnish case-control material (326 AD cases and 463 controls) and single nucleotide polymorphisms (SNPs). In addition, we measured CSF tau, ptau and AB42, in a subgroup of AD cases and estimated levels of these biomarkers in different genotype and haplotype carriers. Genotyping of the SNPs was done using TaqMan Allelic Discrimination Assays and the CSF levels of AB42, tau and ptau were measured by an ELISA method according to the manufacturer's instructions. Preliminary results show that strong pairwise linkage disequilibrium according to the Haploview program (http://www.broad.mit.edu/mpg/haploview/) was found between SNPs and haplotype estimation suggested one haplotype block. Both single and haplotype frequencies of SNPs showed a significant difference between AD and controls in women older than 65 years. Data from biomarkers and its relation to genotype and haplotype carriers remain to be estimated.

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DJ-1 Protects Against Dopamine Toxicity: Implication for Parkinson's Disease

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Background: Mutations in DJ-1 lead to early onset autosomal recessive PD. Recently, changes in DJ-1 protein have been demonstrated in patients with sporadic PD. The metabolism of dopamine induces formation of toxic reactive oxygen species (ROS) and their accumulation is implicated in the special sensitivity of midbrain dopaminergic neurons to oxidative stress and degeneration in PD.

Aim: To examine whether DJ-1 is protective against dopamine toxicity and to elucidate the protective mechanism.

Methods: Human neuroblastoma SH-SY5Y cells were transfected with plasmids inducing overexpression of DJ-1 or SiRNA for DJ-1. Cells were exposed to dopamine, to the pro-oxidants 6-hydroxydopamine and rotenone and to the antioxidant N-acetyl cysteine. Protein and mRNA levels were quantified by Western blot and real-time PCR, respectively. Viability and intracellular ROS accumulation were monitored by the MTT and DCF assays.

Results: Exposure of human neuroblastoma cells to dopamine as well as to other pro-oxidants led to rapid upregulation of DJ-1. Dopamine-induced upregulation of DJ-1 was abolished by antioxidant treatment. Overexpression of DJ-1 increased cell resistance to dopamine toxicity, and reduced intracellular ROS. Contrary effects were achieved when DJ-1 levels were reduced by SiRNA. Moreover, we found that DJ-1 affects intracellular dopamine homeostasis and reduced free intracellular dopamine.

Conclusions: These observations suggest a novel mechanism in which ROS, generated by increased cytoplasmic dopamine, lead to rapid upregulation of DJ-1, which in turn protectively reduced free intracellular dopamine. This might explain why mutations in DJ-1 trigger PD and suggest that DJ-1 may represent a novel therapeutic target for PD.

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5,10-Methylenetetrahydrofolate Reductase C677T Gene Polymorphism Can Influence Age of Onset PD in Chinese Population

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Background and Aim: Recent studies have shown that that TT genotype of the 5,10-Methylenetetrahydrofolate reductase gene (MTHFR) C677T polymorphism is associated with an increased risk for Parkinson's disease (PD) in Caucasian. We performed a study to investigate: (1) whether the genetic polymorphism is associated with the development of PD in a Chinese population; and (2) the genetic polymorphisms could be a predictor of levodopa-induced adverse effects (LDIAEs)in PD patients.

Methods: This genetic case-control study included 94 PD patients treated with levodopa and 146 controls of Chinese origin, matched by sex and gender. The C/T polymorphism of the MTHFR was identified by conventional PCR and FRLP resulting in three genotypes (CC,CT and TT).

Results: There were no differences of the allelic and genotypic frequencies of the MTHFR C677T polymorphisms in PD patients from the controls. Analysis of age of onset in PD patients with MTHFR C677T polymorphism showed a trend of early age for 3.8 years of onset among PD patients carrying with T allele. The genetic influence was particularly significant in old-onset PD patients (onset age > 60 years). Analysis of the difference in the genetic polymorphism in PD patients stratified by LDIAEs revealed it was not associated with the risk to develop dyskinesia, "on-off"/"wearing off" and psychosis induced by levodopa.

Conclusions: The findings revealed that T allele of the MTHFR C677T polymorphism was associated with an early age of onset PD in Chinese population.

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Biochemical Characterization of Leucine Rich Repeat Kinase 2 (Dardarin)

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Background and aims: Leucine rich repeat kinase 2 (LRRK2) belongs to Ras/GTPase family of multi-domain proteins containing an N-terminal leucine rich repeat domain, a mixed lineage kinase domain and a C-terminal WD40 domain. Mutations in LRRK2 appear to be the most frequent cause of familial Parkinson's disease (PD) and sporadic PD. This combined with the pleomorphic pathology in LRRK2 mutation carriers have led to a heightened interest in understanding the effects of familial PD mutations in LRRK2 gene on functional activity of the protein. In the present set of studies we set out to evaluate the effects of the most frequent FPD mutation on kinase activity of LRRK2 as well as its impact on cellular differentiation and survival.

Methods: Kinase activity of wild type and mutant LRRK2 was assessed using myelin basic protein as the substrate. SH-SY5Y cells stably expressing LRRK2 were used as the cellular model of dopaminergic neurotoxicity.

Results: Comparison of wild-type LRRK2 with G2019S mutant LRRK2 expressed and purified from either the Sf9 cells or HEK293 cells demonstrated approximately 1.8-fold augmentation in kinase activity in the mutant protein. This increase in kinase activity was observed in both autophosphorylation and myelin basic protein phosphorylation assays. We also evaluated the effects of these mutations on differentiation of SH-SY5Y cells stably expressing wild type or mutant LRRK2.

Conclusions: The data provide further evidence of the pathologic role of familial mutations that cause an increase in kinase function by demonstrating their effects on differentiation of SH-SY5Y cells into dopaminergic phenotype.

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Clinical and Functional Description of a New Form of Autosomal Recessive Familial Parkinson´S Disease With Late Onset

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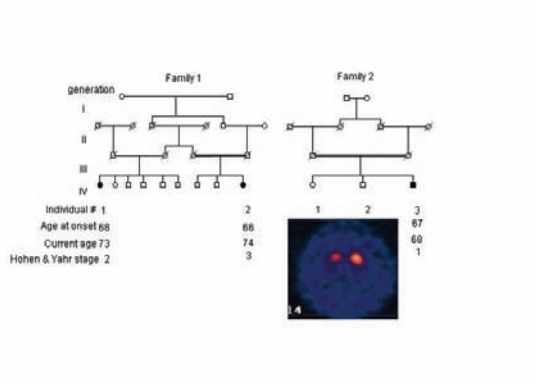
We report the clinical data of two families with autosomal recessive Parkinson disease coming from a genetic isolate region, in which known causative genes for recessive Parkinson disease (PARKIN, DJ1, PINK 1) were ruled out by homozygosity search. LRRK2 frequent mutation G2019S and α-synuclein were also examined and discharged as well, as the cause of a pseudorecessive pattern of transmission. These families present a late onset benign Parkinson disease that represents a new form of recessive familial Parkinson disease. Functional study with iodine-123-betaIoflupane Spect (Datscan®, GE) of affected cases showed a bilateral although asymmetrical uptake in putamen and caudatus nucleus, demonstrative that a presynaptic lesion was responsible for the clinical. 123–IBZM Spect was normal, ruling out a postsynaptic lesion.



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Identification of Novel Alzheimer Disease Genetic Risk Loci in a Population Isolate Using a Genome-Wide Scan of 500,288 SNPS

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Background: Although mutations in three genes have been shown to cause early-onset familial Alzheimer Disease (AD), only the apolipoprotein E (APOE) locus has been confirmed as a risk locus for late-onset familial and sporadic AD (LOAD). The presence of the APOE-e4 allele is attributed to a dose-dependent increased risk of AD. However, epidemiologic studies indicate that only about 50% of LOAD patients carry an APOE-e4 allele, therefore additional genetic factors are involved in determining the risk for AD.

Aims: We hypothesized that additional genetic loci involved in conferring risk for LOAD could be identified through newly-developed genome-wide association methods.

Methods: Affymetrix GeneChip Mapping 500K SNP arrays were used in a pooled genome-wide association analysis to look for risk loci in a series of LOAD patients and controls collected from Trøndelag, Norway. Interesting regions identified by a novel algorithm developed at Tgen (genepool) were followed up by individually genotyping patient and control samples included in the pools, as well as in two additional independent replication series. Once a region was confirmed in the replication series, additional SNPs were chosen using data from the HapMap project to further delineate the region harboring risk variant(s).

Results: The APOE locus was strongly detected by the genepool algorithm, providing proof of concept in the study design. In addition, more than five novel loci identified from

the initial scan have been verified and are currently being fine-mapped.

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Further Characterization of a Family With Corticobasal Syndrome (CBS) and Frontotemporal Dementia With Parkinsonism (FTDP) Due to Progranulin Mutation

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Background: Corticobasal Syndrome (CBS) is a neurodegenerative disorder characterized by an asymmetric akinetic-rigid syndrome, apraxia, and dementia. Most cases are sporadic; however, rarely, there have been familial cases of CBS occurring along with frontotemporal dementia (FTD). *Purpose: *The current study provides further characterization of the clinical, genetic and neuropathological features of a family with CBS and FTDP due to mutation in progranulin (PGRN).

Methods: Two family members were recruited through a longitudinal dementia study and underwent clinical assessment including history and physical examination, standardized behavioural neurology assessment, neuropsychological battery, MRI and SPECT. The third family member was residing out of country and only tissue samples and clinical reports were available. The normal control group consisted of 200 unrelated subjects of North American origin. Mutation analysis of PGRN was carried out by direct DNA sequencing. RT-PCR was used to identify mRNA transcripts. Neuropathological examination was conducted using standard techniques.

Results: This Canadian family of Chinese origin had two family members with CBS, while one newly assessed patient had FTDP.

Genetic analysis identified a novel IVS7+1G>A mutation which segregated with disease and was absent in 200 normal controls. RT-PCR confirmed the absence of mutant PGRN transcript suggesting a haploinsufficiency model of disease. Neuropathological examination revealed tau-negative, ubiquitin-positive cytoplasmic and intranuclear inclusions.

Conclusion: This study extends the evidence for genetic, pathological and phenotypic heterogeneity of CBS and FTD spectrum disorders. We are expanding the information on the family by genotyping additional family members and by providing further neuropathological characterization.

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Are Mitochondrial Haplogroups H and U Act to Increase the Penetrance of Alzheimer's Disease?

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Alzheimer's disease (AD) is the most common form of dementia in the elderly in which interplay between genes and the environment are supposed to be involved. Mitochondrial DNA (mtDNA) has the only non-coding regions at the displacement loop (D-loop) region that contains two hypervariable segments (HVS-I and HVS-II) with high polymorphism. mtDNA has already been fully sequenced and many subsequent publications have showed polymorphic sites, haplogroups and haplotypes. Haplogroups could have important implications to understand association between mutability of the mitochondrial genome and disease.

To assess relationship between mtDNA haplogroup and AD, we sequenced the mtDNA HVS-I in 30 AD patients and 100 control subjects. We could found that haplogroups H and U are significantly more abundant in AD patients (P= 0.016 for haplogroup H and P=0.0003 for haplogroup U), Thus, these two haplogroups might act synergistically to increase the penetrance of AD disease.

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Lack of Association Between Mitochondrial A4336G/Haplogroup and Parkinson's Disease

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we have studied 20 unrelated PD patients and 113 control subjects for mitochondrial mutation A4336G and mutations in the complete regions of ND1, tRNALeu, ND2 and 16s rRNA by sequencing method. We also investigated common deletion in the blood samples of these patients. Our study suggests that the A4336G mutation were not associated with an increased risk of PD in Iranian population, but other mtDNA mutations may contribute the risk factor to idiopathic PD. Lack of association was found between mtDNA haplogroups and PD, so we did not find evidence for the involvement of specific inherited mitochondrial haplogroups in conferring both risk of and protection from the common form of PD in Iranian population.

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Effect of Epidermal Growth Factor Gene Polymorphisms on Schizophrenia and Parkinson's Disease Patients in Korean Population

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Several lines of evidence suggest that growth factors, cytokines, and neurotrophic factors may play an important role in the pathophysiology of both schizophrenia and Parkinson's disease. Epidermal growth factor (EGF) is a 6-kDa polypeptide growth factor not only involved in promotion of proliferation and differentiation of mesenchymal and epithelial cells but also known as a neurotrophic factor affecting the brain development. Analyzing the genetic difference of EGF gene between schizophrenia and Parkinson's disease might provide us with possible clues to understand molecular mechanisms of development of these diseases. A61G functional polymorphism on 5' untranslated region and 5 nonsynonymous exonic polymorphisms (K430R, I707M, D783V, T841M and E919V) of EGF gene were chosen and analyzed in this study. Among the four single nucleotide polymorphisms (SNPs), the rare C allele of EGF -382A>G was significantly different between schizophrenia and parkinson's disease in two alternative models: Codominant [P=0.03, odds ratio (95%CI), 1.49 (1.03-2.14)], dominant [P=0.005, odds ratio (95%CI), 1.88 (1.21-2.93)]. We also fund that the rare C allele of EGF +66707A>G I707M was significantly different between schizophrenia and Parkinson's disease in two alternative models: Codominant [P=0.04, odds ratio (95%CI), 1.46 (1.01-2.10)], dominant [P=0.01, odds ratio (95%CI), 1.76 (1.14-2.73)]. In haplotype analysis revealed significant results haplotype ht2 [A-G-G-A]. These results showed that these two diseases have different genetic characteristics.

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Matrix Metalloproteinase-1 Gene Polymorphism is Different Between Parkinson's Disease and Schizophrenia

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Matrix metalloproteinase-1 (MMP-1) is a zinc-dependent protease that degrades the interstitial types I, II, and III collagens, important in several diseases. To determine the genetic difference of MMP-1 gene between Parkinson's disease and schizophrenia, we investigated polymorphisms of MMP-1 gene in 188 Parkinson's disease patients, 218 schizophrenia patients, and 1682 healthy control in Korean population. Thirteen polymorphisms in MMP-1 gene were selected for this study. Among the 13 single nucleotide polymorphisms (SNPs), the rare A allele of -1121G>A, and +2508G>A (Ala216Ala) were found to be significantly different between Parkinson's disease and schizophrenia in the following models; Codominant [P=0.03, odds ratio (95%CI), 1.50(1.03-2.18)] in -1121G>A; Codominant [P=0.003, odds ratio (95%CI), 1.91(1.25-2.90)] and dominant [p=0.0008, odds ratio (95%CI), 2.15(1.38-3.35)] in +2508G>A (Ala216Ala). Similarly MMP-1 +7865T>C was significantly different between Parkinson's disease and schizophrenia. In haplotype analysis, two haplotype blocks were identified and haplotypes block2-ht2 [Dominant model; p=0.03, odds ratio (95%CI), 0.64(0.42-0.96)] and block2-ht3 [Co-dominant model; p=0.01, odds ratio (95%CI), 1.69(1.11-2.58); Dominant model; p=0.006, odds ratio (95%CI), 1.88(1.20-2.95)] were significantly different between Parkinson's disease and schizophrenia. These results suggest that MMP-1 gene polymorphisms are association with differences in genetic characteristics of Parkinson's disease and schizophrenia.

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Vascular Endothelial Growth Factor Gene Polymorphisms in Korean Patients With Parkinson's Disease and Lung Cancer

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Lower incidence of smoking-related malignancies, especially lung cancer, in patients with PD has been reported in several studies. Analysis of genetic differences between PD and lung cancer might provide us with possible clues to understand molecular mechanisms of pathophysiology of PD. Vascular endothelial growth factor (VEGF) has been implicated in neuronal survival, neuroprotection, growth, differentiation, and axonal outgrowth. There is increasing evidences of a pivotal role of VEGF in progressive damage of nigrostriatal dopaminergic neuron. The present study was undertaken to test the hypothesis that polymorphic variations in the VEGF gene has a association with development of PD. A cohort of 188 Parkinson's disease patients and 321 lung cancer patients was included in this study. Nine single nucleotide polymorphisms (SNPs) were selected for large scale genotyping based on LDs and frequencies (frequency > 0.05). There was significant differences between PD and lung cancer in one haplotype (ht2) co-dominant (P=0.40, OR=1.14), dominant (P=0.93, OR=1.02) and recessive (P=0.05, OR=2.68). However, there were no significant differences in tested SNPs and other haplotypes. The present study shows that genetic variations in the VEGF gene are different between PD and lung cancer. These results suggest that VEGF genetic

polymorphisms might be a possible molecular clue for low incidence of lung cancer in patients with PD.

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Dopamine Transporter Imaging in Subjects Carrying LRRK2 G2385R Mutation

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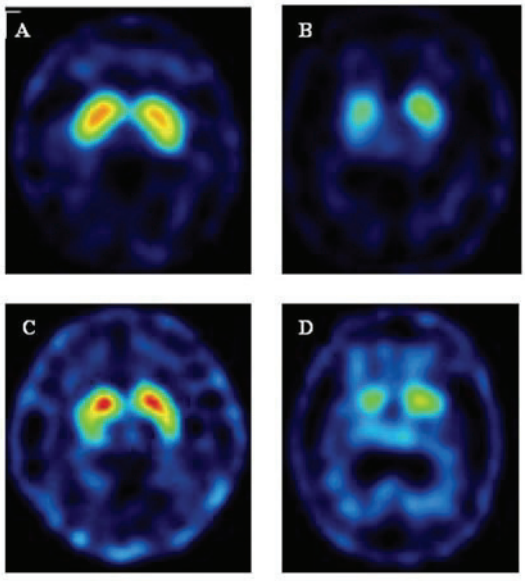
Background: The mutation in the Leucine-rich Repeat Kinase 2 (LRRK2) gene has been documented as an important cause of autosomal dominant Parkinson's disease (PD). Heterozygosity for the G2385R variant had a high incidence in Taiwanese PD patients, and might act as a putative risk factor for sporadic PD.

Methods: Using SPECT imaging with 99mTc-TRODAT-1 (TRODAT), we analyze the dopamine transporter (DAT) function in eight G2385 carriers.

Results: TRODAT uptake decreased in five G2385R carriers. Figure 1 shows a normal TRODAT binding in a 46-year-old female carrier (A). The TRODAT uptake decreased in a 63-year-old carrier (B), compared with the imaging in a 62-year-old healthy subjects without G2385 mutation (C). The decline of TRODAT uptake was most pronounced in a PD patient (D: Hoehn and Yahr stage 3).The uptake ratio of TRODAT in the posterior putaman significantly declined in the G2385R carriers than this in the age-matched healthy subjects.

Conclusions: Our study suggested that the DAT function had been modified in some G2385R carriers.



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The Cathepsin D (224C→T) Polymorphism Confers An Increased Risk to Develop Alzheimers Disease in Men

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Cathepsin D, the major human lysosomal protease, seems to be involved in β-amyloidogenesis in Alzheimer´s disease (AD). Since it has been shown that a single-nucleotide-polymorphism (224C→T, exon 2) of the cathepsin D gene leads to an increased expression of the proenzyme, 23 genetic association studies have been performed to reveal the impact of the polymorphism on the risk of AD. Taken together, the findings are controversial, suggesting the polymorphism to play a minor role in the etiology of AD. However, little is known about gender-specific differences. Therefore, we performed a population based genetic association study with 433 subjects (186 AD, 247 controls). Screening of the cathepsin D 224C→T polymorphism shows a significantly higher T-allele frequency among male Alzheimer patients (32%) when compared to male controls (15%; $p < 0,01$). In males the T-allele confers a 2.72-fold increased risk to develop AD (OR: 2,72; 95% CI: 1,23-6,00). In contrast, there was no significant difference in T-allele distribution in women (female Alzheimer patients 14%; female controls: 21%; $p=0,11$; OR: 0,58; 95% CI: 0,30-1,13). APOE 4 status and age did not have any additional effect on the morbidity risk, although we could confirm APOE 4 alone to have a significant impact on the AD risk (OR: 2,5; 95% CI: 1,6-3,7). Thus, our results show that the cathepsin D 224C→T polymorphism confers an increased risk for AD in men, but not in women. Further investigation should be performed to evaluate the role of gender by re-analyzing the data given so far by a meta-analysis.

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Apoe E4, Age at Onset and Education in a Norwegian Population With Alzheimer's Disease

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Background: Apolipoprotein E (ApoE) is essential for the maintenance and repair of neurons, and is a component of plaque and neurofibrillary tangles, the pathological hallmarks of Alzheimer's disease (AD). Hetero- and homozygote APOEe4 carriers have increased risk for AD compared to carriers of alternative alleles.

The e4-allele both increases the risk of developing AD and lowers the age at onset of the disease.

Material and method: The population of central Norway is genetically relatively homogeneous, and is the focus of a

study to examine genetic and metabolic aspects of dementia. Healthy persons in the same age group as patients were recruited as controls. Patients diagnosed with AD (n=362) and controls (n=344) were genotyped for ApoE.

Results: Using homozygote e3/e3 as a reference group, homozygote e4/e4 were found to have Odds Ratio (OR) = 13.3 for AD. Genotype e3/e4 had OR = 3.15 for AD, and subjects with e2/e4 were found to have OR=2.60 for AD.

Age at onset of symptoms lowers significantly with each Apo e4-allele, from 76.5 years with no ApoE e4-alleles, via 73.8 with one apoE e4-allele to 70.3 in patients with two ApoE e4-alleles. Men had significantly lower age at onset than women, in spite of significantly longer education.

Conclusion: Carrying one or two APOEe4 alleles is a strong risk factor for AD. Men have lower age at onset than women, in spite of higher education level.

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Influence of Behavioural Symptoms on Resource Use

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Background and Aims: Several behavioural symptoms occur over the course of dementia, at various stages, affecting both patients and caregivers. The aim of this study was to determine their influence on resource use.

Methods: A cross-sectional analysis of a cohort of 349 dementia patients living in the southwest of France. The behavioural symptoms screened were: delusion, hallucination, agitation, depression, anxiety, euphoria, apathy, disinhibition, irritability and motor behaviour. Hospitalisation, ambulatory care, treatments and social services data were collected retrospectively over the last three months. The use of resources was compared between patients with and without symptom using the Mann-Whitney and Fisher's exact tests.

Results: All symptoms had a prevalence over 10% except euphoria and disinhibition. Patients with delusion, hallucination, agitation, anxiety and motor behaviour used more social services (home for elderly). More informal caregiving time was required for patients with delusion, hallucination, agitation, apathy, irritability and motor behaviour. More hospitalisations occurred in patients with anxiety. Antipsychotics were used more frequently in patients with agitation, delusion, hallucination and irritability. There were no direct associations with ambulatory care resource uses. Depression, euphoria and disinhibition showed no major influence on resource use.

Conclusions: Delusion, hallucination and agitation were the symptoms with the most influence on resource use as a whole (institutionalisation, caregiving time and antipsychotic treatments). Anxiety, irritability and motor behaviour also showed influence on some resources. The question of causality between symptoms and associated resources remains still unresolved, but will be addressed by follow-up of the cohort at six and twelve months.

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Effect of Hyperhomocysteinaemia on Response to Rivastigmine in Patients With Parkinson's Disease Dementia

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Background: Hyperhomocysteinaemia has been associated with increased risk of developing Alzheimer's disease and other dementias.

Objective: To investigate the effect of hyperhomocysteinaemia on rate of decline on placebo and response to rivastigmine in patients with Parkinson's disease dementia (PDD).

Methods: Prospective analysis of a subpopulation of patients from a 24-week randomized placebo-controlled trial of rivastigmine in PDD. Efficacy and tolerability outcomes were compared in patients with elevated plasma homocysteine (≥14 µmol/L) versus normal/low homocysteine (<14 µmol/L) at baseline. Efficacy variables included Alzheimer's Disease Assessment Scale cognitive subscale (ADAS-cog), Alzheimer's Disease Cooperative Study Clinicians Global Impression of Change (ADCS-CGIC), Alzheimer's Disease Cooperative Study Activities of Daily Living scale (ADCS-ADL), Neuropsychiatric Inventory (NPI) and Delis-Kaplan Executive Function System (D-KEFS) letter fluency test.

Results: Of 342 patients with plasma homocysteine assessments at baseline, 247 (72.2%) had elevated and 95 (27.8%) had normal/low homocysteine. Placebo-treated patients with elevated homocysteine declined on all efficacy measures while those with low/normal homocysteine remained stable. Compared with placebo, significantly greater responses to rivastigmine were seen in patients with elevated homocysteine on the ADAS-cog ($p<0.001$), ADCS-CGIC ($p=0.010$), ADCS-ADL ($p=0.025$), NPI-10 ($p<0.001$) and D-KEFS letter fluency test ($p=0.003$). No significant differences versus placebo were seen in patients with normal or low homocysteine. Motor symptoms reported on UPDRS Part III scores were not significantly different between rivastigmine and placebo groups in either sub-population.

Conclusions: Hyperhomocysteinaemia is common in PDD, and patients with this condition may have a more aggressive course of disease and derive greater benefits from rivastigmine therapy.

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FE65 A3 a Novel Alternatively Spliced Human Variant Isoform

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Several Alzheimer's amyloid precursor protein (APP) binding proteins have been identified, among them FE65, which is found in the cytoplasm and the nucleus. The FE65 family comprises three members: FE65, FE65L1 and FE65L2.

FE65L1 and FE65L2 are ubiquitously expressed. Two transcript variants have been identified for FE65. Transcript variant 1, FE65 a1 (NM_001164) represents the longest transcript and encodes the longest isoform (E9). This isoform is exclusively expressed in neurons. Transcript variant 2, FE65a2 (NM_145689), encodes a protein that maintains the reading frame but is a shorter isoform. This encoded isoform (delta E9) is widely expressed in all non-neuronal cells, but is not expressed in differentiated neurons. Immunoreactivity studies revealed that the highest level of expression is observed in regions of the hippocampus, but high levels of expression are also detected in the cerebellum, thalamus and selected brain stem nuclei. APP and FE65 colocalize in the endoplasmic reticulum/Golgi, endosomes, in intranuclear sites corresponding to splicing factor compartments, in cell-substrate adhesion sites, in synaptic sites and in distal domains of neuronal growth cones, particularly actin-rich lamellipodia. In the study here described the C terminal domain of APP was used as bait in a Yeast Two Hybrid screen, and positive clones were stringently screened and sequenced. Further validations were carried out to confirm the interaction with APP. A novel alternatively spliced variant of FE65 (FE65 a3) was thus identified. The resulting protein has a distinct N terminus. The occurrence and putative consequence of this novel Fe65 isoform will be discussed.

Acknowledgments: Supported by the European Union VI Framework Program (Project APOPIS), Fundação para a Ciência e Tecnologia of the Portuguese Ministry of Science and Technology and Centro de Biologia Celular, Universidade de Aveiro.

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Prongf and Its Binding to the TrkA, P75NTR and Sortilin Receptors

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The neurotrophins are a family of proteins that provide trophic support to neurons of the central and peripheral nervous system. They mediate their actions through a common p75NTR neurotrophin receptor, as well as specific tyrosine kinase receptors (Trks). Nerve growth factor (NGF), the prototypic neurotrophin, binds to TrkA and p75NTR, promoting cell survival. Cholinergic neurons of the basal forebrain are particularly vulnerable in Alzheimer's disease and loss of these neurons early in the disease is associated with memory loss and cognitive impairment. The cholinergic cells are unique in the brain in that they express both TrkA and p75NTR. However, it was recently shown that the NGF precursor; proNGF is the dominant form of NGF in the brain. proNGF has been shown to bind to p75NTR, together with a co-receptor sortilin, to bring about apoptosis. Therefore the presence of proNGF and its binding to these receptors leads to speculation that this may be implicated in cholinergic neuron loss.

In order to fully characterise the binding of proNGF compared to mature NGF to the receptors, we have produced recombinant human proNGF, in wild-type and cleavage resistant form, without tags or extraneous amino acids. We have also produced cell lines stably expressing the TrkA, p75NTR and sortilin receptors individually as well as in combinations. This has allowed a full characterisation of the binding of proNGF and mature NGF to the receptors using receptor binding assays

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Presenilin1 Prevents Neurodegeneration by Stimulating PI3K Signaling

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We recently reported that presenilin1 (PS1) promotes survival of confluent fibroblasts by activating the PI3K/Akt signaling (Baki et al.2004, EMBO J. 23(13):2586-96.). The PI3K/Akt signaling pathway is of critical importance for neuronal function and survival. In addition to its well established antiapopototic role, this pathway has been implicated in synaptic potentiation and memory formation. Using primary neuronal cultures from PS1 wild-type and PS1 knockout embryonic brains we observed that loss of PS1 results in neuronal degeneration and premature cell death. At the biochemical level PS1-/- neurons show impaired PI3K/Akt signaling, underphosphorylated (overactivated) GSK-3, and increased caspase-3 activation. Re-introduction of PS1 into PS1-/- neurons stimulated PI3K/Akt signaling, Akt-dependent phosphorylation of GSK-3 and suppressed activation of apoptotic caspase-3. Moreover, exogenous PS1 was able to prolong the life of PS1-/- primary neurons, independently of gamma-secretase activity, but not in the presence of pharmacological inhibitors of the PI3K/Akt pathway, indicating that the survival effect of PS1 in primary neuronal cultures is mediated by PS1/PI3K/Akt signaling. Indeed, pharmacological inhibition of PI3K promoted degeneration of PS1+/+ neurons, whereas a constitutively active form of Akt was able to rescue PS1-/- neurons. Importantly, all PS1 FAD mutations examined so far suppressed PI3K/Akt signaling, induced caspase 3 activation and prevented phosphorylation/inactivation of GSK-3. Our data indicate the importance of PS1/PI3K signaling for neuronal physiology, whereas the deregulation of this signaling by FAD mutations emphasizes the importance of this mechanism for the development and/or progression of Alzheimer's Disease pathology.

(Supported by the NIH 1RO1-NS047229-02 and NIRG-04-1011 research grants.)

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The Amyloidogenic Pathway in Human Brain Vascular Smooth Muscle Cells is Triggered by Oxidative Stress

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Cerebral amyloid angiopathy, associated to most cases of Alzheimer's disease (AD), is characterized by the deposition of amyloid ß-peptide (Aß) in brain vessels. The origin of vascular amyloid deposits is still controversial: neuronal vs vascular. We demonstrate that primary cultures of human cerebral vascular smooth muscle cells (HC-VSMCs) share with neurons all the secretases involved in amyloid ß-protein precursor (APP) cleavage. Moreover, VSMCs are also able to produce Aß1-40 and Aß1-42. Oxidative stress, a key factor in

the etiology and pathophysiology of AD, up-regulates BACE1 expression, as well as Aß1-40 and Aß1-42 secretion in HC-VSMCs. This process is mediated by c-Jun N-terminal Kinase and p38 MAPK signaling and appears restricted to BACE1 regulation as no changes in other secretases were observed. In conclusion, oxidative stress-mediated up-regulation of the amyloidogenic pathway in human cerebral vascular smooth muscle cells may contribute to the overall cerebrovascular dysfunction observed in AD patients.

Supported by the Spanish Ministerio de Sanidad (FIS-PI041242; Red-HERACLES) and Generalitat de Catalunya (GSR2005-266).

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Overexpression of Amyloid Precursor Protein With the 670/671 Mutation Modulates Nicotinic Receptor-Mediated Intracellular Calcium Signaling in Rat Hippocampal Neurons

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The 670/671 APP mutation – also called Swedish or APPswe - results in a 3- to 4-fold increase in the production of the amyloid peptide and development of Alzheimer's disease pathology in its human carriers. Externally added high concentrations of amyloid 1-42 have been shown to bind to the alpha7 nicotinic acetylcholine receptor subtype (nAChRalpha7) and modulate nicotinic electrical currents. alpha7 is the nicotinic receptor subtype with the highest calcium permeability.

By means of confocal calcium imaging we assesed the effect of endogenously over-expressed APPswe on intracellular calcium responses in primary hippocampal neurons derived from our newly established APPswe transgenic rat model. 10 uM nicotine evoked a fast and transient increase in cytosolic calcium concentration in hippocampal cultures from control animals, but failed to evoke any response in cultures derived from our transgenic animals. There were no differences in responses to 100 uM NMDA.

We will further investigate whether the observed results depend on changes in the nicotinic receptor population or modulation of nicotinic receptor function as well as which nicotinic receptor subtype is responsible for the effect we have seen. In parallel we have started investigating calcium responces in a human neuroblastoma cell line (SHSY-5Y) overexpressing APPwt, APPswe and the secreted APP derivate sAPPalpha, in order to elucidate which APP derivate has the most profound effect on nicotine-dependent intracellular calcium signaling.

Our results show that APPswe affects cholinergic-dependent calcium signaling in neurons.

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Hypoxia Modulates Gamma-Secretase Complex and APP in SK-N-BE Cells

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Background and aims: Ischemic cerebrovascular diseases, usually involved in hypoxia, have been reported to increase the risk of dementia such as Alzheimer's disease (AD). Gamma-secretase has been identified to participate in the secretion of beta-amyloid peptides (Ābeta, and its alteration would contribute to the AD neuropathology. The aim of the study was to investigate on the effect of hypoxia (0, 2, 6, 24h) on the mRNA and protein levels of gamma-secretase complex components in human neuroblastoma SK-N-BE cells. At the same time, we also examined the effect of hypoxia on gamma-secretase complex substrate, Amyloid Precursor Protein (APP).

Methods: Neuroblastoma SK-N-BE cells culture in normal and hypoxic conditions were used. Gene expression was analyzed by a real time PCR method, while protein expression by Western Blot.

Results: We demonstrated that hypoxia alters some components of gamma-secretase complex, both at transcriptional and translational level, according to the different elapsed times after the hypoxic stimulus. Interestingly, PSEN2, APH1 as well as APP mRNA showed a significant increase, just two hours after hypoxia. Moreover, we observed a subsequent down-regulation, both at transcriptional and translational level, in the following treatment times.

Conclusions:Thus, APP and gamma-secretase complex modulation in the neuronal cell, in a so early time after hypoxia, may be a linkage in the pathophysiology between cerebrovascular and Alzheimer diseases.

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Gamma Secretase Localization and Function at Synapses

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Familial cases of AD (FAD) result from mutations in APP and presenilin 1 and 2 (PS1 and 2) proteins. PS are thought to be the catalytic subunit of gamma secretase, and thus function in the generation of amyloid peptide from APP. However, the gamma secretase also cleaves other type I transmembrane proteins, including Notch, Cadherin and ErbB4. Other binding partners of PS1 have also been identified. One of these, NPRAP (delta catenin), has been shown to interact with ABP (AMPA receptor binding protein), by our lab. Thus, the secretase could have a more ubiquitous function in degrading proteins than initially thought. We therefore asked if gamma secretase is present and active at synapses and if it plays a role in modifying synaptic architecture and function through the cleavage of cadherins,

which are synaptic cell adhesion molecules. We find by immunocytochemistry that PS1 and nicastrin, two gamma secretase components, are present at synapses of hippocampal neurons in culture and colocalize with gamma secretase substrates, including cadherin and ErbB4, and with other synaptic proteins, including AMPA receptors. Synaptic localization was confirmed by subcellular fractionation. Furthermore, we find that gamma secretase can be active at synapses, as indicated by cadherin cleavage in synaptosomes. These data suggest that gamma secretase is synaptic and thus may modify synaptic architecture and function through cleavage of synaptic substrates. A deregulation of this protease, such as by FAD mutation, could pathologically activate secretase function at synapses, leading to synapse degradation and loss.

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Inhibition of Abeta Peptide Production and APP-Induced Neurodegeneration in Novel Neuronal Models of Alzheimer's Disease

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Alzheimer's disease is a progressive neurodegenerative disorder characterized by the accumulation of b-amyloid peptide (Ab) generated from amyloid precursor protein (APP) by b- and g-secretases. While elevated Ab appears to induce cell death in human brain, most preclinical models do not exhibit measurable neurodegeneration despite obvious manifestation of amyloid pathology, exemplifying the need for surrogate models representing the full scope of Alzheimer's pathology. We have established neuronal APP processing assays using primary cortical cultures from transgenic mice (Tg2576, Swedish mutant APP) as well as cortical brain slices. Neurons from these mice secreted high levels of Ab into conditioned media and this cleavage activity was effectively blocked by inhibitors of b- and g-secretases, measured by ELISA. These assays proved highly efficient platforms to determine the efficacy of inhibitor molecules in neuronal tissue contexts. To compare the responses of wildtype and Swedish APP, we have developed a high-content brain slice explant platform biolistically transfected with a fluorescent reporter protein (YFP) along with wt or swAPP. This system allowed clear visual identification of APP expressing neurons in a three-dimensional tissue structure. Remarkably, we were able to detect time-dependent neuronal degeneration associated with APP overexpression, simulating disease-associated neuropathology in vitro. Immunoblot analysis revealed robust signals of APP cleavage products corresponding to apparent C99 and Ab. Inhibition of b-secretase resulted in significant reduction of C99 and Ab, while a g-secretase inhibitor resulted in a depletion of Ab accompanying accumulations of C99. We are currently evaluating the impact of secretase inhibitors on neuronal survival.

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[3H]GSK535217A, a Novel Radioligand That Binds Bace-1 Expressed in Rodent Cerebral Cortex and Human Bace-1 Transiently Expressed in HEK293 Cells

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Amyloid beta (Abeta peptide) is believed to play a key role in the pathogenesis of Alzheimer's disease and its production depends on the proteolytic cleavage of Amyloid Precursor Protein (APP) by 2 proteases, beta-site APP cleaving enzyme (BACE-1) then gamma-secretase. Consequently inhibition of BACE-1 is considered to be an attractive therapeutic approach for the treatment of Alzheimer's disease. We have developed a selective BACE-1 inhibitor radioligand, [3H]-GSK535217A that binds to human BACE-1 transiently expressed in HEK293 cells and to rodent BACE-1 endogenously expressed in rat and mouse cerebral cortex. Binding selectivity was confirmed using brain tissue from BACE-1 knock-out mice. [3H]-GSK535217A was also used in ex vivo binding studies to assess the potency of peripherally administered BACE-1 inhibitors. Our results suggest that this novel radioligand is a useful tool to help understand the relationship between inhibition of BACE-1 binding, CNS drug exposure and preclinical efficacy following administration of BACE-1 inhibitors in vivo.

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Trail Pathway in Beta Amyloid Neurotoxicity

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Tumour-necrosis-factor-related apoptosis-inducing ligand (TRAIL) is a novel cytokine characterized by selective killing activity against tumour cells. We recently found that beta-amyloid-induced apoptosis in human neuronal cell line was mediated via induction of TRAIL. The specificity of such effect was demonstrated by data showing that TRAIL-neutralizing monoclonal antibody protects neuronal SH-SY5Y cells from beta-amyloid neurotoxicity. Moreover, exposure of neuronal cell line to TRAIL leads to cell death, indicating that this substance per se is endowed with neurotoxic properties. Similarly to beta-amyloid and TRAIL, activation of the death domain adaptor protein FADD results in neuronal cell death. Lack of FADD function, by over-expression of its dominant negative, rescued cells from either TRAIL-, or beta-amyloid-induced neurotoxicity, supporting the hypothesis that these three molecules share common intracellular pathways.

Interestedly, the TRAIL death receptor DR5 was demonstrated to be a key factor in TRAIL death pathway. In fact, while TRAIL expression was enhanced dose-dependently by concentrations of beta-amyloid ranging from 10 nM to 1 microM, only the highest toxic dose of beta-amyloid (

25microM) induced the increased expression of DR5 and neuronal cell death. In addition, the increased expression of DR5 receptor after beta-amyloid treatment was sustained by p53 transcriptional activity, as demonstrated by the data showing that the p53 inhibitor Pifithrin alfa prevented beta-amyloid -induced both DR5 induction and cell death. These data suggest a sequential activation of p53 and DR5 upon beta-amyloid exposure.

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Cell-Surfatce Binding Characteristics of The Soluble Amyloid-Beta Precursor Protein

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The Abeta peptides, found in senile plaques accompanying Alzheimer's disease, are formed by amyloidogenic processing of the amyloid-beta precursor protein (APP). Considerable effort has been devoted to understanding APP processing in relation to neurodegeneration and dementia, but the biological function of APP itself and its mammalian paralogs, APLP1 and 2, remains unclear. Among other proposed functions, evidence exists that the soluble APP ectodomain (sAPP) is involved in cell proliferation, neuroprotection, neurite outgrowth, and synaptogenesis. These multiple sAPP-mediated cellular effects suggest that one or more receptors or binding partners for sAPP exist that link extracellular sAPP to the cell interior. These putative sAPP receptors are of great interest, but they remain elusive. To address this question, we analyzed the binding properties of sAPP to cells relative to heparin-dependent and heparin-independent binding sites. We showed that, depending on the cell line examined, sAPP-binding is mediated not only via its heparin-binding domains but also via a protein-protein interaction of a cell surface receptor. Interestingly, binding of sAPP persists in B103 cells which do not express any APP family member. Thus, independent of APP homodimerization and heterodimerization with APLP1 and 2, additional sAPP cell surface receptors exist. sAPP consists of several individual folding units including the growth factor-like domain and the E2-domain. To determine the contribution of the sub-domains towards a heparan sulfate-dependent and/or protein-dependent binding, binding studies were preformed utilizing the individual folding units. Preliminary results suggest differential binding of the individual sub-domains with regard to both heparan sulfate- and protein-dependent binding mechanisms.

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Hyperhomocysteinemia in Parkinson's Disease

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Object: Elevated plasma homocysteine (Hcy) levels have been associated with cognitive impairment via neurotoxic (hyperstimulation NMDA receptor) and vascular effect. The aim of the study is to verify whether Hcy levels are correlated with cognitive impairment, white matter brain lesions (WMBL) in PD patients and to evaluate the effects of L-Dopa on plasma Hcy concentrations.

Patients/methods: Fifty patients (20 non demented PD patients, 30 demented PD patients treated with L-Dopa) who fulfilled UK Brain Bank criteria for PD were recruited. Control group was 50 health subjects with no sex/age differences.

The patients were evaluated using the Hoehn&Yahr stage, MMSE, Mattis dementia rating scale (MDRS), Frontal Assessment Battery (FAB). WMBL on magnetic resonance imaging were evaluated on Magnetom Vision 1.5 T at proton density and T2 weighted scans. Using a standardized scale white matter lesions were rated from proton density and T2 weighted axial images. Statistical analysis was tested using Spearman correlation and significance was assessed by Mann-Whitney U test.

Results: All demented patients had significantly higher total WMBL scores compared with controls ($p<0.001$) and non demented patients ($p<0.01$). Plasma Hcy level was positively correlated with total WMBL scores ($r=0.48$, $p<0.01$), negatively with MMSE, MDRS, FAB scores ($r=0.6$, $p<0.001$). Hcy concentrations were significantly higher in the L-Dopa treated group (19.7±4.9 μmol/L; $p<0.001$) compared with Dopamine-agonists group (14.5±2.1 μmol/L) and control (10.6±2.3 μmol/L).

Conclusion: We suppose the potential implications of hyperhomocysteinemia in mechanisms of neuronal damage may realize via dual tonic NMDA-glutamate receptor stimulation by homocysteine and glutamate in PD.

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BA1-42 Interacts With Receptor for Advanced Glycation End Products (RAGE) and Induces Apoptosis in Rat Neuromicrovascular Endothelial Cells Culture

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Background. Alzheimer's disease (AD) is thought to involve oxidative damage, either as a primary case or as a consequence of disease progression and neurotoxic effects of bamyloid (bA), major component of the senile plaques, are at least in part mediated by free radicals. Significant cerebral microvascular pathology in AD indicate that the neurovascular dysfunction could have a major role in the pathogenesis of AD. However, the precise mechanism of why bA is toxic to vascular endothelial cells is still unclear.

Methods. We use rat neuromicrovascular endothelial cells (NECs) as an in vitro model for vascular dysfunctions investigating by rat bA1-42 peptide (10-7M) induced. We evaluated cellular oxidative stress (by means of superoxide dismutase (SOD) assay, nitric oxide (NO) release, and nitric oxide synthase (NOS) activation), apoptotic rate (by TUNEL assay, caspase 8 and caspase 3 activation) and RAGE involvement in bA effect.

Results. We found that bA peptide induces a time-dependent increase of SOD and NO concentration, and the activation of NOS. Moreover we show that bA peptide induces an apoptotic pathway by the activation of caspase-3 and of

caspase-8, suggesting the possibility that the apoptotic pathway is induced by cross-linking and activating receptors as Receptor for Advanced Glycation End products (RAGE). In fact, when we inhibit bA-RAGE interaction, either toxicity, NO release and apoptosis bA-induced were reduced.

Conclusions. Oxidative stress and NO are involved in the bA-induced cytotoxic effect on NECs. Moreover the apoptotic pathway seems to be activated by the cross-linking of bA to RAGE.

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Covalently Cross-Linked Oligomers of Abeta and Neurotoxicity

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Background and Aims: Abeta peptide is toxic but the mechanism(s) are the subject of debate. A number of different hypotheses have been proposed, with recent interest directed at the formation of soluble oligomers of Abeta and their interactions with lipid membranes. Our aims are to elucidate factors affecting Abeta toxicity. Methods: Investigate the interactions of Abeta with a range of co-factors and examine the effects on Abeta toxicity. Additionally we have prepared Abeta peptides where key residues have been altered and examined changes in the biochemistry of the peptide, which have then been correlated to the neurotoxic activity of Abeta. Results: Our data indicates that specific metallated forms of Abeta are more toxic than non-metalled forms. The consequences of Abeta metal interactions include the formation of soluble covalently cross-linked oligomeric species. For Abeta to be toxic it must associate with the cell surface membrane, oligomeric metallated Abeta has a higher affinity for lipid membranes than non-metalled Abeta. Once bound to the lipid there is a correlation with the ability of the peptide to induce lipid peroxidation via the formation of peptide centred radicals.

Conclusions: Metal-induced oxidative modifications to Abeta and lipid peroxidation can be prevented by inhibiting Abeta/metal interactions. We have developed a class of metal-protein attenuating compounds that inhibit Abeta/metal interactions, these compounds have shown efficacy in cell and animal models of AD as well as therapeutic potential.

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Mapping Determinants for A-Beta Production in PS1 and PS2

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Production and deposition of A-beta peptide in amyloid plaques contributes the hallmark pathology of Alzheimer's disease in brains of affected patients. The A-beta peptide is produced by sequential cleavage of APP by enzymes referred to as beta- and gamma-secretases. Inhibition of the proteases responsible for A-beta production from APP is an active area of drug development for therapeutic intervention in AD. Gamma secretase, a multisubunit complex comprised of Presenilin, Nicastrin, Aph-1 and Pen-2, mediates a unique intra-membrane cleavage of APP, producing the carboxy-terminus of A-beta peptide. Two Presenilin homologs, referred to as PS1 and PS2, comprise the catalytic core of gamma secretase. Observations from KO mice reveal that Presenilin 1 comprised gamma secretase contributes to ~80% of total A-beta production, while PS2 comprised gamma secretase contributes to ~20% of A-beta. We employed transformed fibroblasts from Presenilin double-KO mice transiently transfected with APPsw + PS1, or APPsw + PS2, to map determinants for A-beta production in the presenilins using chimeric constructs. PS1 and PS2 mediated A-beta production in double-KO cells closely parallels A-beta production in KO mice, in agreement with published studies, and validating the cellular model. Our studies with chimeric Presenilin molecules reveal a bi-partite domain, mapping to the amino-terminus of PS1, which confers high A-beta production to PS2 in a context specific manner. The details of our studies mapping determinants for differential A-beta production in the Presenilins will be presented.

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APP Isoform-Specific Differences in Metabolism

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Alzheimer´s amyloid precursor protein (APP) can occur in several different isoforms, including APP751, which is the most abundant isoform in non-neuronal tissues, and A PP695, commonly referred to as the 'neuronal specific' isoform. To date, isoform-specific differences have not been significantly addressed. In the work here described, endogenous APP isoforms and APP cDNAs fused to green fluorescent protein (GFP) were used to permit isoform-specific monitoring. Studies were performed in terms of intracellular processing and targeting. Our work unravelled differences in the turnover rates of the immature isoforms, with APP751 having a faster turnover rate than APP695 (0.8h and 1.2h respectively, for the endogenous proteins, and 1.1h and 2.3h for the transfected proteins). That is, the APP751 matures relatively faster. Further, we showed that although APP751 responded to both okadaic acid (OA) and phorbol 12-myristate 13-acetate (PMA), as determined by sAPP production, PMA induced a more robust response. In contrast, for the APP695 isoform, PMA produced a strong response but OA failed to elicit such an induction in sAPP production. Thus, APP isoform specificity is involved in phosphorylation-dependent events. In essence, it appears that the APP695 isoform is processed/metabolized at a slower rate and exhibits a differential response to OA when compared to the APP751 isoform. The relevance of isoform-specific processing in Alzheimer´s Disease needs to be further addressed, given that APP695 isoform is abundant in neuronal tissues and that isoform-specific alterations in expression levels have been associated with the pathology.

ACKNOWLEDGMENTS

Supported by the European Union VI Framework Program (Project APOPIS), Fundação para a Ciência e Tecnologia of the Portuguese Ministry of Science and

Technology and Centro de Biologia Celular, Universidade de Aveiro.

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The Role of the NPTY Domain on APP Subcellular Distribution and Abeta Production

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The neurotoxic Abeta peptide is derived by proteolytic processing from the Alzheimer's amyloid precursor protein (APP), whose short cytoplasmic domain contains several phosphorylatable amino acids. The latter can be phosphorylated 'in vitro' and 'in vivo', and in some cases phosphorylation appears to be associated with the disease condition. Moreover, Lee et al (2003) demonstrated that 7 of the 8 potentially phosphorylatable residues in the intracellular domain of APP were phosphorylated in AD patients, namely Tyr653, Ser655, Thr668, Ser675, Tyr682, Thr686 and Tyr687. The latter lies within the 682YENPTY687 domain, a typical internalization signal for membrane-associated receptor proteins, as well as a consensus sequence for coated-pit mediated internalization. Using APP-GFP fusion proteins to monitor intracellular pathways, the role of the NPTY domain was addressed by mimicking Tyr687 constitutive phosphorylation (Y687E) and dephosphorylation (Y687F), respectively. Contrasting effects on subcellular APP distribution were observed. Y687E-APP-GFP was targeted to the membrane but could not be detected in transferrin containing vesicular structures, and exhibited a concomitant and dramatic decrease in Abeta production. In contrast, Y687F-APP-GFP was endocytosed similarly to wild type APP, but was relatively favoured for beta-secretase cleavage. Overall, Tyr687 appears to be a critical residue determining APP targeting and processing via different pathways, including secretory, endocytic and retrograde transport. Significantly, from a disease perspective, mimicking Tyr687 phosphorylation resulted in a hitherto undescribed inhibition of Abeta production. Our results provide novel insights into the role of direct APP phosphorylation on APP targeting, processing and Abeta production.

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The Role of Metal Ions in the Fibrillogenesis of Beta-Amyloid

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Metal ions are widely recognized as a key factor for conformational changes and aggregation of Alzheimer's disease amyloid (Abeta). The purpose of this study is to compare the effects of Abeta and Abeta-metals (Al, Zn, Cu, Fe) in human SHSY5Y neuroblastoma in terms of cell viability (MTT assay), cellular metal levels (ICP-MS), membrane structure properties (fluorescence anisotropy) and mitochondria function (determination of respiratory control ratio).

No significant toxic effects are observed in neuroblastoma cells after 24h treatment with Abeta and Abeta-metals (Zn, Cu, Fe), except a significant reduction of cellular viability after treatment with Abeta-Al complex. In agreement with these data, a strong increase in the neuroblastoma content of different metal ions (Zn, Cu, Fe) is detected and this effect could be important as a putative mechanism of Abeta-Al complex toxicity.

The effects of Abeta-Al as well as other Abeta-metal complexes are also evaluated in terms of fluorescent anisotropy in order to consider the possible alteration in the membrane structure properties. In this connection, treatment with Abeta-Al is able to increase specifically the membrane fluidity with respect to other Abeta-metal complexes.

Moreover, the toxic role of Abeta-Al is also demonstrated in isolated rat brain mitochondria where Abeta-Al induces a significant alteration of coupling mitochondrial respiration with respect to other Abeta-metal complexes (Abeta-Zn, Abeta-Cu, Abeta-Fe).

Based on these findings, involvement of Al in Abeta aggregation and consequently increasing neuroblastoma toxicity is clearly demonstrated.

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Sensitive Detection of Abeta Protofibrils by Conformation Specific ELISA

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Background: Soluble oligomeric amyloid-beta (abeta) is expected to be involved in the pathogenesis of Alzheimer's Disease (AD) as they mediate neurotoxicity both in vitro and in vivo. The protofibrillogenic Arctic Alzheimer mutation (APP E693G) provides strong clinical support for abeta protofibrils as a pathogenic species. Monoclonal antibodies with specificity for this form of abeta are therefore a potentially effective tool for diagnosis and treatment of AD.

Objective: To develop conformation selective, monoclonal IgG antibodies with high affinity for abeta protofibrils and use them for detection of abeta protofibrils in biological samples.

Methods: Antibodies were raised against abeta protofibrils and characterized with inhibition ELISA. One of these antibodies was used in a sandwich ELISA to measure abeta protofibrils in biological samples.

Results: The monoclonal IgG2a antibody mAb158 had selective affinity for abeta protofibrils. A sensitive abeta protofibril specific sandwich ELISA based on mAb158 was established and enabled detection of abeta protofibrils in biological samples without interference from abeta monomers or APP. Cell cultures and transgenic mice with a combination of the Arctic and Swedish (APPKN670/671ML) mutations were used as models for increased abeta protofibril formation. When compared to models carrying only the Swedish mutation, the mAb158 sandwich ELISA analysis revealed a significant increase of abeta protofibrils in the Arctic samples, proving that abeta protofibrils are formed not only in vitro, but also in vivo.

Conclusions: This assay provides a novel tool for investigating the role of abeta protofibrils in AD and has the potential of becoming important in diagnostics of AD.

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Gamma-Secretase Inhibitors Affect Processing and Secretion of Abeta and C-Terminal APP Fragments in Association With Exosomes

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Alzheimer's disease (AD) is the most common form of dementia in humans, of which the key pathological hallmark is the deposition of the 40-46 amino acid beta-amyloid peptide (Aβ) in the brain. Aβ peptides are derived from sequential cleavage of the amyloid precursor protein (APP) by β- and -secretases. Previous studies have shown that C-terminal fragments (CTF) of APP can accumulate in endosomally derived multivesicular bodies (MVB). These intracellular structures contain intralumenal vesicles which, when the MVB fuses with the plasma membrane, are released from the cell as exosomes. Exosomes are known to contain many membrane proteins and can be purified from the media of cultured cells. Here we have used CHO cells overexpressing wild-type human APP (695 amino acid isoform) to examine the pathway of APP processing and investigate whether exosomes contain contain Aβ, APP and APP CTF's. Using preparative ultracentrifugation to prepare exosomes from these cells we show, using a variety of antibodies against specific regions of APP, that they contain APP-CTF's, as well as Aβ. In addition, inhibition of γ-secretase using either DAPT or L-685,478 results in a significant increase in the amount of α-secretase cleavage further increasing the amount of APP-CTF's contained within these exosomes. These results provide further evidence for the existence of a novel pathway in which APP fragments can be released from cells.

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Ubiquilin-1 Variant May Be Link to Alzheimer's Disease Through Modulation of BACE1 Activity

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Alzheimer's disease (AD) is a devastating neurodegenerative disease associated with aging. Although a familial genetic predisposition has been clearly identified by mutations in the amyloid precursor protein (APP), presenilin-1 (PS1) and presenilin-2 (PS2) genes, the majority of ad cases are sporadic with unknown etiology. Beta-amyloid peptide (AÎ²) deposition in the brain of ad patients is considered a central event in the pathogenesis of both genetic and sporadic ad forms. AÎ² peptide is produced following cleavage of the amyloid precursor protein (APP) by two distinct secretases, namely, beta-site amyloid protein precursor cleaving enzyme 1 (BACE1) and the Î³-secretase, which contains at least, PS1 or PS2, nicastrin, APH-1 and PEN-2. We have used the yeast-2-hybrid method to screen a human brain CDNA library to identify BACE1 biochemical partners. Among the positive candidates, 4 independent clones encoding the ubiquilin-1 (UBQLN1) were obtained. The BACE1-UBQLN1 interaction was confirmed by co-immunoprecipitation and we showed that these partners co-localize in cell compartments. Interestingly, an exon 8 deletion genetic variant of UBQLN1 (UBQLN1Î"8) has been associated to ad patients from different families. We found that bace1 failed to interact with UBQLN1Î"8 resulting in an increased BACE1 stability and consequently, an increased in app processing. Our results suggest that UBQLN1 IS implicated in the regulation of BACE1 activity and we propose a biological mechanism by which UBQLN1Î"8 variant may be linked to AD.

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Purification and Characterization of the Subunit Architecture of Gamma-Secretase

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Background and aims: Alzheimer´s disease γ-secretase is a complex composed of presenilin (PS), the catalytic subunit, nicastrin (NCT), which serves as substrate receptor, and APH-1 and PEN-2. In human cells, two PS homologues, PS1 and PS2, and two APH-1 homologues, APH-1a and APH-1b exist, which are part of distinct γ-secretase complexes. Basic questions regarding the subunit organization, the subunit stoichiometry, the presence of additional components in γ-secretase complexes and their molecular interactions are largely unresolved. Most of these studies will be facilitated by, if not even require, pure preparations of endogenous γ-secretase. Methods, results and conclusions: To begin to address these questions endogenous human γ-secretase was purified from HEK293 cells by ammonium-sulfate precipitation, lectin-affinity and ion exchange chromatography. By this procedure all essential γ-secretase complex subunits were isolated in stoichiometric amounts. Interestingly, the novel modulator components TMP21 and CD147 were isolated only in tiny amounts suggesting that these components may be stoichiometric components of only a small fraction of γ-secretase complexes. Upon reconstitution in lipid vesicles, purified γ-secretase produced Aβ peptides in physiological ratios, which could be modulated by the NSAID sulindac sulfide. Chemical crosslinking of the purified PS1/APH-1a γ-secretase complex as a model suggested close neighborhood of the PS1 NTF and CTF, the PS1 NTF and PEN-2, the PS1 CTF and APH-1a, and of NCT and APH-1a. Interestingly, dimer formation of PS1 was not observed favoring the view of a 1:1 stoichiometry of the PS1 NTF and CTF catalytic subunit within the complex.

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Intraneuronal Beta-Amyloid Accumulation is Associated With MAP2 and Tau Microtubule Pathology

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Background and aims: Aβ and tau aggregates are hallmarks of Alzheimer's disease (AD) neuropathology, although their potential relationship remains unclear. We previously reported that Aβ42 accumulates in multivesicular bodies and smaller endosomal vesicles of neurons, especially in distal processes and synaptic compartments, prior to plaques in AD transgenic mice. Since accumulating intraneuronal Aβ42 was associated with subcellular pathological alterations, we further explored Aβ-induced alterations in microtubules in transgenic mouse models of AD.

Methods: Aβ accumulation was studied in relation to the microtubule associated protein, MAP2, and tau in APP mutant (Tg2576) and APP, PS1 and tau mutant (3xTg-AD) transgenic mouse brains with aging by immunohistochemistry and immuno-electron microscopy.

Results: In Tg2576 mice localized Aβ42 accumulations within neurites were associated with microtubule pathology evident by reduction in MAP2 even prior to high molecular weight (HMW) Aβ oligomerization. MAP2 was consistently absent in dendrites accumulating HMW Aβ oligomers. Phosphorylation of tau was observed in processes of aged Tg2576 mouse brains, especially near sites of Aβ42 monomer or low molecular weight (M/LMW) oligomers within neurites surrounding plaques. In 3xTg-AD mice, aberrant tau phosphorylation and paired helical filaments developed in Aβ42 accumulating neurites and cell bodies. In both AD transgenic mice reduction of MAP2 and elevation of phosphorylated tau with aging occurred especially at sites of Aβ42 M/LMW oligomer accumulations within neurites.

Conclusions: Our results support the scenario that intraneuronal Aβ42 accumulation is involved in microtubule pathology even prior to HMW Aβ oligomerization and plaque formation, especially within neuronal processes and synaptic compartments.

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Abeta1-40-Dependent Signaling Kinase Cascades Are Accelerated by Divalent Iron in Primary Neuronal Cells

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Losses of neuronal cells in Alzheimer's disease (AD) patients has been attributed in part to oxidative stress (OS). There is currently scant information as to the mechanism by which metal ions alter cellular signaling cascades either via an Aβ-dependent or -independent action. To examine that, primary cerebral cultures were treated either separately or with a combination of Fe2+ (5 μM) and Aβ1-40 (5 μM). Several OS parameters including free radicals formation and lipid peroxidation, respectively were measured by dichlorofuorescein staining and thiobarbituric acid assays. Additional parameters included mitochondria function, caspase activation and TUNEL staining. A cytotoxic combination of Fe2+ and Aβ1-40 caused delayed ERK activation, enhanced p38 MAPK and caspase activity. Akt, Ser-136 BAD phosphorylation and several protein kinase C (PKC) isoforms were down-regulated. Addition of Aβ1-40 without iron enhanced in contrast PKC levels, stimulated Akt and enhanced Ser-136 BAD phosphorylation suggesting a potential anti-apoptotic function of the peptide. A number of inhibitors including GF, a PKC inhibitor, wortmaninn and LY, enhanced Aβ1-40/Fe2+-induced toxicity, while U0126, a MEK inhibitor, and SB, a p38 MAPK inhibitor, prevented cell damage and apoptosis. The oxidative stress component of the Aβ1-40/Fe2+-induced toxicity could be attenuated by NAC and catalase antioxidants, by the iron chelator deferroxamine and by a pentameric Aβ1-40 analog known to be an Aβ1-40 aggregation inhibitor. These findings further support the possibility that metal ion chelation, antioxidants and pharmacological inhibition of pro-apoptotic kinase cascades may be beneficial in reducing the severity of Alzheimer's disease.

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Surface Plasmon Resonance and Nuclear Magnetic Resonance Studies of Abad-Aβ Interaction

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Background and aims: Aβ binding alcohol dehydrogenase (ABAD) is an NAD-dependent mitochondrial dehydrogenase. The ABAD-Aβ interaction is likely a direct link between Aβ and mitochondrial toxicity in Alzheimer's disease.

Methods: In this study, surface plasmon resonance (SPR) and NMR was employed to characterize ABAD-Aβ interaction.

Results: A van't Hoff analysis revealed that the ABAD-Aβ association is driven by a favorable entropic change (ΔS = 300 ± 30 J mol–1 K–1) which overcomes an unfavorable enthalpy change (ΔH = 49 ± 7 kJ mol–1). Therefore, hydrophobic interactions and changes in protein dynamics are the dominant driving forces of the ABAD-Aβ interaction. This is the first dissection of the entropic and enthalpic contribution to the energetics of a protein-protein interaction involving Aβ. SPR confirmed the conformational changes in the ABAD-Aβ complex after Aβ binding, consistent with differences seen in the crystal structures of free ABAD and ABAD-Aβ complex. Saturation transfer difference (STD) NMR experiments directly and unambiguously demonstrated the inhibitory effect of Aβ on the ABAD-NAD interaction. Conversely, NAD inhibits the Aβ-ABAD interaction.

Conclusion: Binding of Aβ and NAD to ABAD are likely mutually exclusive. Thus, Aβ binding induces conformational and subsequently functional changes in ABAD, which may have a role in the mechanism of Aβ toxicity in Alzheimer's disease.

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HtrA2/Omi Protease Regulates APP Metabolism Through Endoplasmic Reticulum-Associated Degradation

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Yeast two-hybrid assay was used to identify proteins that bind to the N-terminal, cysteine-rich region of APP. One of the isolated clones was identical to HtrA2/Omi protease, a stress responsive chaperone/protease mostly found in the mitochondria. Coimmunoprecipitation of HtrA2 with APP from cell lysates and mouse brain extracts showed that HtrA2 associates mostly with the immature form of APP. In an in vitro cleavage assay using purified recombinant proteins, HtrA2 partially cleaved APP generating N-terminally truncated forms of APP when partially active and degraded APP to completion when fully activated. APP overexpression in cells increased HtrA2 expression whereas HtrA2 RNAi or knockout increased production of APP C-terminal fragments and secretion of Aβ without significantly altering APP holoprotein levels. Furthermore, we show that in addition to mitochondria HtrA2 localizes to the cytosolic side of endoplasmic reticulum (ER) membrane and contributes to the ER-associated degradation of APP together with the proteasome. Inhibition of the proteasome by epoxomicin resulted in accumulation of cytosolic, ubiquitinated APP and increased association of immature APP with HtrA2 and Derlin-1 in microsomal membranes. Importantly, HtrA2 contributed to degradation of APP in microsomal membranes when proteasomal activity was low. Our results suggest a novel function for HtrA2 as a regulator of APP metabolism through ER-associated degradation.

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Amyloid Oligomers Induce Tau Oligomerization Ans Fibrillization in Vitro

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Alzheimer's disease (AD) is characterized histopathologically by the presence of amyloid plaques and neurofibrillary tangles. A central issue in the pathogenesis of is the relationship between amyloid deposition and neurofibrillary tangle formation. In both cell culture and transgenic mouse models tau pathology was linked to Aβ. The emerging evidence supports the hypothesis that amyloid pathology lies upstream of tau pathology, but the pathway(s) and the mechanism details still unclear, how Aβ peptides induce tau pathology in vivo or which aggregation state of Aβ most significant for tau aggregation.

Here, we used seeding and cross-seeding experiments to test the effect of different Aβ and α-synuclein species on Tau firilization. Preformed oligomers from Aβ42 and α-synuclein induced tau oligomerization and eventually fibrilization. Fibrillar and soluble Aβ42 and α-synuclein failed to cause tau aggregation. Tau oligomers formed by cross-seeding were capable of seeding soluble tau and causing its aggregation, surprisingly, tau oligomers (pre-fibrillar) prepared by seeding and cross-seeding were toxic to cells at 1-2μM concentration.

Supported by: AHAF for (R.K.), NIH and Hillblom foundation for (C.G.G.).

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Abeta Pathology in Parkinson's Disease

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Introduction: In Alzheimer's disease (AD) Abeta peptide, under the influence of the Apolipoprotein E (APOE) genotype, deposits in extracellular plaques and as cerebral amyloid angiopathy (CAA). In Parkinson's disease (PD) the presynaptic alpha-synuclein protein forms cytoplasmic Lewy bodies and cortical LBs have been implicated in the pathogenesis of PD dementia. As recent evidence suggests potential 'crosstalk' between the Abeta peptide and alpha-synuclein leading to enhanced fibrillisation of the latter, we wished to assess morphologically the relationship between LB pathology and Abeta deposition in PD. Material and methods: The frequency and severity of Abeta plaque load was assessed semi-quantitatively, CAA was graded using previously described scales, in addition to quantitative assessment of LB density in four brain areas of 40 PD cases and 20 age-matched controls. The APOE genotype of each case was also established. Results: A significantly higher proportion of PD cases (57.5%) had Abeta parenchymal plaques than controls (25%), although there was no difference in the frequency and severity of CAA. Although there was no difference in the frequency of the APOEε4 allele between the groups, it significantly correlated with increased plaque density and CAA severity in both groups. The number of cortical LBs correlated with Abeta plaque load in the temporal lobe. Conclusions: Abeta deposition in brain parenchyma more often occurs in PD than in age-matched controls and the severity of Abeta load appears to correlate with the severity of cortical LB pathology in the temporal lobe. Further studies are required to validate these observations.

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The Insect Homologue of the Amyloid Precursor Protein (APPL) Regulates Neuronal Migration During Embryonic Development

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Like vertebrate APP, the insect APP-like protein (APPL) is abundantly expressed within the developing nervous system, but its normal functions remain controversial. We have investigated the role of APPL as a guidance receptor for migrating neurons in the enteric nervous system (ENS) of Manduca sexta, a preparation that permits migrating neurons to be manipulated in vivo. During ENS development, identified neurons (EP cells) migrate along well-defined

pathways before innervating the gut. The migratory EP cells strongly express APPL, while APPL processing and trafficking coincide with specific phases of their differentiation. Human APP has been shown to regulate the heterotrimeric G protein Goα. Manduca Goα also co-immunoprecipitates with APPL, and the two proteins co-localize within the EP cell processes. Goα activation inhibits EP cell migration in a calcium-dependent manner, suggesting that it may regulate neuronal responses to inhibitory guidance cues. To test whether APPL might act as a Goα-coupled receptor, we inhibited APPL expression with antisense morpholinos. This treatment caused aberrant migration by the EP cells into regions that are normally inhibitory to migration. Treating the ENS with synthetic ectodomain fragments of APPL (to interfere with endogenous ligands) caused similar effects. These results suggest that during development, APPL may act as a Goα-coupled receptor for guidance cues that normally prevent neurons from migrating into inappropriate domains. We are currently testing whether APPL regulates Goα activation in the EP cells, and we are using an expression cloning strategy to screen for potential ligands of APPL in the ENS. Supported by NIH AG025525

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Memantine Enhances the Decreased [3H]Dopamine Release in the Presence of Amyloid-Beta 1-42 Peptide in Rat Corticostriatal Slices

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Alzheimer disease is characterized by cognitive deficiency, impaired performance of daily activities and psychiatric symptoms. Basic pathological features include extracellular deposition of amyloid-beta peptides. Clinical and postmortem studies suggested that the cholinergic neurotransmission is affected in the patients. While treatment was previously limited to cholinesterase inhibitors, memantine, an NMDA-receptor antagonist is approved nowadays. We have investigated the effect of memantine on the release of [3H]dopamine in the presence of amyloid-beta 1-42 peptide. Rat corticostriatal slices were loaded with [3H]dopamine, submerged in a two-compartment bath so that the corticostriatal glutamatergic afferentation was preserved between cortical and striatal regions. Electrical stimulation of cortical regions increased the release of [3H]dopamine in the striatal parts. Amyloid-beta peptide (10 nM) significantly decreased the electrically-evoked release of [3H]dopamine. It has been suggested that amyloid-beta peptide binds to nicotinic acetylcholine receptors containing alpha7 subunits and blocks them in a non-competitive way. Memantine (1 uM, 10 uM) alone did not change either the spontaneous or the electrically-evoked release of [3H]dopamine. Application of memantine in the presence of amyloid-beta peptide (10 nM) partially reversed the suppressed release of [3H]dopamine. These results suggest that the amyloid-beta peptide impedes the cortically-evoked release of [3H]dopamine in the striatal region of corticostriatal slices and this depressed release can be partly restored by the weak NMDA blocker memantine. The detrimental effect of amyloid-beta peptide on striatal dopamine release might contribute to the cognitive deficiency in vivo. The beneficial outcome of memantine therapy might be partly related to its effect on striatal dopamine release.

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Oxidative-Dependent Stimulation of G-Proteins in Human Control and Alzheimer's Disease Brain by V717G (London) FAD Mutant of APP714-723

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Amyloid precursor protein (APP) is known to interact with signalling molecule Go and V717 FAD mutants of APP can cause Go-mediated neuronal apoptosis and death (Nishimoto, 1998). We found that V717G mutant of APP714-723, (transmembrane fragment of V717G (London) FAD mutant of APP770) significantly stimulates the [35S]-GTPγS binding (G-proteins activity) in the temporal cortex membranes from post-mortem human brain. The 10 μM peptide enhanced the radioligand binding by 500 %. In Alzheimer's disease (AD) temporal cortex, the stimulatory effect of V717G -APP714-723 on G-proteins was 2.5 times lower than in control. In control sensory postcentral cortex, the peptide stimulation of G-proteins was 1.5-fold less than in temporal cortex whereas in AD, the effect showed no remarkable regional difference. Glutathione, desferrioxamine (DFO) and 17-β-estradiol dramatically depressed the strong stimulatory effect of V717G -APP714-723 on [35S]-GTPγS binding to control temporal cortex, the effect of DFO being most potent. In AD, the protective effect of antioxidants was significantly lower than in control. Critical role of methionine-722 (M-722) in V717G-APP714-723-induced oxidative stimulation of G-proteins in control temporal cortex was found. M-722 oxidation to methionine sulfoxide, M722S(O), resulted in 2-fold lower stimulatory effect of the peptide than that of unoxidized parent peptide; the antioxidants depressed the V717G-M722S(O)-APP714-723-induced stimulation of G-proteins less than the V717G-APP714-723 stimulation.

We suggest that the V717G -APP714-723-induced G-protein stimulation, differently expressed in human control and AD brain, implies the oxidative-dependent mechanism that is influenced by relationship of (per)oxidizing substrates and antioxidants in the control and AD brain regions.

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Collapse of Endoplasmic Reticulum as a New Mechanism Mediating Beta-Amyloid Peptide-Triggered Neurotoxicity

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Neuronal loss via different modes of neurodegeneration is a key issue for Parkinson's disease (PD) and Alzheimer's disease (AD) Increasing lines of evidence have demonstrated that accumulation of unfolded proteins in neurons leads to

neuronal apoptosis in both PD and AD. The mis/un-folded proteins accumulated in the endoplasmic reticulum (ER) triggers unfolded protein responses (UPR), in which UPR primarily attempts to stop further accumulation of mis-folded proteins. The aim of the study is to investigate whether exogenous Aβ peptide triggers UPR and how it will affect the ER.

Our research group has been focusing on protein translational control in neurodegeneration. One of the gateways to regulate protein translational control is UPR. While UPR provides a protective mechanism for neurons, we are surprisingly to note that extracellular Aβ peptide can trigger the release of Ca2+ from the ER without inducing UPR. Further investigation using live-cell imaging technology elucidates that ER was collapsed in neurons after challenge by Aβ peptide. Aggregation of neurons was partly mediated via remodeling of cytoskeleton proteins and protease. As a result of ER aggregation, there were increased numbers and size of lysosomes in neurons. However, this event does not occur in experimental model of PD as UPR occurs to inhibit the accumulation of mis-folded proteins.

Taken together, induction of UPR is a self-defense mechanism to protect neurons. We provide evidence here to propose a new mechanism showing that lack of UPR because of the collapse of the ER plays a role in Aβ peptide-triggered neurodegenerative process.

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The Effects of Peripheral and Systemic Inflammation on Amyloid Deposition and Glial Activation in APP/Tg Mice

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We have been investigating the role of anti-inflammatory and pro-inflammatory agents on glial activation and neuropathology in amyloid-precursor-protein transgenic (APP/Tg) mice. We observed substantial reductions in reactive microglia and Aβ levels after 15 days of daily peripheral administration of minocycline, an anti-inflammatory drug, in young animals, while the effect of anti-inflammatory drug was negligible in the older mice. Conversely, CFA and the Th1-polarizing adjuvant Quil A, were able to induce progressive increases in glial activation from 7 to 14 days at sites of amyloid-β (Aβ) deposition in cerebral microvessels, and microglial activation was associated with increased levels of APP and Aβ. However, in wt mice glial activation was not apparent at either time point after administration of the pro-inflammatory agents. Although APP/Tg mice have modestly elevated levels of some pro-inflammatory cytokines, we also found that they have significant elevations in several anti-inflammatory cytokines, as measured by multiplex cytokine analysis, which were reduced when mice were exposed to pro-inflammatory agents in the periphery. Interestingly, the proinflammatory response to injection of LPS into the CNS was significantly delayed in APP/Tg mice compared to wild type mice. However the maximal cytokine and chemokine response was greater in the APP/Tg mice. Our results support the hypothesis of V.H. Perry (Nat Rev Neurosci 2003) in which systemic inflammation exacerbates the “primed? inflammatory state of microglia in the aging central nervous system, which contributes to neuronal injury and cognitive decline.

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Oxidized Proteins Block Chaperones Irreversibly (Role in the Induction of Amyloidoses)

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The goal of this work was to study the role of oxidative stress and chaperones in processing of different kind of amyloidoses. Our experiments showed that oxidation of proteins results in the emergence of protein species capable of blocking the chaperones irreversibly. For example, oxidation of the sulfhydryll groups of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with hydrogen peroxide results in the formation of the misfolded forms of the protein that bind irreversibly to the chaperonin GroEL. As a result, chaperonin-dependent folding of other polypeptide chains is completely prevented. Presumably, similar processes may occur in vivo. For example, we demonstrated that inactive oxidized forms of GAPDH accumulated in different brain tissues of the transgenic mice with Alzheimer's disease. Considering that the content of this enzyme constitutes 5-15% of the total content of soluble cell proteins, the processes connected with oxidation of this enzyme can play a significant role in the inhibiting of chaperones and, consequently, in the induction of different types of amyloidoses. It was shown that misfolded forms of proteins irreversibly block chaperones and inhibit chaperone-assisted folding of native proteins. Oxidative stress and chemical modifications can provoke the formation of misfolded forms of proteins. Thus, experiments with artificial protein systems allowed us to clarify the nature of the development of prion diseases and amyloidoses and general mechanisms of the formation of protein aggregates involved in the development of many diseases, and also to outline the way of prophylaxis and specific therapy. The financial support: INTAS (03-51-4813) and RFBR (№ 05-04-48955-a, № 04-04-81038).

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Inflammation and Oxidative Stress Are Induced in Cells Exposed to A**β** Peptide

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Inflammation and Alzheimer have been poorly assay in brain and in primary culture. This prompted us to investigate their mechanism action in brain and in primary cells in culture. Astrocytes, neurons and microglia in primary culture, as well as mixer cultures were used to understand the relationship between different cells in brain. Cells treated with beta amyloid (Aβ) at different times were compared with control cells. We here found that Aβ increased oxidative stress which in turn, caused phosphorylation of MAP kinases and subsequently induced cell death. A potent astrogliosis was

detected in mixer culture (astrocyte and neuron) whether the toxic is present. Also we investigated the involvement of pro-inflammatory and anti-inflammatory cytokines and the receptor expression of IL-1RI, TLR-4 and IL-10. We detected an unbalance between pro- and anti- inflammatory cytokines in primary culture of neurons, astrocytes and in mixer cultures after Aβ toxic-action. We here try to determine the mechanism of inflammatory action in Alzheimer disease using cells in primary culture.

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Amyloid Beta Peptide Induces Caspase 3 Activation and Metabolic Alterations in Human Erythrocytes

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Amyloid beta accumulation in brain is thought to be important in causing the neuropathology of Alzheimer's disease (AD). Since vascular deposition of A beta is also implicated in AD, the interaction of erythrocytes with these toxic peptides gains importance. This implies that A beta may be involved in several reported alterations in erythrocytes from AD subjects. In order to investigate a possible role for plasma A beta in AD, we have initiated detailed studies on the effects of A beta on erythrocyte energetic metabolism. It is known that human erythrocyte metabolism is modulated by the cell oxygenation state. Among other mechanisms, competition of deoxy-hemoglobin and some glycolytic enzymes for the cytoplasmic domain of band 3 (cdb3) is probably involved in modulation. This metabolic modulation is connected to variations in intracellular NADPH and ATP levels as a function of the oxygenation state of the cell, and, consequently, it should have physiologic relevance. Metabolic differences between erythrocytes incubated at high and low oxygen saturation disappear following to 24 hours exposure to A beta. Spectrophotometric and Western blotting analysis show that caspase 3 is concurrently activated. Pre-incubation of A beta-treated erythrocytes with a specific inhibitor of caspase 3, partially restores the oxygen-dependent modulation. These preliminary results indicate that, human erythrocytes following to exposure to A beta show a complete loss of the oxygen-dependent metabolic modulation, which is partially restored by caspase 3 inhibitor. Future experiments will be addressed to relate these events with caspase 3-dependent cdb3-cleavage and with the pathways linked to eryptosis.

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isoAsp Formation of APP Alters Cleavage by the Potential ß-Secretases BACE-1 and Cathepsin B

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The formation of isoaspartate (isoAsp) residues under physiological circumstances can lead to a loss of function and a rapid degradation of the respective protein. The enzyme L-isoaspartyl methyltransferase (PIMT) transfers an active methyl group to the free alpha-carboxyl group of isoAsp resulting in an O-methyl ester. The isoAsp methyl ester rapidly breaks down to form a cyclic imide arising from deamidation or aspartate isomerisation. With each cycle of methylation, 15 - 30 % of the atypical peptide bond is converted to a normal peptide bond. Consequently, the repair capacity of this enzyme is related to many physiological and pathophysiological functions such as cerebral ischemia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, glaucoma, neuropathies, skin ageing and autoimmune disorders.

These aspects prompted us to investigate the ability of recombinant PIMT to repair peptides known to be involved in the pathogenesis of different diseases.

Moreover, we have determined the influence of isoAsp on the ability of different proteases to cleave important physiological substrates, e.g. amyloid precursor protein (APP). Our in vitro results prove, that specificity and rate of the potential ß–secretases BACE-1 and cathepsin B are both, different and significantly altered at the ß–secretase cleavage site of APP-derived peptides if isoAsp (…KM↓isoDAE…) is present instead of Asp672 (…KM↓DAE…). These results suggest that isoAsp might have an important role on the proteolytic formation of Aß and that PIMT might be a therapeutic target promoting the repair of such protein structure changes.

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Crystal Structure of Amyloid Beta Fragments

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Amyloid beta adopts a helix-turn-helix conformation in organic solvents and detergents. However, the atomic-level structure of amyloid beta in aqueous solution has not been determined. Here, we tried to determine crystal structure of amyloid beta fragments, 10-24, 28-40 and 28-42, using fusion proteins with ribonuclease HII from a hyperthermophile, Thermococcus kodakaraensis. Crystal structure analysis revealed that amyloid beta (28-42) forms a beta-conformation whereas amyloid beta (28-40) and amyloid beta (10-24) have disordered conformations. These results suggest that beta-structure formation of amyloid beta fragment (28-42) within full-length amyloid beta (1-42) in aqueous solution would nucleate and induce overall beta-sheet fibrils. On the other hand, it is difficult to form such a beta-sheet within amyloid beta (1-40) because of lack of the C-terminal residues, leading to lower aggregative ability and neurotoxicity of amyloid beta (1-40) than amyloid beta (1-42).

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A Novel Cytometric Assay for AICD Production Based on Differential Retention of Fluorescent Probes

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Background and Aims: Alzheimer's disease is characterized by senile plaques composed primarily of amyloid-b-peptide. The latter is released from the amyloid precursor protein (APP) by the sequential proteolytic cleavage by b- and g-secretases. Cleavage of APP by g-secretase results also in the release of APP intracellular domain (AICD), that remains cytosolic and is involved in signalling to the nucleus. In order to estimate g-secretase activity, we developed a new tool measuring AICD production in living cells.

Methods and Results: The assay was performed with two fluorescent reporter substrates for g-secretase activity, APP-GFP and C100-GFP that are efficiently cleaved by b- and/or g-secretase, leading to the release of AICD-GFP into the cytosol. The intracellular localization of the two probes in multiple cell models and how this feature was changing with time and upon different treatments was also studied. The differential retention of the fluorescent probes upon plasma membrane permeabilization is suitable for detecting molecular events as proteolytic activities or changes in intracellular compartimentation due to protein release or specific conformational modification.

The strategy, capable to convert subcellular spatial signals into steady "all-or-none" fluorescence intensity responses, avoids the need and the limits of high resolution microscope imaging or biochemical techniques.

Conclusions: Based on a change in their intracellular repartition, we here describe the application of this class of fluorescent probes in cellular assays by flow cytometry where g-secretase activity was measured. This approach may provide a rapid and simple tool for a functional characterization of drugs modulating AICD production directly in cells.

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The Amyloid Precursor Protein Intracellular Domain (AICD) Modulates Cytoskeletal Dynamics and Expression of Genes Which Are Upregulated in Alzheimer's Disease

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Amyloidogenic processing of the amyloid precursor protein (APP) results in the generation of ß-amyloid, the main constituent of Alzheimer plaques, and the APP intracellular domain (AICD). Recently, it has been demonstrated that AICD has transactivation potential, however, the targets of AICD dependent gene regulation and hence the physiological role of AICD remain largely unknown. We analyzed transcriptome changes during AICD dependent gene regulation using a human neural cell culture system inducible for expression of AICD, its coactivator FE65, or the combination of both. Induction of AICD was associated with increased expression of genes with known function in the organization and dynamics of the actin cytoskeleton including α2-Actin and Transgelin (SM22). AICD target genes were also found to be differentially regulated in the frontal cortex of Alzheimer's disease patients compared with controls as well as in AICD/FE65 transiently transfected murine cortical neurons. Confocal image analysis of neural cells and cortical neurons expressing both AICD and FE65 confirmed pronounced changes in the organization of the actin cytoskeleton including the destabilisation of actin fibres and clumping of actin at the sites of cellular outgrowth. Our data point to a role of AICD in developmental and injury-related cytoskeletal dynamics in the nervous system.

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Identification of Interacting Proteins With Fibrillar **B**-Amyloid, Amylin and NAC Peptides by Co-Precipitation Assay

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According to our knowledge many peptides are involved in amyloidogenesis. β-amyloid (Aβ), amylin and NAC all belong to this peptide class. All of these peptides have been associated with several neurodegenerative diseases.

Aβ is overexpressed in Alzheimer's disease, accumulates in neuritic plaques and aggregates also intracellulary (Bückig et al., 2002). Aβ peptide is generated by multiple proteolytic processing of amyloid precursor protein. Aβ peptides form fibrils which are able to interact with various molecular partners. In this work we have studied the fibrillar form of Aβ1-40.

NAC is a minimum of 35 amino acids long peptide, which forms fibrils, too. This peptide is the hydrophobic region of α-synuclein amino acid sequence 61-95. NAC is pivotal for forming α-synuclein aggregates.

Human amylin is a 37 amino acid long peptide. May and coworkers (1993) reported that human amylin was toxic to rat hippocampal neurons. Beaumont and coworkers (1993) have identified amylin binding sites in rat brain. It seems that amylin toxicity depends on its ability to form aggregates. Lorenzo and coworkers (2000) reported that the pattern of proteins binding to fibrillar amylin is quite similar to the pattern of proteins binding to fibrillar Aβ.

In this work we identified proteins which bind to the fibrillar Aβ1-40, amylin and NAC peptides. Triton X-100 soluble proteins were extracted from synaptic plasma membrane, microsoma, mitochondrial and cytosol fraction. Interacting proteins were isolated by co-precipitation assay and we used fibrillar crystalline as negative control. Proteins were identified by LC-MS/MS mass spectrometry after "in solution" tryptic digestion.

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Amyloid Beta-Petide Mediates Triose Phosphate Isomerase Activity Impairment and Aggregation by Nitric Oxide in Neuronal Cells

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Amyloid ß-peptide (Aß) fibrils contained in senile plaques are known to be a source of superoxide anion, which induces oxidative stress in neurons. Superoxide anion reacts with high affinity with nitric oxide present in neurons, giving rise to peroxynitrite, which is one of the most reactive molecules in biological systems. The harmful effect of peroxynitrite is its capacity to nitrotyrosinate proteins. In the present work, we have demonstrated that Aß is producing the nitrotyrosination of triose phosphate isomerase (TPI) in neuronal cells. TPI is a key enzyme of the glycolytic pathway. The nitrotyrosination of TPI yields to a reduction in the bioavility of trioses needed for the Krebs's cycle by inhibiting the activity of the enzyme. We have found that at least one crucial Tyr residue in the catalytic center of the enzyme is being nitrotyrosinated. Long time exposures of TPI to peroxynitrite produce the aggregation of the enzyme in large structures as observed by transmission electron microscopy. The aggregation of TPI turns the monomers to ß-sheet structures, seen by thioflavin staining, which are not able to be degraded by the proteosoma. TPI obtained by immunoprecipitation from cerebral cortex from AD patients show the same pattern of aggregation, which was not observed in samples from healthy individuals.

Supported by the Spanish Ministerio de Sanidad (FIS-PI041242; Red-HERACLES) and Generalitat de Catalunya (GSR2005-266).

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Protective Effects of Stilbene Molecules Against Beta-Amyloid-Induced Mitochondria Dysfuctions in IMR-32 Cells

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Amyloid beta(1-42) peptide is considered responsible for the formation of senile plaques that accumulate in the brains of patients with Alzheimer's Disease (AD). In the last years considerable attention has been focused on identifying natural molecules, i.e polyphenols and phytoestrogens that prevent or almost retard the appearance of Aβ-related neurotoxic effects. Our recent studies evidenced a neuro-protective mechanism mediated by two stilbenes through the modulation of apoptotic gene family expression. Because mitochondrial dysfunction is one of the major biochemical event linked with Alzheimer's disease, in this study, we investigated the effects of resveratrol, representing the best known of the stilbene-family, and other similar molecules on beta-amyloid-induced mitochondria dysfunctions on human neuroblastoma cells (IMR-32). The obtained results show that beta amyloid treatment led to i) release of cytochrome c, ii) mitochondrial oxygen consumption decrement and iii) mitochondrial membrane potential alteration. All these pro-apoptotic effects are partially o completely abolished by the treatment with resveratrol or other stilbenes. At the light of these preliminary observations we retain that besides the common therapeutic strategies developed to treat AD (i.e. anti-inflammatory, antioxidant, and antiamyloid approaches), mitochondrial antioxidant therapy could be a lot efficacious in reducing pathological events without adverse effects, promising as a treatment for AD patients.

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Modulation of APP Processing by Hypoxia and Its Effects on Calcium Signalling in Cortical Astrocytes

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Our previous studies have shown that prolonged hypoxia (1-2.5% O2, 24h) leads to remodeling of Ca2+ signaling in primary cultures of cortical astrocytes (J Biol Chem (2003) 278, 4875-4881). Importantly, these effects are tightly coupled to APP processing, since inhibition of A-beta formation at least partially reverses this remodeling of Ca2+ signaling (J Neurochem (2003) 88, 869-877). These findings are of tremendous potential clinical importance given the predisposition of individuals suffering hypoxic / ischemic episodes to Alzheimer's disease. We have therefore investigated the effects of hypoxia on both beta and gamma secretases in astrocytes. Western blots indicated that Presenilin-1 was present in astrocytes, and was markedly up-regulated by 24h hypoxia. Beta secretase was also found to be present in these cells, but hypoxia was without effect on its activity, as determined by a FRET-based assay. Fluorimetric measurements of [Ca2+]i revealed that apparent mobilization of Ca2+ from endoplasmic reticulum stores was enhanced under conditions of hypoxia. We have previously demonstrated that this is due to Ca2+ loading of mitochondria and inhibition of plasmalemmal Na+ /Ca2+ exchange, both factors inhibiting efficient Ca2+ buffering following mobilization (J Biol Chem (2003) 278, 4875-4881). Crucially, these effects were partly restored by pharmacological inhibition of beta or gamma secretase. Thus, hypoxia promotes A-beta formation by astrocytes, and this accounts in part for the deleterious effects on Ca2+ homeostasis in these cells. Such effects are likely to contribute to the pro-neurodegenerative consequences of prolonged hypoxia.

Supported by the Alzheimer's Research Trust, The Wellcome Trust and the MRC.

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Isoform Difference in Cathepsin D Mediated ApoE Proteolysis and Its Interaction With Amyloid-Beta Peptide

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Background: ApoE isoforms differentially affect the risk of Alzheimer's disease and apolipoprotein E (apoE) has also been shown to exhibit isoform-specific differences in binding to the amyloid-b peptide (Ab). Although the mechanism by which apoE genotype modifies the risk of the disease is unknown, proteolytic fragments of apoE have been suggested to play a role. There is evidence that apoE can be proteolyzed by different proteases and we have found evidence that aspartic protease(s), such as cathepsin D, may contribute to the proteolysis of apoE in AD brain homogenates.

Method: We tested whether there are isoform differences in the susceptibility of full-length apoE4 and apoE3 to proteolysis by cathepsins D and E using recombinant human apoE and purified enzymes. We also tested whether fibrillar or oligomeric amyloid-b peptide (Ab) would alter apoE proteolysis. Full-length apoE3 and apoE4 were incubated with purified enzyme (cathepsin D or cathepsin E) and the extent of proteolysis was monitored using Western blotting.

Results: There is an isoform difference in susceptibility to proteolysis by cathepsin D with apoE4 being more susceptible than apoE3 (3 hrs incubation at pH 4.5). Preliminary data reveal no obvious isoform difference in apoE susceptibility to proteolysis by cathepsin E. Additional experiments, in which fibrillar or oligomeric Ab were added to the mixture showed evidence of inhibition of the breakdown of both full-length apoE3 and apoE4 by cathepsin D.

Conclusion: Full-length apoE may exhibit isoform differences in susceptibility to proteolysis, which may also be affected by the presence of Aß.

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Soluble Amyloid B1-40 Modulates 'A'type K+ Channels by Augmenting the Association of KChIP3 With the Kv4.2 Subunit

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We have previously shown that the Alzheimer's disease related peptide amyloid beta protein (Aβ) increases expression of 'A'-type K+ channel currents in rat cerebellar granule neurons (CGN) (Ramsden et al., 2001). These earlier studies used a soluble synthetic, human form of Aβ1-40 however the endogenous, rat Aβ differs from human by 3 amino acids. This study investigated the effects of recombinant human Aβ1-40 and compared it to Aβ1-40 with a rodent sequence. Additionally we investigated its effect on the Kv-interacting channel protein (KChIP3) which associates with Kv4.2 and presenilin.

Dissociated cultures of CGN were prepared from 6-8 day old rats as reported previously (Ramsden et al., 2001). Whole-cell patch clamp measurements of K+ channel currents were carried out using quasi-physiological intra- and extracellular solutions (Ramsden et al., 2001). Recombinant human and rat Aβ1-40 were diluted to a concentration of 10nM and applied to cultures for 24 hours. Immunoblotting was carried out on Aβ treated CGNs using anti-KChIP3 and anti-Kv4.2 polyclonal antibodies.

The inactivating, 'A'-type, component of the K+ current density/voltage (I-V) relationship was particularly sensitive to rat Aβ1-40 (3.4-fold increase, n=19 control, treated cells, $p < 0.05$) and human recombinant Aβ1-40 (2.6-fold increase, n=17 control, treated cells, $p < 0.05$). Protein expression of KChIP3 significantly increased by 30% in Aβ treated cells, however Kv4.2 expression only increased by 17% (n=4 control, treated cells, $p<0.05$).

These data suggest that Aβ1-40 modulates K+ channel current, by increasing the association of KChIP3 with Kv4.2, augmenting protein trafficking of this channel complex.

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Differential Apoptotic Response of Primary Human Cerebral Endothelial Cells to Oligomeric Assemblies of Amyloid Beta Genetic Variants

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Amyloid deposition in small and medium cerebral vessels, defined as Cerebral Amyloid Angiopathy (CAA), is associated with numerous microvascular changes and loss of endothelium. The most common CAA-associated proteins are related to familial variants of the Alzheimer's Aβ peptide being mutations clustered at positions 21-23 and 34 particularly linked to vascular pathology. Unknown is the molecular basis by which these amyloid subunits oligomerize, assemble into insoluble fibrils, and form the amyloid deposits that eventually replace the normal vessel wall architecture. Current knowledge suggests that the tendency of Aβ to form oligomeric and/or fibrillar structures is central for the induction of neurotoxicity, however only very limited data is available for cells composing the vessel wall. In our studies, primary human cerebral endothelial cells (EC) were challenged with well-defined oligomeric assemblies of the AβE22Q (Dutch) and AβL34V (Piedmont) variants, and their response compared to that of wild type Aβ40 and Aβ42. Circular dichroism, thioflavin T binding, Western blot analysis under native and denaturing conditions and atomic force microscopy showed an array of secondary structures and different aggregation/fibrillization kinetics, being Aβ42 the one with the highest β-sheet content and fibrillogenic tendency. Annexin-V immunofluorescence and quantitative evaluation of the generation of nucleosomes (histone-complexed DNA fragments) indicated that apoptosis preceded fibril formation correlating with the presence of oligomeric amyloid assemblies. These data indicate that the presence of similar structures do not necessarily evoke the same cellular

response and suggest that the toxic effect of these oligomeric assemblies goes beyond mere multimerization.

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Physiological Modulation of Neuronal Ion Channel Activity by Amyloid ß Peptides

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Much of the focus of research into amyloid ß peptides (Aßs) has been on their role in neurodegeneration. Relatively high (20-25µM) concentrations of aggregated Aßs are toxic in assays of cell death. In contrast, the physiology of the peptides, present at a concentration of 1-10nM in cerebrospinal fluid in non-Alzheimer sufferers, has been largely ignored. We have found that Aß can alter the activity of voltage gated ion channels in the physiological range. Thus, 1-10nM Aß1-40 increases the functional activity of both voltage-gated K+ and Ca2+ channels in dissociated cultures of neurones from the central nervous system. Surprisingly, these effects were due to the unaggregated, soluble form of the peptide rather than the aggregated peptide. When aggregated peptide was applied to cells, no effect on functional ion channel expression was observed, or a slight decrease in current was seen. This suggests that the soluble peptide is important in modulating ion channel activity in neurones and that aggregation leads to a loss of function. Consistent with this, inhibition of endogenous Aß production causes a reduction in the K+ channel current. This effect is observed when Aß production is reduced by inhibitors of either ß- or gamma- secretase and can be recovered by co-application of Aß1-40 but not Aß1-42. These data suggest that Alzheimer's disease may be associated with a loss of function of Aß activity as Aß production is shifted to the 1-42 size form and exacerbated by aggregation of the peptide.

Supported by the Alzheimer's Research Trust and MRC.

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Purification of Cell-Derived Amyloid-Beta Protofibrils With Immunoaffinity Chromatography

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Background/Aims: In recent years there has been an increasing interest in soluble amyloid-beta oligomers as a major pathogenic species in Alzheimer's disease (AD). The idea is supported by the enhanced protofibril formation by the Arctic Alzheimer (APP E693G) mutation. The protofibril is a distinct intermediate of the fibrillization reaction, which has been suggested as a potential target for new therapeutic drugs. However, the development of protofibril-specific therapeutics requires a detailed knowledge of the protofibril. The aim of the project is to enable purification and extraction of secreted amyloid-beta protofibrils from conditioned cell culture media using a protofibril selective antibody. Our hypothesis is that naturally produced amyloid-beta protofibrils from cultured cells are more similar to amyloid-beta protofibrils in the brain of AD patients and therefore more relevant objects of study than synthetic amyloid-beta protofibrils. Methods: Protofibrils secreted by cultured cells (SHSH-5Y, expressing APP with the Arctic and Swedish mutation) were purified by immunoaffinity chromatography (ÄKTAprime – system) using a protofibril selective monoclonal IgG. The eluate was collected in fractions and measured by a protofibril selective ELISA. Results: Preliminary results show an increased protofibril concentration after the purification procedure. Conclusions: Our data indicate that it is possible to isolate naturally secreted protofibrils by employing a protofibril selective antibody in a liquid chromatography system. The results suggest that the purification procedure can be used in applications for producing naturally secreted protofibrils for studying its characteristics, which in the future can be valuable for evaluating a protofibril therapeutic antibody.

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Study of the Mechanism Behind the Neuro-Protective Effect of the Nicotine, AChE-I and SAPP-alpha Agents on Alzheimer Disease Related Amyloidogenesis

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Although it is quite established that beta amyloid (Aß) is involved in the pathology of Alzheimer's disease (AD) it is not yet established which form (s) of the Aß that is most crucial to target in the attempts to effectively treat AD. Nicotine is known as a as a cognitive enhancer exerting its effect via the nicotinic acetylcholine receptor (nAChR) subtypes alpha4 and alpha7. The effects of chronic nicotine exposure on Aß accumulation have been studied in both humans and animal models. The underlying mechanisms for interfering with the AD neuropathology are still not yet known. Nicotine may exert anti-amyloid properties via non-receptor mechanisms.

For testing this statement, we are investigating, the effect of nicotine, as well as different acetylcholinesterase inhibitors (AChE-I) and sAPP-alpha on production of the Aß 1-40, Aß 1-42, in the human embryonic kidney cells, transfected with the human APPswe gene (HEKswe). HEKswe is lacking nAChRs. Using Enzyme-Linked Immunosorbent Assay (ELISA) technique, we have preliminary observed in HEKswe, treated with (-). nicotine (10-4 M), (+).nicotine (10-4 M), and sAPP-alpha (10-9 M), decreased levels of both Aß 1-40 and Aß 1-42. Further studies are aiming to investigate the possible underlying mechanisms for nicotine and other cholinergic compounds with focus on their interfering with the amyloidogenesis.

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The Nature of Monoclonal Antibodies Produced From Mice Immunized With Abeta Protofibrils

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Background: Accumulating evidence indicates that soluble Aβ oligomers are responsible for the neurotoxicity in AD. The Arctic APP mutation (E693G) strengthens the idea that soluble Aβ oligomers are pathogenic in AD. This mutation, found in a Swedish family, is located within the Aβ sequence and enhances the formation of large soluble oligomers of Aβ, i.e. protofibrils. To be able to study protofibrils in their natural environment we have produced antibodies with specificity for this state of aggregation, and which are able to immunologically discriminate between different Aβ conformations.

Aims: To investigate the protofibrils as an antigen with regard to the nature of the monoclonal antibodies produced.

Methods: To create protofibril selective antibodies lots of effort has first been put into understand the nature of this antigen. Using Size Exclusion Chromatography (SEC) on HPLC, Native gels, Western Blot and EM we have been able to create a reliable protocol for making protofibrils, both for Aβ42 Arc and Aβ42 wt. To evaluate the immunological effects of protofibrils we have immunized mice, screened their antibody response by ELISA and investigated the produced monoclonal antibodies -both considering their molecular sequences and their ability to bind Aβ.

Conclusion: By immunizing mice with protofibrils we have succeeded in making monoclonal antibodies with high selectivity for protofibrils. The nature of these monoclonal antibodies can be divided into three subgroups: Low affinity IgM antibodies, high affinity IgM antibodies and high affinity IgG antibodies.

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GxxxG Juxtamembrane and Transmembrane Motifs Control the Amyloidogenic Processing of APP

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The amyloidogenic processing of APP leads to the formation of beta-amyloid peptide (Abeta) and APP Intracellular C-terminal Domain (AICD), two metabolites that are likely to play a key role in the onset and progression of AD. Abeta is the major constituent of senile plaques, and AICD was reported to control the expression of APP target genes. The mechanisms governing the interactions between APP and the secretases play thus a key role in APP processing, and yet they are poorly understood. We analyzed the role of the GxxxG motifs in APP processing. These motifs, which are present in many transmembrane sequences, are known to be essential in mediating membrane protein-protein interactions.

The glycine residues (G) present in the transmembrane and juxtamembrane GxxxG motifs of human APP695 were mutated either to alanine (A) or to leucine (L). We showed that the GG 625/629 LL mutation (APP695 numbering) decreased the levels of extracellular Abeta by 67%. This decrease in Abeta production resulted from an impairment of gamma-cleavage, but not of beta-cleavage,. Furthemore, the detection of shorter Abeta isoforms indicated that this mutation also shifted the position of gamma-cleavage. Strikingly, GG 625/629 LL mutation had no effect on the cellular AICD levels detected either by Western blotting or by Gal4 transactivation assays. We are currently investigating the involvement of GxxxG motifs in APP-PS1 interaction and APP homo-dimerization.

Taken together, our data identify the juxtamembrane/transmembrane GxxxG motifs of APP as a major determinant for amyloidogenic processing.

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Nitric Oxide and Arachidonic Acid Metabolism Mediate Amyloid Beta Peptide Evoked Alteration of NF-KB and Poly (ADP-Ribose)Polymerase Activity and Function

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Our previous study indicated that amyloid beta (Aβ) and non amyloid component (NAC) by significant activation of free radicals in hippocampus induced DNA damage that leads to activation of poly(ADP-ribose)polymerase-1 (PARP-1) and AIF release from mitochondria. PARP-1, depending on intensivity of oxidative stress, could be involved in DNA repair and regulation of transcription or in cells death. In this study the role of Nitric Oxide and Arachidonic Acid metabolism by cyclooxygenases COX(s) and lypoxygenases LOX(s) in Aβ evoked oxidative stress in cells treated or contained different Aβ concentration was determined. Moreover, relationship between Aβ level and PARP-1 activity and function was evaluated. The study was carried out on hippocampal synaptoneurosomal fraction and on control PC12 cells and PC12 cells transfected with human amyloid precursor protein gene (APP wt) and with APP bearing double Swedish mutation (APP sw). The data indicated significant relationship between Aβ level and free radicals concentration, Nitric oxide synthase (NOS), cytosolic PLA2 (cPLA2) and COX2 activities and expression. Inhibitors of neuronal NOS (nNOS), cPLA2 and COX2 protected cells against Aβ evoked oxidative stress and had effect on alteration of NF-kB and PARP-1 function.

Supported by PBZ-MIN-001/PO5/16

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Reduced Phosphatidylinositol-3-Kinase Activation in Alzheimer's Disease Patients

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Background: Tau protein hyperphosphorylation, a relevant step in the pathophysiology of Alzheimer's disease (AD), is mediated, by the glycogen synthase kinase-3ß (GSK3ß). GSK3ß undergoes a negative regulation by the phosphatidylinositol-3-kinase (PI-3-K)/Akt pathway, which is in turn stimulated by a number of extracellular signals including insulin. The coexistence of probable AD and non-insulin dependent diabetes mellitus prompted us to search for abnormalities in the PI-3-K/Akt pathway in AD patients.

Methods: We examined the PI-3-K/Akt pathway in peripheral blood mononuclear cells (PBMCs) isolated from non-diabetic AD (n=17), mild cognitive impairment (MCI) (n=3), and control subjects (n=20) and challenged with either human recombinant insulin (100 µg/ml) or a combination of ionomycin and phorbol 12-myristate 13-acetate (PMA). Levels of phosphorylated Akt and phosphorylated ERK1/2 were determined by Western blot.

Results: Stimulation of the PI-3-K by insulin or by ionomycin and PMA was blunted in PBMCs from all AD/MCI patients, independently of the extent of cognitive decline. No correlation was found between the reduced activation of the PI-3-K pathway and a battery of neuropsychological tests exploring attention, memory, language, intelligence, or visuospatial ability. A defective activation of the MAP kinase pathway was only found in severe AD patients (MMSE score <14) when PBMCs were challenged with ionomycin and PMA.

Conclusions: A reduced activation of the PI-3-K pathway in PBMCs may provide a novel reliable peripheral marker of AD. A reduced PI-3-K activity, with the ensuing dysregulation of GSK3ß in nerve cells, may contribute to the formation of neurofibrillary tangles in the AD brain

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Lithium Down-Regulates Tau in Cultured Cortical Neurons: A Possible Mechanism of Neuroprotection?

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Lithium is a medication for bipolar mood disorders. Its therapeutic mechanism of action remains unclear. Lithium has been reported to have a neuroprotective effect, for example, against beta-amyloid (Abeta) neurotoxicity. Glycogen synthase kinase-3beta(GSK-3beta) can be directly inhibited by lithium and this inhibition might represent a molecular mechanism for the neuroprotection provided by lithium. Tau is a microtubule-associated protein largely expressed in neurons. GSK-3beta is thought to be one of key factors involved in the abnormal phosphorylation and aggregation of tau into neurofibrillary tangles (NFTs), a hallmark of tauopathies such as Alzheimer's disease (AD). In a transgenic mice model of tauopathies, the suppression of transgenic tau was reported to prevent behavioural impairments, and neurodegeneration, without preventing the accumulation of NFTs. Extracellular amyloid plaques are another feature of AD. Recent data suggest that tau could mediate Abeta-neurotoxicity, since neurons depleted with tau were less prone to Abeta-neurotoxicity. Furthermore, chronic treatment with lithium reduced tau lesions and neurodegeneration in transgenic mice overexpressing mutant human tau variants. Here, we raise the possibility that lithium could exert its neuroprotective effect through the modulation of tau protein levels. We show that exposure of cultured cortical neurons to lithium decreased tau protein levels. This decrease was not linked to the activation of proteolytic processes or to neuronal loss, but was associated with a reduction in tau mRNA. Moreover, prior exposure to lithium markedly reduced pre-aggregated Abeta-induced neuronal apoptosis. We suggest that lithium exerts its neuroprotective action by down regulating tau at the transcriptional level.

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in Vivo Efficacy of GSK3beta Inhibitors in The Postnatal Rat Model of Tau Hyperphosphorylation

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Background: Glycogen synthase kinase-3 (gsk3beta) is involved in several neuropathological events associated with alzheimer´s disease (ad), including tau hyperphosphorylation, app processing, and cell death

Aim: In the present study we applied a postnatal rat model of tau hyperphosphorylation to study the efficacy of different classes of small molecule gsk3beta inhibitors such as arylindolemaleimide (sb216763), bis-indole (indirubin-3-monoxime), aminopyrimidine (chir98014), thiazole (ar-a014418), benzazepinone (alsterpullone) in decreasing tau phosphorylation in vivo.

Results: We observed a peak in tau phosphorylation at ser202, ser396, thr205 and thr181 epitopes in the first two weeks of postnatal development in the rat, which decreased in adulthood. Likewise, the increased p-tau levels during early brain embryonic and postnatal development correlate with the increased gsk3beta enzyme activity. In this study we show that all inhibitors tested reduced tau phosphorylation in 12-day old postnatal rats, with brain exposure levels within the ic50 range of these compounds. However, the efficacy of these inhibitors on decreasing phopshorylation of two isoforms of tau, 3r0n and 4r0n, varied according to the inhibitors used. Furthermore, we show that the observed reduction in p-tau levels robustly correlated with the inhibition of enzyme activity. Also, given recent evidence that gsk3b could also be involved in app processing and apoptosis, we also tested and showed an effect of gsk3beta inhibitors to reduce abeta production in hek293 cells stably overexpressing human appswe.

Conclusion: These data suggest that gsk3beta inhibition could be used as an effective therapy to treat both canonical pathogenic pathways underlying ad pathogenesis: tau hyperphosphorylation and abeta over-production.

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Astroglial Activity in the Inferior Olivary Nucleus in Alzeimer's Disease and Aging

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The role of astrocytes in normal and pathological conditions has been studied intensively during the last decades. Yet most studies on Alzheimer's disease (AD) focus on cortical and hippocampal areas which display the classical pathological hallmarks. Little is known about the principal inferior olivary nucleus (PO), which does not display neither neurofibrillary tangles nor neuritic plaques. Our recent study (Lasn, et al., JCMM, 10:145-156, 2006) demonstrated that a few diffuse amyloid deposits do exist in AD brains. Nevertheless, the inferior olivary nucleus in AD patients undergoes degenerative changes, such as significant neuronal and oligodendroglial loss without significant numerical changes of astrocytes. To characterize the astrocytes in the PO qualitatively and quantitatively, we used human control and AD brains for stereology and ELISA measurements. To observe the activity of astrocytes we applied S100B, GFAP and vimentin as the major markers for plasticity and reactivity. We denote a modest up-regulation of S100B in aged controls but significant increase in AD. Furthermore the neuronal number correlated negatively to the amount of S100B positive astrocytes. ELISA measurements confirmed the results of morphology, where the S100B was up-regulated ca 2 folds (197 %) in AD compared to controls. The density of GFAP is increased significantly by ca 120 % in AD while we were not able to visualize the presence of vimentin. Our data suggests that S100B overexpression in PO might contribute to the neuronal pathology in AD.

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Inhibition of BACE1 by a Novel Small Molecule Shows in Vitro and in Vivo Efficacy

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Alzheimer's disease (AD) is a progressive neurodegenerative disease that is the leading cause of dementia. The 39-42 amino acid amyloid b-peptide (Ab), believed to play a central role in the pathogenesis of AD, is produced by the sequential proteolytic processing of b-amyloid precursor protein (APP) by b- and g-secretase. The novel membrane-bound aspartyl protease BACE1 (also called memapsin2 or Asp2) was recently characterized as the enzyme possessing b- secretase activity and therefore, is considered an attractive therapeutic target for the treatment and prevention of AD.

Here we describe the identification of a potent and selective small molecule BACE1 inhibitor whose nanomolar potency was optimized from a micromolar high-throughput screening hit using classical SAR and structure-based design. Compound 1 displayed low nanomolar potency in inhibiting BACE1-mediated cleavage of a peptide substrate and lowering production of Ab secreted by APP recombinantly-expressing cell line; furthermore, this compound displayed selectively against BACE2, Cathepsin D and pepsin. In addition, oral single acute or subchronic administration of compound 1 to Tg2576 mice resulted in a significant decrease in Ab40 in plasma and Ab40/42 in brain. Lastly, compound 1 reversed the cognitive deficits exhibited by Tg2576 mice, as assessed by compound-mediated increased % freezing vs. vehicle in the contextual fear-conditioning model for hippocampal-mediated memory. This represents the first description of a potent, selective and orally active small molecule BACE1 inhibitor that will contribute toward our understanding of APP processing as well as the development of disease-modifying AD therapeutics.

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Effects of Metal Chelators and Metal Divalent Cations on in Vitro Gamma-Secretase Activity

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Gamma-secretase represents a novel class of proteolytic enzyme involved in regulated intramembrane proteolysis of a subset of receptors and adhesion molecules, including the Alzheimer's disease amyloid precursor protein, APP. Gamma-secretase cleavage of the APP stub resulting from ectodomain shedding by beta-secretase leads to the release of APP intracellular domain, AICD and concomitant formation of Abeta amyloid peptides. Therefore, gamma-secretase is an important therapeutic target, and gamma-secretase inhibitors are being developed and undergoing first clinical trials.

The molecular mechanisms of gamma-secretase activation and regulation remain poorly understood. We have investigated the effects of metal ions and metal chelators on gamma-secretase activity in an vitro assay. Gamma-secretase was extracted from brain guinea pig membranes or from human neuroblastoma SH-SY5Y cells and was tested for cleavage of an APP C101-3XFLAG substrate and production of AICD in presence or absence of metal chelators and divalent metal cations. Broad metal chelators, EDTA and phenanthroline decreased gamma-secretase activity by 70-80% whereas, more specific chelators such as the copper chelator, clioquinol, and the zinc chelators, thiorphan, phosphoramidon and ilomastat, had no effect. EGTA improved formation/detection of both AICD and Abeta, suggesting that Ca is not required for activity. Buffer containing 5 mM Mg produced highest activity. We are investigating further the effects of Mg on gamma-secretase complex stability by blue native gel electrophoresis. (supported by the NHMRC)

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Characterization of APP Efficacy and Notch Toxicity With Gamma Secretase Inhibitors

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Background and Aims: Inhibition of gamma-secretase-mediated processing of APP is one approach towards developing a disease modifying therapeutic for the treatment of Alzheimer's disease. A major liability of gamma-secretase as a therapeutic target is its role in processing other type I transmembrane proteins, most notably the Notch family of receptors. In preclinical models, inhibition of gamma-secretase-mediated Notch processing leads to altered differentiation in the spleen, thymus and gastrointestinal tract. Here, we report on our efforts to balance APP efficacy with Notch toxicity.

Methods: Cell-based models expressing APP or Notch substrates were used to characterize inhibitor potency and substrate-dependent effects. Acute and chronic rat studies were used to evaluate inhibitor Abeta efficacy and Notch toxicity.

Results: Using cell-based models, we show that APP substrate levels affect both inhibitor potencies and the generation of Abeta rises. These substrate-dependent effects also appear to occur in vivo, highlighting the importance of the animal model used. We also present evidence that APP efficacy can be separated from GI toxicity in vivo.

Conclusion: Our results illustrate that despite the complex nature of gamma-secretase as a therapeutic target, the development of safe inhibitors may be possible.

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Functional Target Validation in Cell Disease Models of AD

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Target-based drug discovery starts with the identification of target genes and their respective protein products associated with or controlling a disease-relevant phenotype.

In this respect, drug discovery has been driven largely by a genomics-based approach and, more recently, a proteomics approach has also been incorporated into this process. Once candidates are obtained from the above mentioned approaches, the development of effective novel therapeutic agents faces many significant challenges, such as demonstrating that a putative target plays a critical role in disease progression. The use of cellular models has been proven to be a valuable tool in these studies. Obviously, the closer the cellular models resemble the disease situation, the better the target profile will be. In such effort primary culture of rat cortical neurons have been largely used as models of neurodegenerative diseases, but they present some technical drawbacks that limited so far their use in the development of efficient functional target validation assays. They suffer from low transfection efficiencies and are extremely sensitive to environmental variations occurring during the delivery and expression of foreign genes.

We developed a luciferase reporter-based assay system allowing the sensitive and reproducible functional analysis of putative targets genes (modulated by overexpression or RNAi) involved in Amyloid beta-mediated neurotoxicity.

Our expectation is that the implementation of these reporter-based technologies to primary cell cultures may enable a fast and reliable conversion of potential genomic targets into functionally validated molecules, resulting in practicable gene-based drug discovery pipelines and useful instruments to unravel molecular mechanisms of neurodegeneration.

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Altered Subcellular Location of Phosphorylated Smads in Neurons of Alzheimer´S Disease Brain

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Background and aims: Many growth factors and cytokines as for example transforming growth factor beta 1 (TGF-b1) are elevated in Alzheimer´s disease (AD) giving rise to activated intracellular mitogenic signaling cascades. Activated mitogenic signaling involving the mitogen-activated protein kinases (MAPKs) and other protein kinases might alter the phosphorylation states of structural proteins such as tau resulting in hyperphosphorylated deposits. Many intracellular signaling proteins itself are potential targets of misregulated phosphorylation and dephosphorylation. Recently, a crosstalk between MAPKs and Smad proteins, both involved in mediating TGF-b1 signaling, has been reported. Although TGF-b1 has previously been shown to be involved in the pathogenesis of AD the role of Smad proteins has not been investigated.

Results: In this study we, thus, analysed the subcellular distribution of phosphorylated Smad2 and Smad3 in the hippocampus of both normal and AD brains. Here we report on strong nuclear detection of phosphorylated Smad2 and Smad3 in neurons of control brains. In AD brains these phosphorylated proteins were additionally found in cytoplasmic granules in hippocampal neurons, within amyloid plaques and attached to neurofibrillary tangles.

Conclusions: Our data suggest an important role of Smad proteins in the pathogenesis of AD.

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Orally Active Peptidomimetic BACE Inhibitors

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Background: Alzheimer's Disease is a progressive neurodegenerative disease and the major cause of dementia in

the Western world. The amyloid cascade hypothesis states that an imbalance between the generation and the removal of amyloid peptides is the ultimate cause of amyloid peptide aggregation, plaque formation, microgliosis, synaptic dysfunction and neuronal loss in the affected patients. BACE (beta-site amyloid precursor protein cleaving enzyme) is an aspartyl protease catalyzing the first of two required cleavages of APP (amyloid precursor protein) to generate amyloid peptides.

It has been shown that BACE enzymatic activity is absolutely needed for amyloid peptide generation, and BACE knockout mice are viable and show a very mild phenotype only. Therefore BACE represents an attractive therapeutic target for the treatment of Alzheimer's Disease.

Methods: Peptides related to the BACE cleavage site in APP served as starting point for our approach to find inhibitors of the enzyme. Optimization of the original structures resulted in potent compounds with biopharmaceutical properties suitable for in vivo testing in a transgenic mouse model.

Results: We could demonstrate that compound 1 reduced products of the amyloidogenic pathway in transgenic mouse brain after high iv doses, whereas compound 2 showed efficacy also after oral application.

These experiments showed that inhibition of BACE indeed results in reduction of amyloid generation, and identified some key issues to overcome for further improvement of BACE inhibitors.

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Inhibition of Glutaminyl Cyclase Reduces Pyroglutamyl-Abeta, Abeta and Improves Memory in Animal Models

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Abeta-depositions in brains of AD-patients preferentially consist of pyroGlutamyl-N-modified Abeta in contrast to the Abeta-composition found in non-demented, aged persons.

pGlu-peptides are potent seeds of Abeta(1-42) oligomerization and are neurotoxic. Such pGlu-peptides are exclusively formed by Glutaminyl Cyclase (QC, EC 2.3.2.5). Previously, we found in vitro and in situ that QC-inhibitors prevent generation of Abeta(N3pGlu) and Abeta(N11pGlu).

Consequently, the enzyme inhibitor P150/03 was used to elucidate whether QC-blockage reduces (i) formation of Abeta(N-pGlu)-peptides in vivo, (ii) total Abeta-deposition and (iii) memory loss of transgenic (tg) animals.

First, we investigated the impact of P150/03 in a traumatic brain injury (TBI) rat model, injecting pharmacological doses of Abeta(1-40) or Abeta(3-40) into the deep cortex of hemispheres in presence or absence of inhibitor.

Second, young swAPP-tg mice were treated orally for 6 month with different doses of P150/03, placebo and ibuprofen.

Third, aged swAPP-tg mice underwent behavioral analysis after treatment for 5 month with different doses of P150/03.

In general, the application of the QC-inhibitor reduced the formation of Abeta(N3pGlu) in all models, but also diminished the total Abeta-load in young, 6 months treated swAPP-tg mice.

Although, the impact of P150/03 on total Abeta was only marginal in aged swAPP-tg mice, there was a statistically significant improvement of contextual memory in a conditioned fear test setting.

Thus, we conclude a treatment paradigm based on QC inhibition reducing proteolytically stable and highly amyloidogenic Abeta(N-pGlu) species may be beneficial also in humans diminishing the total Abeta-burden and may improve memory in the patients.

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Small Heat Shock Proteins Colocalize With The Pathological Lesions of Hereditary Cerebral Hemorrhage With Amyloidosis (Dutch) and Induce Cytokine Secretion

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Background: Hereditary cerebral haemorrhage with amyloidosis of the Dutch type (HCHWA-D) is characterized by an E22Q mutation within the amyloid-β protein (Aβ), which generates a toxic form of Aβ that results in severe cerebral amyloid angiopathy (CAA) and diffuse senile plaques (SPs). Small heat shock proteins (sHsps) are molecular chaperones that are associated with SPs and CAA in Alzheimer's disease (AD). Although sHsps have a high homology, interaction with Aβ strongly depends on 1) the specific sHsp, 2) the aggregation state of Aβ and, 3) the specific Aβ peptide.

The aim of this study was to investigate association of sHsps with pathological lesions of HCHWA-D brains, and the role of sHsps in local inflammatory processes observed in AD and HCHWA-D.

Methods and Results: Using immunohistochemistry, we showed association of Hsp20, HspB8 and HspB2 with CAA in HCHWA-D brains, whereas both sHsps were only found in SPs in AD. Surprisingly, sHsps that are present extracellularly, such as Hsp20, HspB2 and HspB8, induced interleukin 6 (IL-6) production in cultured pericytes and astrocytes, whereas this effect was absent for αB-crystallin, Hsp27 and Aβ1-40, Aβ1-42 and Aβ1-40 with the Dutch mutation. In addition, IL-6 production induced by these sHsps was antagonized by dexamethasone, but not by cyclooxygenase (COX-1 and COX-2) inhibitors.

We conclude that specific sHsps species may be involved in the of SP and CAA pathogenesis in AD and HCHWA-D, and that sHsps associated with these lesions, rather than Aβ,may be key mediators of the inflammatory response in AD and HCHWA-D.

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All-Trans-Retinoic Acid as a New Modulator of Secretases

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Background: All-trans-retinoic acid (ATRA) is known to control cellular growth and differentiation processes, is involved in synaptic plasticity and learning and has been shown to modulate alpha- and beta-secretases at the levels of transcription, translation and activity. By influencing secretases and therefore affecting amyloidogenic pathways, ATRA could be of interest for further investigation in respect to Alzheimer's Disease.

Objectives and methods: Examination of the effect of ATRA on transcription, translation and activity of alpha-, beta- and gamma-secretases using human (IMR-32) and murine (N2a) neuroblastoma as well as human embryonic kidney (HEK-293) cell lines.

Results: ATRA-induced differentiation in IMR-32 but not in N2a cells correlated with increased activity and translocation of PKCalpha and PKCbetaII and showed a similar profile IMR-32 and HEK-293, but not in N2a cells. Additionally, we found increased DNA-binding activity of the transcription factors NF-kB in all cells, but increased AP-1 activity in N2a cells only. Furthermore, we found up-regulation of RAR-RXRbeta DNA-binding activity in all cell lines that, however, neither occurred through PKCalpha nor PKCbetaII.

Whereas in IMR-32 and HEK-293 cells levels of alpha- and beta-secretases increased upon ATRA-treatment, they remained largely unchanged in N2a cells. These data correlated with differing translocation patterns of ADAMs and BACE I. Expression and location of Presenilin 1 and 2, however, were not affected by ATRA.

Conclusions: We conclude that the ATRA-regulated balance of secretases shows a tendency towards the anti-amyloidogenic pathway, which is cPKC- and cell type-dependent.

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Intraperitoneally Administered Pentapeptide Defends Against Abeta1-42 Induced Neuronal Excitation in Vivo

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Amyloid-beta peptides (Aβ1-40, and Aβ1-42) have determinative role in the pathogenesis of Alzheimer's disease (AD). Inhibition of the misfolding and aggregation of soluble Aβ species by peptide based molecules seems to be promising in the therapy of AD. In our former study, several pentapeptides proved to be protective against the N-methyl-D-aspartate (NMDA)-response enhancing effect of Aβ1-42 in vivo. One of them, the most effective Leu-Pro-Tyr-Phe-Asp-amide (LPYFDa) was tested in this work under other circumstances, because it was administered intraperitoneally, not directly into the brain.

We have utilized in vivo extracellular single-unit electrophysiology combined with iontophoresis on hippocampal CA1 neurons of Wistar male rats. LPYFDa was able to block the Aβ1-42 induced neuronal excitation. A temporal window have been observed, within the compound was effective. We should assume that LPYFDa penetrates the blood-brain barrier (BBB) and resists to proteolytic enzymes.

We applied blood-brain barrier permeability study with tritium-labeld LPYFDa in order to visualize, that the peptide reaches the brain. It showed, that [3H]-LPYFDa readily crosses the BBB. These data shows that peptide based molecules may serve as leads for the design of drug candidates for the therapy of Alzheimer's disease.

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Design, Synthesis and Study of Peptides Against Protein Misfolding Diseases: A Proteomic and Algorithmic Approach

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Protein misfolding diseases are caused by the folding of proteins into self-associating structures resulting in high resistance to degrading enzymes and consequently, accumulation. The assembly of the 42-43 amino acid long beta amyloid peptide into such structures is a typical pathological hallmark of Alzheimer's Disease (AD). Our preliminary proteomic studies have revealed that the fibrillar beta amyloid 1-42 peptide binds to a huge number of proteins derived from rat synaptosomal membrane fractions. Based on these results we aimed to find similar subsequences of the beta amyloid-binding proteins by mathematical methods, and use them as a basis set in the design of new, putative neuroprotective compounds against AD. We developed a new computer program which applies a novel and efficient algorithm to examine the possible subsequences according to their similarity. To measure this feature of the subsequences, we utilized the widely known BLOSUM amino acid scoring matrix which is based on statistical observations of highly conserved regions of proteins. This mathematical method resulted in a frequency table giving the occurrence of every natural amino acid in each position of the subsequence. The most frequent amino acids were combined into pentapeptide sequences and synthesized by solid phase peptide synthesis. The effectiveness of the pentapeptides was studied by in vitro viability test (MTT) and both in vitro and in vivo electrophysiological methods; two of them exhibited outstanding protecting activity and can serve therefore as lead compounds in rational drug design against AD.

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Changes in Ex-Vivo Gamma Secretase Activity and Brain and Plasma Abeta40 and Abeta42 in Rats Following Administration of BMS-299897

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Reduction of brain beta amyloid by inhibition of its formation by gamma secretase is a widely favoured strategy for the disease modifying treatment of Alzheimer's disease. The gamma secretase inhibitor BMS-299897 has been shown to reduce Abeta40 and Abeta42 in the brain and plasma of APP over-expressing mice and Abeta40 in the brain, plasma and CSF of guinea pigs.

In this study we have acutely dosed male Sprague-Dawley rats with BMS-299897 (30mg/kg, intraperitoneally) and profiled changes over time in ex-vivo gamma secretase activity and brain and plasma Abeta40 and Abeta42.

Gamma secretase activity, measured by conversion of C100 to Abeta40 by brain homogenates, was decreased from 30 to 90 minutes in BMS-299897-treated rats compared to vehicle-treated rats.

Brain Abeta40 was reduced within 30 minutes of dosing and the reduction was sustained for 15 hours after dosing. In plasma there was total clearance of Abeta40 at 30 minutes, but a return to the baseline level by 3 hours.

In contrast, following an initial reduction at 30 minutes, plasma Abeta42 was substantially elevated from 1.5 to 6 hours after dosing. Brain Abeta42 was also elevated from 3 to 6 hours after dosing.

These data show that the effects of inhibiting gamma secretase may differ between model organisms and highlight the complexities of the mechanisms of Abeta40 and Abeta42 clearance and transport between tissue compartments.

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Resveratrol Ameliorates Amyloid-Beta 1-42 Induced Neurodegeneration Through Enhacement of Antioxidative and Anti-Inflammatory Properties

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Background and aims: Amyloid beta-peptide 1-42 (Aβ1-42) has strongly been implicated in neuroinflammation, immune activation and oxidative stress and hence has been considered to be central to pathogenesis of Alzheimer's disease (AD). Resveratrol (trans-3,5,4′-trihydroxystilbene, RSVL), a naturally occurring polyphenol synthesized by a variety of plant species, and present at high level in red wine, has been reported to possess antioxidative, anti-inflammatory and antimutagenic activities. Recent epidemiological studies have shown that moderate red wine consumption is significantly correlated with a reduction in the incidence of age-related macular degeneration, AD and stroke. Herein, we examined whether RSVL could alleviate the neurotoxic consequences of exposure of cultured neuronal and microglial cells to Aβ1-42.

Methods: We investigated the neuroprotective effect of RSVL on Aβ1-42-induced neurotoxicity and reactive oxygen species in murine hippocampal HT22 cell line. Also, we tested if RSVL attenuates microglial activation by lipopolysaccharide in BV2 microglial cell line.

Results: RSVL significantly attenuated the ROS production and neurotoxicity induced by Aβ1-42 in HT22 cells. It also potently suppressed the production of pro-inflammatory cytokine tumor necrosis factor-alpha and interleukin-6 in BV2 cells.

Conclusions: Based on these findings, the authors speculate that RSVL or its related compounds may provide an effective means of treatment for AD through attenuation of Aβ-induced oxidative stress and/or neuroinflammation.

Acknowledgement: This work was supported by the Second-Phase of BK(Brain Korea) 21 Project in 2006.

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A Novel Thiophene Sulfonamide Inhibitor of APP Gamma Secretase With Minimal Activity at Notch Receptors

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Gamma secretase is responsible for the intra-membranous cleavage of the Alzheimer's Precursor Protein (APP), the Notch receptor, and several other substrates. While inhibition of this protease results in potentially therapeutic reductions in the levels of neurotoxic Aβ peptide, severe side effects might result from inhibiting Notch processing. We previously identified the Notch-sparing gamma secretase inhibitor (GSI), 4-chloro-N-[(1S,2S)-1-(hydroxymethyl)-2-methylbutyl]-benzenesulfonamide by HTS screening. Optimization of the potency of this lead by SAR studies afforded the Notch-sparing GSI 5-chloro-N-[(1S)-2-ethyl-1-(hydroxymethyl)butyl]thiophene-2-sulfonamide, a novel GSI that selectively inhibits cleavage of APP. This compound inhibits Aβ production in vitro and displaces reference GSIs from enriched gamma-secretase enzyme preparations with low nM potency. Cellular assays of Notch function and fragment generation reveal that this compound is selective for the inhibition of APP cleavage. Parenteral administration of this compound in guinea pig causes a dose-dependent accumulation of the gamma-secretase substrate Ct-99 and reductions of total Aβ in the brain. In the Tg2576 transgenic mouse, this compound causes a robust reduction in brain Aβ levels and reverses memory deficits that are correlated with Aβ load. These data suggest that this GSI is a potent and selective therapeutic moiety for AD.

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Design, Synthesis and Pharmacological Evaluation of Some 4-Nitrophenol Aryloxy Derivatives as Potential Drugs for Alzheimer's Disease

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Anti-amnesics are a heterogeneous group of compounds of diverse chemical composition and biological function that facilitate learning and memory or overcome natural or induced cognitive impairments. Supporting the cholinergic theory of Alzheimer's disease (AD) the treatment of AD has been dominated by the use of acetylcholinestrase inhibitors (AChEIs). These drugs compensate for the death of cholinergic neurons and offer symptomatic relief by inhibiting acetylcholine (ACh) turnover and restoring synaptic levels of this neurotransmitter. The only FDA-approved drugs for treating AD include indirect cholinomimetic agents with the pharmacological profile of AChEIs (tacrine, donepezil, galanthamine and rivastigmine). An introspection of these compounds reveals the correlation of the compounds with the structure of endogenous neurotransmitter ACh and is considered in postulating the design strategy for the compounds included in the current research work.

Very few literature references are available on the antiamnesic activity of compounds possessing the nitro group. The present research work was designed to synthesize a series of five such compounds with the insertion of p-nitrophenol group. Pharmacological evaluation was done using elevated plus maze model and biochemical evaluation using Ellman's method. Results show that these drugs inhibited AChE activity in a dose dependent manner. The compounds have shown significant antiamnesic activity as compared to reference drugs. This research has paved the way to continue search for such new more effective cognition enhancers to develop drugs with novel pharmacological profiles and maximal therapeutic benefits.

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Investigation of the Role of ADAM Family Members on Ectodomain Shedding of BACE1

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Alzheimer's disease (AD) affects over 18 million people and is characterised by neuritic plaques in the brain, comprised mainly of amyloid β peptide (Aβ) and intracellular neurofibrillary tangles. Aβ is produced by sequential processing of amyloid precursor protein (APP) by β- and γ-secretases, respectively. β–secretase has been identified as BACE1 (β-site APP cleaving enzyme-1), a type-I membrane-associated aspartyl protease. As the rate limiting step in Aβ production, the cellular mechanisms regulating BACE1 are of interest. Like many membrane proteins, BACE1 is subject to ectodomain shedding by an as yet unidentified protease. Phorbol ester stimulation and inhibition by hydroxamic-acid based inhibitors are characteristic features of ectodomain shedding by the ADAM (a disintegrin and metalloprotease) family of metalloproteases. GXW4023 and GXW0264 are ADAM10 selective and equipotent ADAM10 and TACE/ADAM17 hydroxamic-acid based inhibitors, respectively. Treatment of HEK293 cells stably expressing BACE1 with the ADAM10 selective inhibitor showed a greater reduction in BACE1 ectodomain shedding compared to the general inhibitor. RNA interference knock down of endogenous TACE resulted in a 60% reduction of TACE levels, however, no effect on BACE1 shedding was observed. The role of ADAM10 and other catalytically active ADAM family members in the ectodomain shedding of BACE1 are being investigated, in order to gain insight into the cellular mechanisms involved in the control of this key enzyme.

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Nmr Studies of the Binding of Neuroprotective Pentapeptides and Thioflavine-T to Beta Amyloid 1-42 in a Controlled State of Aggregation

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One possible treatment against protein misfolding diseases, like Alzheimer's Disease, is the application of aggregation inhibitors (AI) to inhibit the formation of misfolded protein conformations, or more probably to counteract the harmful effect of the neurotoxic protein aggregates by covering their surface. Therefore, it is crucial to characterize the synthesized AIs with respect to their ability to bind to the protein aggregates.

Here we present a simple and fast method to determine the binding affinity of several pentapeptides exclusively to beta amyloid 1-42 fibrils. The peptides are proven to be efficient neuroprotective agents against beta amyloid 1-42 mediated neurotoxicity both in in vitro viability assays and in vivo electrophysiology experiments. The method is based on the 1H-NMR spectroscopic monitoring of the change in the signal of the binding substance during the course of the experiment. The samples contain pre-formed beta amyloid 1-42 fibrils prepared carefully under standardized circumstances, and the pentapeptides in determined molar ratio.

Moreover, the binding of Thioflavine-T in the presence of the pentapeptides could also be studied with this method. On the basis of our results we can conclude that great care must be exercised when applying the widely used spectrofluorometric assay for the characterization of the efficiency of the AIs against aggregated beta amyloid 1-42, because the binding equilibria of the dye and the AI can possibly interfere with each other.

The elaboration of the methodology for beta amyloid oligomers is currently underway in our laboratory.

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Analysis of Neprilysin (NEP) Phosphorylation

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Background: Brain amyloid ß-peptide (Aß) levels are determined, on one hand, by its proteolytic generation from the ß-amyloid precursor protein (ßAPP) through the combined activities of ß- and γ-secretase. On the other hand, the degradation of Aß is mediated by several zinc metallo-proteases. In particular, the type II membrane protease neprilysin (NEP) has been shown to play an important role in Aß catabolism. NEP undergoes posttranslational maturation by glycosylation of its lumenal domain containing the active site. In addition, it has also been shown that NEP can be phosphorylated in vitro by casein-kinase-2.

Objective: To investigate whether NEP contains additional phosphorylation sites and the kinases involved.

Methods: In in vivo/vitro experiment the incorporation of [32P]-phosphate was analysed. Hek293 cells, stably expressing myc-tagged NEP, and GST-fusion-proteins, containing the N-terminal cytoplasmic domain of NEP, employed for the respective experiments.

Results: In Hek293 cells, the mature form of NEP is preferentially phosphorylated. This effect can be augmented by treatment with okadaic-acid, a protein-phosphatase-2A and -1 inhibitor. Specifically, the N-terminus seems to be phosphorylated since in vitro experiments with recombinant N-terminus showed strong phosphate incorporation by protein-kinase-A (PKA) and casein-kinase-1 (CK1).

Conclusion: Since protein phosphorylation takes place predominantly in the cytosolic compartment and the N-terminus is phosphorylated in vitro by CK1 and PKA, it is likely that the N-terminus is phosphorylated in vivo. This phosphorylation could be involved in the regulation of maturation, trafficking and stability of NEP thereby affecting Aß degradation.

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Stress in Adulthood Partially Improves Early Alzheimer's-Like Changes in Double Transgenic TASTPM Mice. 1: Short-Term Memory, Amyloid and Endocannabinoids Changes

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Stress in adulthood contributes to the later development of Alzheimer's disease (AD). We assessed the effects of adulthood stress on the development of early AD-related pathological markers in double transgenic mice overexpressing amyloid (TASTPM mice). A first group of 4 month-old TASTPM and C57Bl/6 mice was assessed for the acquisition, short-term memory and extinction of contextual fear conditioning (CFC). A second group of 4 month-old TASTPM and C57Bl/6 mice was subjected to 5 weeks of repeated psychological stress (novel cage) and then compare with unstressed mice for performance in the CFC. In all mice, levels of soluble and unsoluble amyloid and levels of endocannabinoids were measured in selected brain areas in the hippocampus and frontal cortex. We found that repeated novel cage stress prevented the onset of short-term memory deficit seen in 5,5 months old TASTPM mice, without reversing the deficit in extinction already seen at 4-month old. Regional levels of soluble and insoluble amyloid were reduced by stress in TASTPM, but in a region-dependant manner. However, chronic stress did not influence the age-related increase in regional brain endocannabinoids levels seen between 4 and 5.5 months in TASTPM mice. The role of the endocannabinoid system in the early stages of AD-like pathology needs to be further investigated. These results show that environmental manipulations can partially delay the onset of Alzheimer's like pathology and their use can lead to the identification of novel therapeutic targets.

Supported by a UoN BRC strategic fellowship to MCP and GlaxoSmithKline who provided the mice

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Stress in Adulthood Partially Improves Early Alzheimer's-Like Pathology in Double Transgenic TASTPM Mice. 2: Long-Term Memory, Plaque Load, MRI Markers

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Stress in adulthood contributes to the later development of Alzheimer's disease (AD). We assessed the effects of adulthood stress on the development of early AD-related pathological markers in double transgenic mice overexpressing amyloid (TASTPM mice). 4 month-old TASTPM and C57Bl/6 mice were subjected to 5 weeks of repeated psychological stress (novel cage) after which they were assessed for the long-term memory and extinction of contextual fear conditioning (CFC; acquisition prior to the stress exposure). In vivo MRI at 7T was then used to determine changes in regional brain volumes and in regional T2 relaxation times. The presence of amyloid plaques in the brain was assessed using immunohistochemistry. Long-term memory was preserved in 5.5 month old TASTPM and unaffected by stress in both mouse lines. The volume of the hippocampus and posterior cingulate cortex did not differ in TASTPM or with stress. Our first results on plaque load indicate a reduction with stress in the hippocampus of TASTPM mice. T2 relaxation time is sensitive to the presence of amyloid, therefore, we expect that changes in this MRI marker will predict changes in amyloid pathology (MRI data under analysis). Together our studies show that repeated novel cage stress in adulthood can partially improve early AD-like changes in TASTPM, allowing for the development of sensitive early markers of the pathology.

Supported by a UoN BRC strategic fellowship and Research Imaging Fund to MCP and GlaxoSmithKline who provided the mice

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Immune Complexes of Auto-Antibodies Against Abeta1-42 Peptides in Cerebrospinal Fluid

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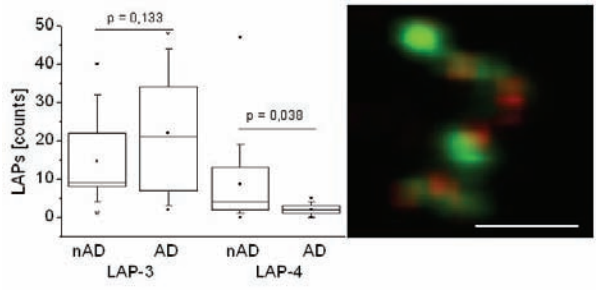
Cerebrospinal fluid (CSF) of Alzheimer's dementia (AD) patients and non-AD controls contains large Abeta-peptide binding particles (LAPs). LAP frequency showed high inter-individual variation but were not confined to one of the two groups. Molecular imaging by confocal microscopy revealed that LAPs are heterogeneous super-aggregates that could be subdivided morphologically in 4 main types (LAP1-4). LAP-4 type, resembling a "large chain of pearls", was detected in 42.1 % of all non AD controls but it was virtually absent in AD patients. LAP-4 type could be selectively removed by protein A beads, a clear indication that it contained immune globulins in addition to Abeta1-42. We observed a close correlation between LAPs and IgG concentration in CSF in controls but not in AD patients. Double labeling of LAPs with anti-Abeta, and anti-IgG antibodies confirmed that LAP type 4 consisted of Abeta and IgG aggregates. Our results assign a central role to the immune system in regulating Abeta 1-42 homeostasis by clustering this peptide in immuncomplexes.



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Morphological Changes in Hippocampal and Striatal Neurons in Tg2576 Mice: A Possible Plastic Mechanism Supporting Enhanced Procedural Learning

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Alzheimer's Disease progressively impairs declarative and spares procedural memories. A sequence of synaptic changes compromises the functional capacity of neuronal networks inducing the cognitive deficit. The A synaptic hypothesis (Tanzi, 2005) suggests the pathogenic role of non-fibrillar A oligomers specifically acting at the synapses.

In Tg2576 mice (Hsiao et al., 1996), the progressive fibrillization of A peptide induce age-dependent cognitive and pathological alterations. We previously reported a cognitive impairment in declarative memory (Middei et al., 2006) and a parallel enhancement of procedural memory, in aged (15-months old) Tg2576 mice. We also reported an intact LTD in cortico-striatal pathway of these mice (Middei et al., 2004) suggesting that striatal functions may compensate for hippocampal alteration induced by AD pathology. To further investigate this hypothesis, we here analyze the time course of synaptic changes in these mice. We suggest that loss of hippocampal synapses over time might trigger a compensatory response in the striatal area of Tg2576 inducing changes in the number and functionality of striatal synapses.

Using a Golgi Cox staining technique, we performed a quantitative analysis of dendritic spines number, length and head area in hippocampal CA1 pyramidal neurons and Dorso-lateral-striatum (DLS) spiny neurons of young (3 months) and old (15 months) Tg2576 and wild type mice.

We report an early synaptic dysfunction in Tg2576 mice selectively affecting hippocampal areas. In parallel with this hippocampal alteration, we also show a compensative increase of synaptic number and, presumably, activity in DLS area.

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The Role of Hypoxia in Amyloid Precursor Protein Processing in Transgenic Mice

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Recent studies reveal that hypoxia is associated with pro-amyloidogenic amyloid precursor protein (APP) processing. Here we report that repeat hypoxia possesses a significant impact on hippocampal amyloid (A-beta) levels and amyloid plaque formation in double transgenic mice (A/P) that carry both mutated APP (APPswe) and presenilin-1 (PS1-A246E). We treated AD mice (9 months, female) with repeated acute hypoxia.for 60 days and found that hypoxia can increase the expression of APP and A-beta production. Western blot showed that hypoxia can increase the expression of Beclin1 and reduce the protein level of Cathepsin D. It has been reported that Lysosomal protease cathepsin D is associated with Alzheimer disease (AD) and Beclin1 is a marker of macroautophagy, both of which are involved in the A-beta production. Our studies provide evidence that hypoxia can affect the expression and processing of APP and suggest that hypoxia a risk factor for AD. Further study is required to quantitatively determine the plaque formation in hippocampus.

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BGC20-1259 Protects Against Cell Death Induced by Serum Deprivation in Chick Cortical Cultures

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BGC20-1259, a multi-functional compound with both acetylcholinesterase and serotonin reuptake inhibiting properties (RS-1259, Abe et al., J. Pharmacol. Sci., 2003) is in clinical development for Alzheimer's Disease. Its ability to protect against neuronal cell death and increase neurite outgrowth was investigated using primary cultures of embryonic chicken cortical neurons. Cells were incubated in the presence of either BGC20-1259, the acetylcholinesterase inhibitor rivastigmine or the serotonin reuptake inhibitor citalopram. The number of viable, apoptotic and necrotic cells and degree of neurite outgrowth were then determined using a range of endpoints under control conditions (5% foetal calf serum FCS supplementation) and under low serum conditions (2% FCS), either in the absence or presence of staurosporine (1 uM) to promote apoptosis.

Under control conditions, all three compounds had no effect on neuronal viability up to 50 uM. Low serum conditions markedly induced neuronal cell death. Under these conditions, BGC20-1259 was neuroprotective and increased neurite outgrowth in a concentration-dependent manner (100 nM - 10 uM), to a significantly greater extent than rivastigmine and citalopram when these agents were given either alone or in combination.

BGC20-1259 also protected against staurosporine-induced apoptosis in a concentration-dependent manner. In contrast, rivastigmine and citalopram were ineffective.

These data indicate that BGC20-1259 is able to protect against neuronal cell death induced by both low serum stress and staurosporine. The ability of BGC20-1259 to inhibit acetylcholinesterase and serotonin reuptake is unlikely to explain the novel neuroprotective action of this compound.

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BGC20-1259 Protects Against Neuronal Cell Death Induced by ß-Amyloid 1-42 and Free Radicals

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The ability of BGC20-1259, a multi-functional compound with both acetylcholinesterase and serotonin reuptake inhibiting properties, (RS-1259, Abe et al., J. Pharmacol. Sci., 2003) to protect against neuronal cell death was investigated using chick neuronal cultures and rat hippocampal slice cultures. Telencephalic neurones from chick embryos were cultured in the presence of BGC20-1259, then exposed to a variety of neurotoxic insults (L-glutamate, MPP+, ß-amyloid1-42). The number of viable cells was determined using the MTT assay. Additionally, the ability of BGC20-1259 to protect against free-radical toxicity induced by duroquinone (100 µM for 3 hr) was investigated in rat hippocampal slice cultures using propidium iodide fluorescence imaging. Effects of BGC20-1259 were compared with the acetylcholinesterase inhibitor donepezil.

In control chick neuronal cultures, neither BGC20-1259 nor donepezil affected cell viability per se up to 50 µM. All three lesions induced appreciable neuronal toxicity. BGC20-1259 (0.5 and 1 µM) increased cortical cell viability in cultures exposed to ß-amyloid1-42 compared to the lesioned control. Donepezil also offered some protection but only at higher concentrations (5 - 100 µM). In contrast, neither BGC20-1259 nor donepezil were found to protect against L-glutamate or MPP+-induced cell death.

However BGC20-1259 (1 and 10 µM) protected neurons in CA1 area of the hippocampus against duroquinone-induced free radical damage, whereas donepezil (5 µM) was ineffective.

These data indicate that BGC20-1259 is able to protect against ß-amyloid1-42 neurotoxicity and free-radical stress which may be unrelated to the ability of BGC20-1259 to inhibit acetylcholinesterase.

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Structure-Based Design of Acylguanidine BACE1 Inhibitors

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Alzheimer's disease is a progressive neurodegenerative disease characterized by gradual and increasing loss of cognitive function and behavioral abnormalities. The formation of beta-amyloid plaques and neurofibrillary tangles are recognized as the key pathologies of the disease. Changes in the levels of various key neurotransmitters has been noted in patients with Alzheimer's disease and may represent the earliest biochemical casualty, preceding or signifying the onset of the disease. Increasing evidence implicates the amyloid beta-peptide (AB, 39–43 residues) in the neurodegenerative pathogenesis of AD. AB is liberated in vivo through sequential proteolytic cleavage of the membrane-bound beta-amyloid precursor protein (APP) by beta- and gamma-secretases. Inhibition of secretases responsible for AB formation may stop or slow the progression of AD by preventing production of these toxic peptides.

The design and synthesis of potent and selective inhibitors of beta-secretase (BACE1) was based on an HTS campaign which identified several novel hits. Among these, WY-25105 (IC50 = 4 uM; BACE1) was the basis for a strategy supported by X-ray structures of BACE1 co-crystallized with various ligands. Further optimization utilizing molecular modeling and traditional medicinal chemistry SAR allowed for the identification of structurally unique, non-peptide, highly potent (IC50 = 50-100 nM) BACE1 inhibitors. Several examples have demonstrated good activity in cell-based assays, and this poster will highlight the results of this work.

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Neuroprotective and APP Processing Mechanism of Action of the Bifunctional Anti-Alzheimer Drug, Ladostigil

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The novel therapeutic approach, in which drug candidates are designed to possess diverse pharmacological properties acting on multiple targets, has been employed in the design of the bifunctional drug, ladostigil. This drug combines the neuroprotective and monoamine oxidase inhibitory effects of the anti-Parkinson drug, rasagiline, with the cholinesterase inhibitory moiety of the anti-Alzheimer drug, rivastigmine, in a single molecule. This study assessed the dual effects of ladostigil in terms of molecular mechanism of neuroprotection and amyloid precursor protein (APP) regulation, using an apoptotic model of neuroblastoma SK-N-SH cells. Ladostigil dose-dependently decreased cell death via inhibition of the cleavage and activation of caspase-3 through a mechanism related to regulation of the Bcl-2 family proteins, resulted in reduced levels of Bad and Bax and induced levels of Bcl-2 gene and protein expressions. Additionally, ladostigil elevated the brain-derived neurotrophic factor (BDNF) and the glial cell line-derived neurotrophic factor (GDNF) gene expressions. Next, we have followed APP processing and revealed that ladostigil markedly decreased apoptotic-induced levels of holo-APP protein without altering APP mRNA levels, suggesting a post-transcriptional mechanism of action. In addition, ladostigil elevated phosphorylated protein kinase C levels and stimulated the release of the non-amyloidogenic alpha-secretase proteolytic pathway. Similar neuroprotective effects were observed with hydroxyaminoindan, a metabolite of ladostigil. These findings suggest that hydroxyaminoindan may have a role in the neuroprotection activities shown by ladostigil and may contribute to its overall activity, thus providing new perspectives for ladostigil as a potentially valuable drug for the treatment of Alzheimer's disease.

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Effects of Cholinesterase Inhibitors (ChEI) and Memantina Upon Verbal Fluency in Demented Patients

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Introduction: ChEI and memantine are the only drugs for specific treatment of Alzheimer Disease (AD) and other dementias. Studies has observed a better course of disease, but measured with global scales like ADAS-cog. However, the effects on specific cognitive domains are not well known.

Objective: Kwon the ChEI and memantine effects on semantic verbal fluency (SVF) (Isaacs B, et al 1973) in demented patients.

Material and methods: We collecting data on patients send for comprehensive neuropsychological assessment for diagnostic of dementia. The neuropsychological evaluation includes a SVF. All patients have an initial evaluation previous to treatment start and another evaluation after treatment start. A number of patients have a third evaluation some months later.

Results: 52 patients, women: 34 (65,4%), age: 78,3±5,93 years (58-88). Education level: 19 (36,5%) illiterate, 31 (59,6%), basic, 2 (3,8%) secondary or superior. Diagnostics: AD: 73.1%, Vascular: 3.8%, Mixed: 7,7%, Frontotemporal: 1,9 %; Mild cognitive impairment: 11,5%. Drugs: Galantamine: 46,2%; Donepezil: 38,5%; Rivastigmine: 11,5 %; Memantine: 1,9%; Combined treatment 3.8%.

Scores in SVF: First: 8,94 ± 2,92. Second: 9,42±4,32 (with treatment) (52 patients). Third: 7,75±3,41 (12 patients).

Interval between evaluations (in months): Between first and second: 9,81±5.34. Between treatment start and second: 7,46±5.81. Between second and third: 8,63±7,04. Between first and second: 38,5% gets worse, 19,2% equal, 42,3% better score.

Conclusions: Improvement in SVF score in 42,3%.

Improvement in SVF scores between first and second evaluation, after 7 months of treatment.

Worsening SVF score between second and third evaluation after 16 months of treatment

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The Therapeutic Effect Comparision of Donepezil and Music Therapy for Dementia Patients by Means of Brain SPECT

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Eventhough nonpharmacologic neuronal stimulation such as music in patients with dementia has been tried, the therapeutic effect comparison with donepezil and music therapy is rare. Our aims were to assess the effect of music therapy and donepezil, respectively and combination therapy of two by means of Brain SPECT for patients with dementia.

We assessed 30 patients with Alzheimer's and vascular dementia. Among them, twenty patients received well designed Music therapy (MT) 4 times per week for 2 months and donepezil treatment, and 10 patients received donepezil treatment only. We evaluated devised short-formed clinical assessment tools included several tests related to language and memory functions, visuospatial function for music and donepezil therapy group and donepezil only group. We also evaluated brain perfusion single-photon emission tomography (SPECT) in both group. In MT and donepezil group, phonemic word fluency was significantly improved ($p<0.005$) with improvement in MMSE and semantic word fluency. we found a strong positive association between perfusion in combination therapy group than donepezil only.

Our results suggest that the combination therapy of donepezil and music therapy is more effective than donepezil only trial on cognitive function and activity of daily living of dementia patients. The combination therapy of Music and donepezil improve phonemic fluency and executive functions, especially on frontal cognitive functions.

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Behavioural Effects of Memantine: Understanding the Pharmacological Rationale

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Behavioural symptoms are a significant problem in Alzheimer's disease (AD). Symptoms such as agitation/aggression and psychosis reduce patient quality of life, significantly increase the burden on caregivers, and often trigger nursing home placement. However, existing behavioural treatments have been associated with safety concerns, and the FDA has issued warnings for the use of atypical antipsychotics in dementia patients, due to increased mortality risk. The NMDA receptor antagonist, memantine, is indicated for the treatment of moderate to severe AD, and has been shown to improve behavioural aspects of AD, including agitation/aggression, and delusions. Furthermore, memantine has a beneficial effect on cognition and function in AD, and displays a favourable tolerability profile. Therefore, it is relevant to investigate the underlying mechanism linking memantine with the behavioural elements of AD. Frontal and cingulate cortices are proposed as regional substrates of agitation and aggression. One hypothesis proposes that memantine corrects dysfunctional glutamatergic neurotransmission in the frontal and cingulate cortices, thereby normalising pathways responsible for causing agitation. To this end, glutamatergic dysfunction occurs in many cortical regions of patients with AD, perhaps triggered by the glutamate elevation induced by beta-amyloid. However, to date, no study has examined this in relation to agitation/aggression. Agitation/aggression may be linked to the abundance of hyperphosphorylated tau protein in the frontal cortex. Memantine has been shown to reduce tau-phosphorylation via GSK-3b (kinase) or activation of PP2A, which might subsequently lead to reduced agitation. Further investigation into the action of memantine may help explain the process underlying behavioural symptoms in AD.

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Effects of H1 and H2 Histamine Agents and Nicotinic Cholinergic System on Memory Consolidation in Rats

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The cholinergic system plays an important role in learning, memory and attention. On the other hand, histamine plays an important role as a central neurotransmitter in the brain. Neuronal histamine is concentrated in the tuberomammillary nucleus of the posterior hypothalamus, with efferent varicose fibers to almost all parts of the brain. The actions of histamine appear to be mediated by three different types of receptors, which differ in pharmacology, localization and as to the intracellular response, which they mediate. Histamine receptors include postsynaptic histamine H1 and H2 receptors and presynaptic histamine. A number of studies have indicated that the histaminergic system is involved in many physiological functions or responses such as stress, feeding, drinking, aggression and sexual behaviors. There is evidence revealing that neuronal histamine plays an important role in learning and memory behaviors. In the present study, the effects of the histamine and cholinergic systems on memory retention in adult male rats were investigated. Post-training intracerebroventricular injections were carried out in all the experiments. Cholinoceptor agonist, nicotine (1-10 μg/rat) increased memory retention. Administration of histamine (5-20 μg/rat) reduced, but the histamine H1 receptor antagonist, pyrilamine (10-50 μg/rat), and the histamine H2 receptor antagonist, cimetidine (1-50 μg/rat) increased memory retention in rats. The histamine receptor antagonists attenuated the response to histamine. Histamine reduced the nicotine-induced enhancement. The histamine receptor antagonists enhanced the nicotine-induced response. It is concluded that histaminergic and nicotinic cholinergic systems have opposing effects on memory retention.

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Alpha7 nAChR Agonists With Pro-Cognitive and Neuroprotective Properties

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Nicotinic acetylcholine receptors of the alpha7 type have emerged as promising therapeutic targets for treatment of Alzheimer's disease owing to their involvement in cognition, memory and neurodegeneration. By screening chemical libraries in a functional FLIPR-based calcium assay employing the rat alpha7 nAChR stably expressed in a GH4C1 cell line, a novel chemical series of potent small molecule agonists of the alpha7 nAChR was discovered. Examples of this series were selective over alpha1, alpha4 nAChRs and 5HT-3 receptors and showed micromolar antagonistic activity at the alpha3 nAChR. The prototypic compound SEN-WAY-3 is presented in greater detail. In whole cell patch clamp recordings SEN-WAY-3 activated peak currents and maximal total charges similar to ACh and was therefore classified as full agonist with an EC50 of 2.3 micromolar. Effects on cognition were assessed in an animal model of short working memory (passive avoidance) and episodic memory (novel object recognition test). In both cognitive paradigms, SEN-WAY-3 reversed the amnesic effects elicited by scopolamine when acutely administered (i.p.) at a dose of 3 mg/kg. Neuroprotection was demonstrated in rats that were lesioned by quisqualic acid injection in the nucleus basalis magnocellularis (NBM). A sub-chronic treatment with 3 mg/kg of SEN-WAY-3 for 7 days (i.p.) significantly attenuated the decrease in the number of ChAT-positive neurons in this brain region. Taken together, this new series of alpha7 nAChR agonists demonstrates pro-cognitive and neuroprotective effects and may be useful for treating neurodegenerative diseases such as Alzheimer's disease.

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Prevention of the Worsening of Clinical Symptoms in Moderate to Severe Alzheimers Disease in Patients Treated With Memantine

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Alzheimer's Disease is a progressive neurodegenerative disorder, and while improvement is a desirable goal, preventing worsening of the clinical condition is a clinically relevant and realistic treatment outcome. In the moderate to severe stages of the disease, the rate of deterioration is highest and causes the largest burden to the patients, their families, and society. Data from 6 randomised, double-blind, placebo-controlled, 6 months studies were pooled in patients with moderate to severe AD (MMSE score <20). Clinical worsening was defined as those patients whose condition showed a worsening in three domains during the 6-month treatment period: a decline on the ADAS-cog or the SIB, and on the CIBIC-Plus, and on the ADL. Marked clinical worsening was defined as a decline of ≥4 points on the ADAS-cog or ≥5 points on the SIB, and a decline on the CIBIC-Plus, and on the ADL. A total of 1826 patients were included, 867 on placebo and 959 on memantine. There were no clinically relevant differences in demographics or baseline characteristics between treatment groups. Over the course of the 6-month treatment, statistically significantly more placebo-treated patients had clinical worsening compared to memantine. Of these, about twice as many placebo-treated patients had marked clinical worsening compared to memantine-treated patients (21% vs. 11%; $p<0.001$). In this population of moderate to severe AD patients, treatment with memantine was associated with preventing worsening of clinical symptoms in AD.

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Update of Memantine Safety in Short- and Long-Term Treatment of Mild to Severe Alzheimer's Disease

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Objective: Memantine, a moderate affinity, uncompetitive NMDA receptor antagonist, is approved in the US and Europe for the treatment of moderate to severe Alzheimer's disease (AD). The safety and tolerability of memantine was assessed in patients diagnosed with probable AD.

Methods: Short-term safety was assessed in 6 double-blind, placebo-controlled trials (placebo, n=1069; memantine n=1242; 24-28 weeks) of overlapping severity: 3 in mild to moderate AD (MMSE 10-23), and 3 in moderate to severe AD (MMSE 3-14). Long-term safety was assessed in open-label extensions to the double-blind trials (placebo-memantine, n=682; memantine-memantine n=723; 24-80 weeks). Safety parameters included adverse events (AEs), vital signs and clinical laboratory tests. Mean length of treatment in the open-label phase was 1 year.

Results: The six short-term trials revealed that no AEs occurred at a rate ≥5% and at an incidence of at least twice that of placebo. The profile of AEs was similar for both the mild to moderate and moderate to severe AD trials. The safety and tolerability profile in the long-term open-label studies was similar to that reported in the short-term studies. Most AEs were considered mild or moderate in severity and not related to memantine. No clinically relevant differences between memantine and placebo patients in vital signs or laboratory values were observed.

Conclusion: Short- and long-term treatment of AD with memantine is safe and well tolerated.

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Long-Term Safety and Efficacy of Memantine Treatment in Moderate to Severe Alzheimers Disease: Results From a Three-Year Trial

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This 134-week extension study evaluated the long-term safety and efficacy of memantine in moderate to severe Alzheimer's disease (AD) patients, as well as the tolerability of a shorter titration period and once-daily dosing. Patients from two 24-week, double-blind, placebo-controlled lead-in clinical studies (memantine monotherapy study MEM-MD-01; memantine/donepezil study MEM-MD-02) were enrolled in the extension, which consisted of a 4-week double-blind titration period (Phase A) followed by open-label maintenance periods B (24 weeks), C (52 weeks), and D (54 weeks). In Phase A, patients previously treated with memantine were either maintained on current memantine dosing (10 mg b.i.d.) or switched to 20 mg q.d.; patients previously treated with placebo were randomized to one of four groups to investigate two titration schemes (22-day vs. 8-day) and two dosing schemes (10 mg b.i.d. vs. 20 mg q.d.). In Phases B, C, and D, all patients were administered 10 mg b.i.d.. Efficacy was assessed using the Severe Impairment Battery (SIB) and compared to a projected placebo decline (Schmitt, 2002). Results: Long-term memantine treatment was associated with a significantly slower rate of decline on the SIB after one, two, and three years. There were no marked differences associated with b.i.d. versus q.d. titration. AEs were similar in type and frequency between groups, predominantly mild to moderate in severity, and judged unrelated to memantine. Overall, the most frequent AEs were agitation, fall, inflicted injury, and urinary tract infection. These analyses support the long-term efficacy and safety of memantine in the treatment of moderate to severe AD.

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Cognitive and Motors Functions of Demented Patients With Parkinson's Disease Treated by Memantine

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The study has investigated the effect of NMDA-glutamate receptor antagonist memantine on motor, cognitive and psychotic symptoms in prolonged therapy of 19 Parkinson's disease patients with dementia (PDD) compared with control PDD patients group (16 cases). METHODS: Motor, cognitive and psychotic symptoms were assessed at baseline, 3, 6, 12, 18, 24, 30 months of treatment period. Patients of active group received additionally memantine (20 mg per day) to dopaminergic therapy. Control group patients continued treatment only using dopaminergic therapy. Unified Parkinson's disease Rating Scale (UPDRS), Mini-Mental State Examination (MMSE), the Mattis dementia rating scale (MDRS) and Interview for Hallucination in PDD (IHPDD) used for efficacy estimation. All patients were treating with combined therapy (L-Dopa/carbidopa with pramipexole or piribedil) and no significant inter group differences on mean drugs doses, MMSE, Hoehn/Yahr, UPDRS, MDRS and IHPDD. RESULTS: The introduction of memantine beginning from 3 month of the study led to significant improvements in cognitive function (MDRS score, $p<0.05$) and from 6 month decrease psychotic symptoms (IHPDD score, $p<0.01$) compared to control group. These inter group differences continued during the total observation period. Because of dementia deterioration and psychotic symptoms in control group we had to discontinue dopamine agonists in 9 cases, reduced L-Dopa/carbidopa in 16 patients and introduce risperidone 1-2 mg/day with increase motors disabilities (Hoehn/Yahr, $p<0.01$; UPDRS, $p<0.001$ compared to memantine group). CONCLUSIONS: The prolonged therapy by memantine in advanced PDD led to improvements in cognitive functions and preservation of motors functional abilities, as well as the amelioration of psychotic symptoms.

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Treatment Benefits of Memantine in Moderately Severe to Severe Alzheimers Disease Patients With Behavioural Symptoms

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There are recent study results showing the behavioural benefits of memantine in AD and long standing evidence that behavioural disturbance is associated with an accelerated disease progression. To investigate this further, we examined the effects of memantine in patients with specific behavioural symptoms. A post-hoc subpopulation analysis was conducted on pooled data from 3 large 6-month studies of moderately severe to severe AD. Patients were included in the Behaviourally Disturbed Subpopulation (BDS) with baseline NPI sub-item scores of 1 or more on any of these items: agitation/aggression, delusions, or hallucinations. Of 983 patients, 593 (60%) met the BDS entry criterion. Compared to those without these symptoms at baseline, placebo-treated patients in the BDS showed greater decline across all outcomes. However, relative to placebo, there were statistically significant benefits for memantine for all outcomes in the BDS (SIB, CIBIC-plus, ADCS-ADL19 and NPI) at all visits (LOCF, OC). For patients without behavioural symptoms at baseline, statistically significantly fewer memantine-treated cases developed behavioural symptoms during the trials. Compared to placebo, memantine-treated BDS patients were statistically significantly less likely to withdraw, withdraw due to adverse events and withdraw due to agitation, aggression, or psychosis. Whilst these analyses were post-hoc, the results provide further support for the efficacy of memantine across all domains, in moderately severe to severe AD. Furthermore, they highlight the utility of memantine in the treatment and prevention of key behavioural symptoms, with potential safety advantages compared to other pharmacological treatments currently prescribed.

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Cognitive Function in Patientes With Alzheimer's Dementia and Concomitant Cerebrovascular Disease Treated With Galantamine - A One Year Open-Label Phase-Iiib Study

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Background: Galantamine has been demonstrated to be effective and generally safe in patients with Alzheimer's disease and cerebrovascular pathology (AD+CVD) in placebo-controlled trials. The aim of this open-label clinical trial (GAL-GER-5) was to study the long-term effects of galantamine on cognition in patients with AD+CVD.

Methods: Open-label, multi-center clinical trial (phase IIIb). Patients with mild to moderate AD+CVD (meeting NINDS-AIREN criteria) received galantamine (4-12 mg bid) for 12 months. Cognitive function was examined using the AKT ("Alters-Konzentrations-Test") and DemTect. Statistics were based on intent-to-treat population (LOCF, t-test and Wilcoxon-test for dependent samples).

Results: 84 patients (43% with mild, 56% with moderate AD+CVD; mean age±SD 75.5±6.8 years; 58% women) were enrolled. 80% of the patients completed the study. Modal daily galantamine dose was 16mg for 44%, and 24mg for 51% of the patients. After 12 months mean total score in AKT showed a stabilization from 49.0±6.7 (baseline) to 49.2±6.9 ($p=0.7807$) and DemTect increased significantly from 7.8±2.0 to 9.4±3.9 ($p<0.0001$). CGI demonstrated an improvement or stabilization for 71% of patients. 56% of the patients had at least one adverse event (AE). Most frequent AEs with an incidence >5% were nausea and vomiting. 8 patients discontinued due to AEs. 21 patients experienced a SAE with 4 SAEs considered as possibly related to study medication

(heart failure, syncope, aggravated dementia, urinary retention).

Conclusions: This open-label study supports evidence from placebo-controlled trials of the efficacy and safety of galantamine in patients with AD+CVD and suggests similar cognitive effects and safety through 12 months.

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Galantamine Therapy in Dementia Due to Parkinson's Disease

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Dementia and psychosis in Parkinson's disease are related with cholinergic deficit in the cerebral cortex. Cholinesterase inhibitors with additional nicotinic activity, such as galantamine, may be useful in PD patients with dementia (PDD) since stimulation of nicotinic receptors may prevent the down-regulation of dopamine and facilitate dopamine release in the striatum.

Materials/methods: Forty one PDD patients (21 - galantamine group, 20 – control group) with onset of dementia after at least two years with parkinsonism participated in this open-label controlled trial of galantamine with maximum dose 16 mg/day. Cognitive, psychiatric, and motor symptoms were assessed before and after 4, 12 and 24 weeks of treatment with galantamine using clinical assessment as well as rating scales including the Mini-Mental State Examination (MMSE), ADAS-cog, clock drawing test, Frontal Assessment Battery (FAB) and the Neuropsychiatric Inventory (NPI-12), including caregiver distress.

Results: Treated patients by galantamine had better MMSE ($p<0,05$), ADAS-cog ($p<0,05$), clock drawing test ($p<0,05$) and FAB ($p<0,01$) scores than did patients in control group. NPI individual item scores change from baseline at Week 12, 24 showed benefits with galantamine treatment compared with control with significant treatment differences for hallucinations ($p=0,0002$), anxiety ($p=0,04$), sleep disorders ($p=0,04$) and apathy ($p=0,006$). Galantamine therapy was associated with a significant reduction in caregiver distress ($p=0,007$), improvement activities of daily life ($p=0,003$). Gait, freezing and falls were improved in galantamine group, but a mild worsening of tremor was noted in two patients. Adverse event (drooling, postural hypotension, nausea, dysuria) observed in 7 (30%) galantamine treated patients.

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Alzheimers Disease -Safety and Dropout in Long-Term Galantamine Treatment in a Routine Clinical Setting

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Alzheimer's disease (AD) is the major cause of dementia in the elderly and is characterized by an insidious onset and a progressive decline in cognition and practical abilities. Multiple double blind, placebo controlled studies have shown beneficial effects of galantamine treatment on cognition and function. What to expect concerning safety and dropout in a routine clinical setting has not been investigated. The Swedish Alzheimer Treatment Study (SATS) is an ongoing prospective, open, longitudinal, multicentre study evaluating cholinesterase inhibitor (ChEI) treatment in AD. Patients are investigated at baseline, at 2 months and every 6 months for a total period of three years. In this poster we evaluate long-term outcome in terms of dropout and safety in the 278 galantamine treated patients (38% men and 62% females) included in SATS so far. At baseline the mean (±SD) age of the patients was 73.0 (±8.3) years, the duration of disease was 3.0 years, the MMSE score 23.1 (±4.2) and the ADAS-cog score 17.1 (±8.8). The mean dose of galantamine, the time until dropout and the cause of dropout were monitored. As well as dropout, the safety and survivial were investigated in this population of galantamine treated patients in the routine clinical setting.

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Treatment With Cholinesterase Inhibitor and Cholinergic Precursor Alone or in Association Protects Brain in a Model of Hypertensive Brain Damage

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Hypertension represents a main risk factor for stroke and vascular dementia (VaD). The higher susceptibility of hypertensive subjects to cerebrovascular accidents probably depends on the particular sensitivity of cerebral vasculature to hypertension. Cognitive impairment of vascular origin is accompanied by cholinergic dysfunction. Enhancement of deficient cholinergic neurotransmission is considered a strategy for treating adult-onset dementia disorders including VaD. Cholinesterase inhibitors (ChE-Is) and cholinergic precursors represent the only strategies of enhancement of cholinergic neurotransmission assessed in clinics.

The present study was designed to establish if treatment with the ChE-I galantamine (GAL) and with the cholinergic precursor choline alphoscerate (GPC) alone or in association has a cereproprotective effect on spontaneously hypertensive rats (SHR) used as an animal model of hypertensive brain damage.

36 week-old SHR and age-matched normotensive Wistar Kyoto (WKY) rats were left untreated or treated for 4 weeks with an oral dose of 3 mg/kg/day of GAL and GPC (150 mg/kg/day) alone or in association.

Treatment with GAL or GPC countered nerve cell loss, cytoskeletal breakdown and blood brain barrier damage in the frontal cortex as well as in the hippocampus. GAL did not affect hypertrophy and/or hyperplasia affecting respectively cerebrocortical and hippocampal astrocytes, whereas GPC countered astrogliosis. The two compounds in association elicited a more pronounced effect than if administered alone.

The observation that treatment both with GAL and GPC and to a grater extent by the two compounds together countered microanatomical changes occurring in SHR suggest that this association could be investigated for enhancing cholinergic neurotransmission in VaD.

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Six Years' Experience With Acetylcholinesterase Inhibitors in a Clinical Setting

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Background: Evidence for acetylcholinesterase inhibitors (ACIs) available consists mainly of trials conducted by pharmaceutical companies. There are substantial differences between participants in clinical trials and patients in normal clinical settings.

Aims: To evaluate the use of ACIs in a clinical setting.

Methods: Hospital records of 454 patients prescribed ACIs over a 6-year period were analysed. In our catchment area of 50,000 people over the age of 65 approximately 100 new patients per year received treatment with ACIs.

Results: The rate of decline on ACIs was less than half of that expected in an untreated population. A significant difference was found between patients with a baseline MMSE scores greater than 20 and those with a score between 10 and 20.

Conclusions: Our participants were older than in clinical trials and those with significant medical problems and relative contraindications were not excluded. ACIs are as useful in AD as they are in DLB/PDD and there were no differences between donepezil and rivastigmine. A baseline MMSE score of 20-10 might predict better response to treatment.

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Cognitive Effects of a Prolonged-Release Formulation of Galantamine (PRC) in Patients With Alzheimer's Disease (AD) - An Open-Label Phase-Iiib-Study

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Background: Randomized controlled clinical trials have demonstrated the efficacy of galantamine-PRC in the treatment of AD-patients. Objectives of this clinical trial were to further study the overall effect of galantamine-PRC on cognition and function in patients with AD.

Methods: Open-label, multi-center clinical trial (GAL-DEM-3002). Patients with mild to moderate AD (NINCDS-ADRDA criteria) received 16-24 mg/day galantamine-PRC for 6 months. Primary objectives were to examine the effects on cognitive function using ADAS-cog and DemTect. Response-rate at endpoint was defined as percentage of patients with change in ADAS-cog=/<0. Statistical analyses based on intent-to-treat population (LOCF, t-test, Wilcoxon-test for dependent samples).

Results: 133 patients (48% with mild, 52% with moderate AD; mean age±SD 75.4±7.8 years; 68% women) were enrolled with 71% of the patients completing the study. 53% of the patients received 24mg/day galantamine-PRC. After 6 months mean total scores changed significantly, both in ADAS-cog, from 23.3±9.3 (baseline) to 20.4±9.7 ($p<0.0001$) and DemTect from 7.3±2.9 to 9.2±4.3 ($p<0.0001$). The response-rate was 64.2%. CGI demonstrated an improvement or stabilization for 83% of patients. 64% of the patients had at least one AE. Most frequent AEs (>5%) were nausea, vomiting and headache. 28 patients discontinued due to AEs. 15 patients experienced a serious AE with 3 SAEs thereof considered as possibly related to study medication (syncope, hypotension, agitation). 2 deaths (sudden death, renal failure) were rated as unrelated to galantamine-PRC.

Conclusions: This clinical trial supports the evidence from placebo-controlled trials that galantamine-PRC is tolerated and effective in the treatment of AD-patients in a clinical setting.

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Novel Acetylcholine Esterase Inhibitory and Cognition Improving Activities of Scopoletin and Scopolin

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The study was conducted in order to show whether scopoletin (7-hydroxy-6-methoxycoumarin) and chemically related substances might potentially be of therapeutic value in memory disorders characterized by dysfunction of cholinergic neurons, such as Alzheimer's disease. Scopoletin has recently been identified by computer aided virtual screening and in vitro tests as an inhibitor of acetylcholine esterase (AChE).

Scopoletin (i.c.v. and i.p.) and scopolin (i.c.v.) were injected in the rat and the effect on acetylcholine release was investigated. Furthermore, scopoletin was injected i.c.v into normal and cholinergically impaired mice and its influence on behaviour was investigated in T-maze alternation test, object recognition task and open field test.

We show here that scopoletin and scopolin enhance the release of acetylcholine in vivo in rat brain to a similar extent as an equal dose of the clinically approved AChE inhibitor galantamine, scopolin being more effective. As expected from this mechanism of action, scopoletin improved performance of untreated and scopolamine-treated mice in the T-maze alternation test. It also restored the disrupted object recognition in mice with scopolamine-induced cholinergic deficit. Furthermore, the substance influenced performance of the animals in the open field test in a way that is consistent with the known effects of AChE inhibition.

Since scopoletin enhances brain acetylcholine release we suggest that the effects on learning and memory and behaviour are caused by the AChE inhibitory property of the compound. Scopoletin and scopoletin-derived molecules might be useful in disorders associated with degeneration or hypofunction of cholinergic neuron systems.

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The Effect of Pretreatment With L-2-Amino-4-Phosphono-Butyrate, a Pre-Synaptic Glutamate Receptor Agonist on Motor Disorders Induced by 6-Hydroxy-Dopamine

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The effect of L-2-amino-4-phosphonobutyrate (L-AP4), A Glutamate metabotropic agonist sub-type III was tested in-vivo on rats with motor disorders induced by intra-cerebral micro-injection of 6-OH-DA (16 µgrams in 2µL buffer phosphate)

Male Sprague Dawley rats intra-cerebrally micro-injected with 6-OH-DA (16 µgrams in 2µl buffer phosphate) into the nigrostriatal bundle, coordinates: (AP=-1.8mm; L=1.8mm; V=- 6.1mm) has produced a Parkinsonian animals with motor disorders characterized by a significant decrease of 44% in locomotion, 36% in eating behavior and 55% in positive forward pushing, and significant increase in rotational movements, rearing and head bobbing. The changes in locomotion were increased gradually reaching its maximum point after 4 weeks.

Sham control animals were cerebrally micro-injected with the same volume of buffer phosphate have shown no significant changes in locomotion rotations or rearing.

Pre-treatment with L-AP4 (40 ngram/2µl BPS) which was focally micro-injected into the nigrostriatal bundle for 15 min before injection of 6-OH-DA (16 µgrams in 2 µL BPS) has reduced significantly the motor disorders. Locomotion was reversed to normal values, from 8.5±0.51(138) to 11.54±0.78(63)($P \leq 0.05$), % change in body weight was reversed from 2% to 39% and positive response to forward pushing was changed from 21% to 78%. On the other hand rotations, head bobbing and rearing were reduced significantly by 29%($P \leq 0.02$), 38%($P \leq 0.001$) and 65%($P \leq 0.01$) respectively.

These results suggests that agonists of glutamate metabotropic receptors which decrease glutamate release by decreasing Ca++ uptake into presynaptic membranes in glutamatergic neurons in basal ganglia circuits, could be used to prevent the development of Parkinson's disease.

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Effect of NGF Injection on Different Type of Memory in Young and Aged Rats

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The decrease of trophic support for the cholinergic neurons in aging brain may lead to neuronal atrophy and appearance of neurodegenerative diseases such as Alzheimer's disease. Administering neurotrophic factors could represent a strategy for the treatment of these disorders. In the present experiment rats were injected intraventricularly with NGF at either 4 or 28 months of age. In order to check an effect of NGF infusion on different type of memory we examined rats performance in three different behavioral tests: short-lasting training in object-recognition task (recognition memory) in object-location task (spatial memory), and long-lasting training in acquisition and reversal of brightness discrimination test (long-term procedural memory). In aged rats, NGF positively effected cognitive processes related to spatial memory, but was ineffective on memory processes involved in the formation of associations established in recognition memory tasks. In acquisition of discrimination learning the choice accuracy was the same for young and aged, both control and NGF-treated rats. However, in reversal learning NGF-treated aged rats mastered the test much longer and made significantly more errors than all other groups. These indicate that in aged animals exogenous NGF deteriorates storage and recall of memory. Together, the contrasting findings show that NGF administration modulates spatial, recognition and visual memory in different not always beneficial ways.

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Evaluation of the Lashley Iii Maze as An in-Vivo Test for Cognitive Enhancing Drugs

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The gradual loss of cholinergic, and at later stages, glutamatergic neurotransmission is believed to be responsible for clinical symptoms of patients suffering from Alzheimer's Disease (AD), which are loss of short-term memory, orientation, and judgement. Thus, compounds that improve cholinergic functioning, e.g. acetylcholineesterase (AChE) inhibitors, ameliorate cognitive deficits of AD-patients.

To test cognitive enhancing compounds in rodents, specific learning and memory test paradigms have been developed. The Lashley III maze is reported as a paradigm, in which learning and memory performance declines in aged rats, and is enhanced by nicotine. The aim of this study was to evaluate whether the Lashley III maze task is suitable for testing putative cognitive enhancing compounds. Time spent and errors made to reach the goal box were measured after treatment with classical amnesic and promnesic compounds.

Young rats (2-3 months old) learned this maze paradigm within 5 days: using 3 consecutive sessions per day they achieved almost 100% performance. Treatment of rats with nicotine at doses of 0.125 - 0.5 mg/kg i.p. accelerated learning and memory of rats significantly. Learning and memory performance could be blocked dose-dependently by the anti-cholinergic drug scopolamine and the NMDA receptor blocker MK-801.

Overall, the results of the present study shows that the Lashley III maze paradigm might be useful for testing putative cognitive enhancing compounds. Further pharmacological validation of this task by clinically used drugs for symptomatic treatment of AD (e.g. AChE inhibitors) is ongoing.

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The Role of Classical PKC in Spatial Memory Processing

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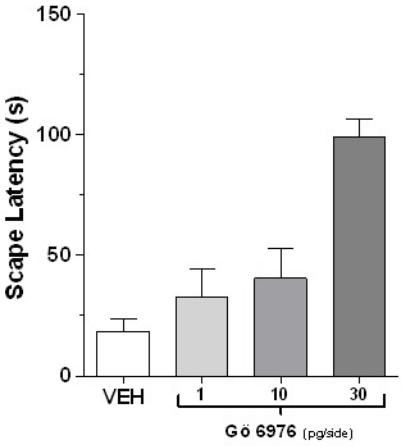
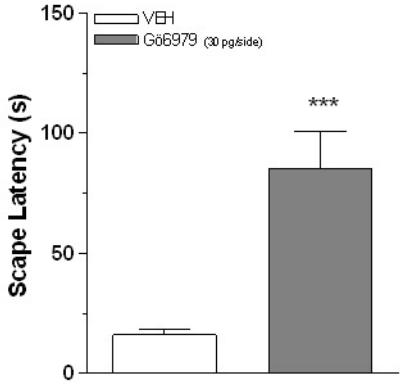
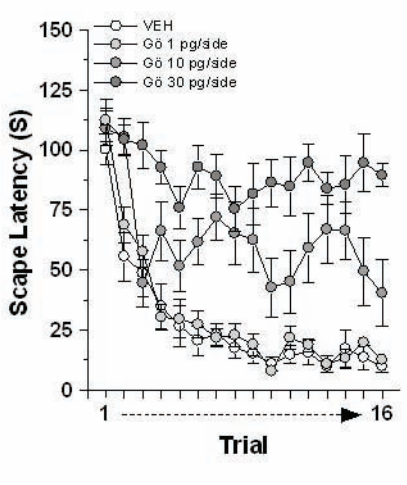
The induction of hippocampal LTP involves the activation of classical protein kinase C (cPKC) isoenzymes to the neuronal membrane.

Furthermore PKC directly interacts with both the A-beta peptides and its precursor peptides APP, and plays a role in memory loss in AD. We analyzed the effect of hippocampal cPKC inhibition on memory acquisition, consolidation and retrieval with the Morris water maze (MWM) spatial learning task in rats. When infused into the CA1 region of the dorsal hippocampus 15 minutes before training, immediately after training or 15 minutes before the test session, the cPKC specific inhibitor Gö6976 blocked acquisition, consolidation and retrieval, respectively. Previously studies have shown that A-beta directly inhibit PKC comparable with GO6976, and inhibits memory consolidation, acquisition and retrieval. The clarification of this mechanism might lead to new therapeutic approaches in AD







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Effects of ZSET1446 on Cognitive Impairment in Senescence Accelerated Mouse at the Age of 8 Months and Aged Rats

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We have previously reported that a newly synthesized azaindolizinone derivative ZSET1446 stimulates acetylcholine release in the hippocampus and cortex and ameliorates impairments of learning induced by intracerebroventricular infusion of amyloid-beta (Abeta)1-40.

In the present study, effects of ZSET1446 were assessed in cognitive impairment, increase in grading scores and deposition of Abeta using senescence accelerated prone mouse strain (SAMP8). ZSET1446 was administered in drinking water at doses of approximately 0.001, 0.01 and 0.1 mg/kg/day from the age of 8 month. SAMP8 at the age of 8 months showed cognitive impairment in a novel object recognition task compared with young SAMP8 (8 weeks). Further, grading scores were gradually increased from the ages of 9 to 12 months and Abeta-immunoreactivity in the hippocampus was increased at the age of 10 months. ZSET1446 ameliorated cognitive deficit and reduced grading scores of SAMP8 after 4, 8, 12 and 16 weeks treatment in novel object recognition task. Further, 8-week treatment of

ZSET1446 significantly reduced the total number of Abeta-positive granules in the hippocampus.

Moreover, effects of ZSET1446 on aged rats at the age of 23 months were also examined. Aged rats showed cognitive deficits in both novel object recognition and water maze tasks. ZSET1446 showed ameliorating effects on cognitive deficits in the aged rats after 4 weeks treatment in drinking water at doses of approximately 0.001 and 0.01 mg/kg/day.

These results suggest that ZSET1446 shows ameliorating effects on SAMP8 and aged rats partly due to the reduction of increase of Abeta-deposition in the hippocampus.

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Gene Expression Profiles of ZSET1446-Treated Rat Hippocampus

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ZSET1446 is a newly synthesized azaindolizinone derivative and we have reported that the treatment of ZSET1446 improves learning deficits induced by β-amyloid, scopolamine and so on. We have found that ZSET1446 stimulates neurotransmitters release including acetylcholine in the rat hippocampus and cortex. It does not affect acetylcholinesterase activity, although the mechanism of ZSET1446 is not clear. ZSET1446 shows affinity for neither muscarinic and nicotinic receptors, nor the other central receptors in binding studies.

In this study, we used differential display method to investigate the mechanism of ZSET1446. Total RNAs were extracted from rat hippocampi after the 5 days daily administrations of ZSET1446. ZSET1446-treated rats showed increases expressions of the genes concerned with mitochondrial ATP production, the growth of neural cells, gene transcription and translation and other various proteins, compared with vehicle controls.

These results suggest that ZSET1446 exhibits multiple mechanisms. Therefore, ZSET1446 stimulates cognitive functions via not only acetylcholine release but also energy metabolism and neural growth. Thus, we have considered that ZSET1446 is worth testing for clinical study aimed for the treatment of dementia such as Alzheimer's disease.

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Prevention of Fiber Formation and Abeta-Mediated Neurotoxicity by Anti-D-Peptide Antibodies

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Immunization with Aβ1-42 has been shown to reduce cerebral amyloid deposition in transgenic mouse models by the induction of both adaptive and innate immunity. In particular, the adaptive immune response is mediated by Aβ-specific antibodies.

It is possible to reduce Aβfibril formation and to overcome inflammation of an amyloid vaccine by using short fragments of Aβ. We have produced antibodies against D- and L-peptides derived from the core Aβ region, which is central to fibrillogenesis and cellular interactions. By this, we attempt to bind soluble rather than fibrillar Aβ, and prevent conformational transitions that lead to the accumulation of toxic oligomeric forms, proto-fibrils and plaques. Such polyclonal antibodies were demonstrated in vitro to prevent fiber formation as measured by a fluorescence assay employing Thioflavin T (ThT), and protect against Aβ-induced neurotoxicity, where anti-D-peptide polyclonal antibodies were more active than the anti-L-peptide counterpart.

To characterize further and confirm these effects, 15 monoclonal antibodies (MAbs) were tested for their ability to prevent β-amyloid formation. Three MAbs demonstrated promising anti-amyloid activity in vitro delaying fiber formation in the ThT assay and inhibiting Aβ-induced DNA fragmentation/condensation in primary rat neurons and human neuroblastoma SH-SY5Y cells. Moreover, co-treament of Aβ with each MAb decreased Aβ-induced toxicity both by reducing caspase 3/7 activity and by increasing cell viability as measured by WST-1 assay. Taken together, these data demonstrate that anti-D-peptide antibodies from the core region of Aβ molecule prevent fiber formation and Aβ-mediated neurotoxicity in vitro and raise the potential for in vivo efficacy.

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The Efficiency of Intravenous Immunoglobulin and Piracetam in Alzheimer Patients

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Background and Aims: To evaluate the efficiency of intravenous immunoglobulin (IVIG) and piracetame by patients of Alzheimer at the initial phase of the disease we examined 16 patients with the symptoms of the disease.

Methods: The patients were divided in two groups of the 8, of the same age and sex. Both groups received 140gr of piracetame for 1 year. One group was administrated additionally a complementary monthly dose of IVIG 0,2gr/kg body weight of IVIG for the duration of the study. All patients underwent a clinical and neurophysiological (Electroencephalograph-EEG, Visual Evoked Potentials, Mini Mental Scale Examination-MMSE) at the beginning and at the end of the treatment.

Results: The comparison of the results of all tests before and after the treatment has shown a significant improvement in the group of patients with the additional treatment with the IVIG.

Conclusions: This leads us to believe that the immunomodulate function of IVIG and piracetame is particularly beneficial in Alzheimer patients and allows for the hypothesis that there may be a disturbance in the immune system of Alzheimer patients.

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Mechanisms of Antibody Delivery to the Brain

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Active immunization against Amyloid-beta peptide (Abeta) with vaccines or passive immunization with anti-Abeta antibodies can decrease the severity of plaque pathology, reduce brain levels of Abeta, and reverse cognitive deficits in mouse models of Alzheimer´s disease (AD). Alternatively to passive immunization with full IgG molecules, single-chain variable fragment (scFv) antibodies can modulate or even prevent the neurotoxicity of Abeta, and inhibit its aggregation in vitro.

To characterize mechanisms of scFv antibody passage from the periphery to the brains of an AD mouse model, we compared various routes of antibody delivery. We found that scFv enter the brain, and that they bind to beta-amyloid plaques in the cortex and hippocampus of transgenic mice. Further studies are underway to evaluate the diagnostic and therapeutic usefulness of different scFv in vitro and in vivo.

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Snapshots of Amyloid Beta 1-42 Conformers by Human Antibody-Displaying Phage Library

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Active species of Aβ assemblies have been a discussion in Alzheimer's disease. To identify the active Aβ conformer, we took an advantage of a human antibody-displaying phage library by taking snapshots of transient molecules under dynamic conformation change. Protein conformation is controlled under various physicochemical conditions in vitro. Confirming these molecular structures by electron microscopy or atomic force microscopy, one can directly react the phage library to the conformation-defined protein molecules in solution or immobilized at plastic plates.

By this approach, we isolated four scFv specific to fibrillar Aβ1-42 as well as six single-chain Fv (scFv) antibodies specific to monomeric Aβ1-42 (Recombinant Antibodies, Boston, 2005).

Recently, Lesne, S et al. have reported that a specific Aβ protein assembly with around 56 kDa in the brain impairs memory (Nature 440: 352-357. 2006). Barghorn, S et al. also reported the globular Aβ1-42 oligomer as a homogenous and stable neuropathological protein in Alzheimer's disease (J. Neurochem. 95, 834-847, 2005).

Based on these findings, we isolated several phage clones that bind to

Barghorn 's globulomer Aβ1-42 but not soluble and fibrillar Aβ1-42. Fibrillar Aβ1-42 specific scFvs inhibit the Aβ1-42 fibril formation in a dose dependent manner, but they showed no inhibitory activity on globulomer Aβ1-42 formation suggesting that fibrillation may be distinct from oligomelization

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A Mimotope-Based Vaccine Approach Leads to Cognitive Improvement in a Mouse Model of AD

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Background: Amyloid β-peptide (Aβ) levels in the brain are increased with progression of Alzheimer's dementia (AD). It is believed that AD is caused by these amyloid peptides. In AD patients levels of soluble Aβ are more closely related to cognitive dysfunction than levels of insoluble Aβ (Luo et al. 1999).

Objective: In this study we tried to correlate the levels of distinct Aβ fractions with cognitive abilities in Tg2576 mice following treatment with the AFFiRiS Mimotope vaccine.

Methods: The Mimotope technology of AFFiRiS is based on molecular mimicry and allows creating antigens for vaccination, which are lacking sequences of the native Aß peptide but which are able to generate antibodies reacting specifically with pathological Aß40/42 molecules. Vaccines are applied by parenteral administration in a vaccine formulation adjuvanted with Alhydrogel. In the present study immune responses were monitored by ELISA. After 3 and 6 consecutive monthly vaccinations a Morris water maze (MWM) task was performed and at the end of the study levels of soluble and insoluble Aß40/42 fractions were assessed.

Conclusions: Mimotope vaccination in Tg2576 transgenic mice leads to the improvement of cognitive abilities in the MWM task which correlates with reduction of Aβ. Thus, the AFFiRiS Mimotope-based vaccine might be a promising novel AD treatment strategy in humans simultaneously eliminating pathologic Aβ and improving cognitive abilities of AD patients.

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Immunizing Mice With Neuronal Tau Protein Induces Tauopathy-Like Pathology and Neurological Deficits

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Objective: To investigate whether immunization with tau-microtubule-associated protein induces a neurodegenerative disease in animals.

Backgound: A possible role of autoimmunity in Alzheimer's-disease(AD) pathogenesis has lately attracted increasing attention. Immunization with Aβ attenuates amyloid pathology, but also induces neuroinflammation. Very little is known about autoimmunity against the other major component of AD – tau aggregation into neurofibrillary-tangles. To investigate this possibility we actively immunized

healthy mice with tau protein and tested whether a neurodegenerative disease is induced.

Methods: We immunized mice with recombinant human tau protein with complete-Freund's-adjuvant(CFA) and pertussis.

Results: Of 11 female animals immunized with tau protein 6 developed neurological signs such as limp tail and limb paralysis, whereas none of CFA-immunized controls showed any clinical disease. In all of the tau-immunized mice we have detected anti-tau Abs in serum, and glial activation as well as tau-related pathology in the spinal cord (and to a lesser extent also in brains). These findings were much more prominent in the clinically affected animals. Mononuclear infiltrates and axonal damage were detected only in the clinically affected tau-immunized animals.

Conclusion: In this study we demonstrated a neurodegenerative disease induced by immunization with the tau-microtubule-associated-protein. To the best of our knowledge this is the first report indicating that tau protein has an immunogenic potential, and can induce neuroinflammation and neurodegeneration, with significant axonal damage and tau-related pathology. These findings may shed light on the possible involvement of tau autoimmunity in neurodegenerative diseases, and point to the potential danger of therapeutic immunization with tau protein.

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Mass Spectrometric and Immunoanalytical Characterization of the Interaction Structure Between Neuroprotective Factor Humanin and Alzheimer's ß-Amyloid(1-40) Peptide

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Humanin (HN) is a linear 24 amino acids peptide recently detected in human Alzheimer's disease (AD) brain. HN has been shown to abolish in vitro neuronal death induced by exogenous β-amyloid (Aß) peptides and by various amyloid precursor protein (APP) and presenilin (PS) gene mutations in familial AD. Due to its high efficacy and specificity, HN represents a potential lead to new therapeutic approaches of AD. However, the molecular mechanism(s) of HN function(s) are still unclear.

The aim of this study was to characterize the interaction structure between HN and Aβ(1-40). HN and four HN-derivatives were synthesized by Fmoc-SPPS and amino acid sequences and homogeneities of RP-HPLC purified peptides ascertained by ESI- and MALDI- mass spectrometry (MS). The complex formation between HN and Aβ(1-40) was studied by affinity-chromatography and high resolution FTICR-MS, as well as by immunoanalytical (ELISA) techniques. The binding sites between the two peptides were identified by applying a proteolytic epitope extraction procedure. The recognition sequence of HN was further characterized by binding analysis of the HN derivatives towards Aβ(1-40), using affinity-MS and ELISA. The interacting region of Aβ(1-40) was further analyzed by a comparative ELISA, using Aβ(1-40) and Aß(1-40)-partial sequences as ligand molecules for HN and by competitive ELISA experiments, in which the influence of different anti-Aβ(1-40) antibodies upon HN-Aβ(1-40) binding was investigated.

The results obtained indicate a high affinity between HN and Aβ(1-40) and provide molecular information on the recognition sites, yielding useful insights for understanding the biological function of HN.

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Epitope Identification and Structure Determination of Aß-Epitope-Specific Antibodies Upon Aß-Immunisation Using High-Resolution Mass Spectrometry

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Antibodies produced upon immunisation with ß-amyloid (Aß) peptides and oligomeric assemblies of Aß have been reported to be therapeutically effective by reducing cerebral Aß deposits and cognitive impairments in Alzheimer's disease (AD). Epitope-excision and -extraction mass spectrometry, used for identification of the epitope recognized by the active antibodies from transgenic AD mice, identified the N-terminal epitope, Aß(4-10) (FRHDSGY) as the specific antigenic determinant. Mass spectrometric studies of the epitope recognized by a monoclonal antibody, anti-Aß(1-17) indicated that the same amino acid residues Aß(4-10) are involved in the antibody interaction. In this work, the functional significance of these residues to the antibodies was investigated by site-directed mutagenesis using synthetic ß-amyloid (4-10) mutants as model peptides. Selective identification of the affinity preserving mutant peptides was achieved by exposing a Sepharose-immobilized antibody column to an equimolar mixture of the model peptides, and the affinity- bound peptides were identified using high-resolution mass spectrometry (FTICR-MS). Replacement of any of the Aß-epitope residues by alanine was found to completely abolish the binding to the monoclonal antibody. The interaction with the polyclonal antibody was preserved in the D7A, S8A, G8A and Y10A mutants indicating F4, R5 and H6 as essential. Details on the differential binding of the mutant peptides to the polyclonal antibody were elucidated by comparative ELISA binding studies. These results provide a basis for the design of improved immunogens containing the Aß(4-10) epitope as new AD vaccine lead structure, that might avoid the inflammatory toxicity observed in first clinical trials by immunization with Aß(1-42).

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Efficacy and Safety of Donepezil in the Treatment of Parkinson's Disease Patients With Dementia

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Objectives: To assess efficacy of donepezil (Aricept®), a reversible non-competitive acetylcholinesterase inhibitor, in dementia associated with Parkinson's disease (PDD), as well as tolerability and safety.

Background: A profound loss of cholinergic neurotransmission occurs in PDD due to loss of ascending projections from basal forebrain nuclei. Usage of agents like donepezil, to enhance cholinergic function, represents a rational treatment strategy.

Methods: 24-¬week, multi-centre, randomised, double¬ blind study, with 3-arm parallel groups (placebo, 5 mg, 10 mg) design.

Inclusion criteria:

- PD (UK Brain Bank criteria)
- Mild - moderately severe dementia (DSM-IV criteria) > 1 year from PD onset
- MMSE 10-26, inclusively

Outcome measures:

Cognition - ADAS-Cog (primary), executive function tests, working memory, attention, visuospatial function;

Clinical global function - CIBIC+ (primary).

Tolerability and safety – standard means documented. UPDRS part III (motor subscale) recorded motor impairments.

Change from baseline in ADAS-cog scores - analysed by ANCOVA including baseline as covariate, countries and treatments as factors. Treatment-by-country interaction was investigated, but not used, in final reported analysis (change from the analysis plan). Primary analysis of CIBIC+ used Cochran-Mantel-Haenszel test, stratified by country.

Results: Tabulated data to be provided.

Efficacy: Primary analysis of ADAS-Cog with treatment-by-country interactions was negative. The more appropriate statistical approach demonstrated statistically significant improvements. CIBIC+ showed statistical superiority for 10 mg donepezil.

Safety and tolerability: Donepezil was well tolerated. Side-effects similar to known donepezil profile, without impact on UPDRS.

Conclusion: Donepezil appears effective and safe in treating mild to moderately severe dementia associated with PD.

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Comparison of Neuroprotective Capability of Selective MAO-B Inhibitor, Rasagiline and Selegiline, Against Lactacystin Induced Nigrostriatal Degeneration

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Background and aims: Nigrostriatal neurodegeneration in Parkinson's disease (PD) may originate from the reciprocal interactions of a restricted number of conditions, such as protein mishandling, mitochondrial defects and oxidative stress. Pharmacological treatments interfering with these pathological pathways may effectively counteract the degeneration. Rasagiline [R(+)-N-propargyl-1-aminoindan] and selegiline are selective and irreversible monoamine oxidase-B inhibitors that possess significant protective properties on dopaminergic (DAergic) neurons in various preclinical models of PD. In present study, the neuroprotective effects of rasagiline and selegiline were compared in an animal model of PD associated with the failure of ubiquitin-proteasome system (UPS).

Methods: C57BL/6 mice were stereotactically injected with lactacystin (1.25 µg/mouse) into the right middle forebrain bundle to generate around 60% loss of nigral DAergic neurons and about 30% inhibition of proteasomal activity. Rasagiline (0.2mg/kg/day) and selegiline (1mg/kg/day) were continuously administrated intraperitoneally 7 days before and 28 days after microinjection. DAergic neuronal depletion, gial activation and proteasomal activation were determined.

Results: Both rasagiline and selegiline exerted a significant neuroprotective effect against lactacystin-induced DAergic neurodegeneration, by attenuating DAergic neurons loss at 55.0% and 34.3%, respectively. They also alleviated microgial activation, although there was no difference between these two drugs. Furthermore, both rasagiline and selegiline showed a moderate but not significant reverse effect against lactacystin-induced inhibition of proteasomal activity.

Conclusion: Our findings indicate that compared with selegiline, rasagiline is more potent in protecting neurodegeneration induced by UPS impairment. The inner mechanism of these neuroprotective properties and the lack of effect on attenuating UPS collapse are under further investigation.

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Activation of Beta2-Adrenergic Receptor Stimulates Gamma-Secretase Activity and Accelerates Amyloid Plaque Formation

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Amyloid plaque is the hallmark and primary cause of Alzheimer disease. Mutations of presenilin-1, the gamma-secretase catalytic subunit, can affect amyloid-beta (Abeta) production and Alzheimer disease pathogenesis. However, it is largely unknown whether and how gamma-secretase activity and amyloid plaque formation are regulated by environmental factors such as stress, which is mediated by receptors including beta2-adrenergic receptor (beta2-AR). Here we report that activation of beta2-AR enhanced gamma-secretase activity and thus Abeta production. This enhancement involved the association of beta2-AR with presenilin-1 and required agonist-induced endocytosis of beta2-AR and subsequent trafficking of gamma-secretase to late endosomes and lysosomes, where Abeta production was elevated. Similar effects were observed after activation of delta-opioid receptor. Furthermore, chronic treatment with beta2-AR agonists increased cerebral amyloid plaques in an Alzheimer disease mouse model. Thus, beta2-AR activation can stimulate gamma-secretase activity and amyloid plaque formation, which suggests that abnormal activation of beta2-AR might contribute to Abeta accumulation in Alzheimer disease pathogenesis.

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Cholinesterase Inhibitors for PD-Related Visual Hallucinations Unresponsive to Atypical Neuroleptics

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Aim of the study: To report clinical effects of cholinesterase inhibitors (rivastigmine and donepezil) on visual hallucinations (VH) emerging in the course of Parkinson's disease (PD) and unresponsive to antipsychotics

Material & Methods: Five patients with PD (with or without dementia) complicated by VH and unresponsive to atypical antipsychotics have been offered a 12-week, open-label trial of a cholinesterase inhibitor.

Results: All 5 subjects have completed a trial with no major adverse effects and, noteworthy, no discontinuations due to adverse events. VH resolved in 4 subjects and were markedly diminished in one person. Neither changes in UPDRS scores nor exaggeration of subjective complaints about extrapyramidal symptoms were noted during treatment.

Conclusion: Cholinesterase inhibitors, rivastigmine or donepezil, might represent a useful alternative to antipsychotics for patients with PD accompanied by VH even in the absence of dementia.

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Self Reported Compulsive Behavior in Parkinson Patients, the Role of Dopamine Agonists

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Background: Impulse control disorders (ICDs) are characterized by an inability to resist the drive to act on an impulse that is potentially harmful. ICDs, including excessive sexuality, excessive gambling and compulsive shopping have been reported in association with the use of dopaminergic medication in Parkinson's disease patients (PDP).

This study compares the prevalence of ICDs in PDP taking a variety of medications, including the dopamine agonists medications pramipexole and ropinirole.

Methods: Surveys were sent to more than 500 confirmed PDP seen at the Spasticity and Movement Disorder Center at Allegheny General Hospital, Pittsburgh, PA. Data obtained included gender, age, handedness, age of onset of symptoms, duration of the disease, current medications (including pramipexole, ropinirole, carbidopa/levodopa, selegeline, amantadine, entacapone). Patients were surveyed for the presence of a variety of symptoms including excessive sexuality, excessive gambling, and compulsive shopping.

Results and Conclusion: Sufficient data for analysis was obtained from 441 surveys. The incidence of reporting any ICDs among the patients not on dopamine agonists medication was 10.7%, compared to patients on ropinirole (13.6%) and patients on pramipexole (24.9%) and these differences were significantly different between groups ($p < .05$). Patients taking pramipexole also were significantly more likely to report 2 or more ICDs (11.1%), compared to either ropenirole (2.3%) or no dopamine agonists (1.3%, $p < 0.001$). Patients reporting 2 or more ICDs also were more likely to be male ($p < .05$), to report a younger age of onset ($p<.05$), and to be younger ($p<.01$). There was no significant difference in disease duration.

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L-Dopa Reverses Acutely the Intracortical Excitability of Alzheimer's Disease Patients

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Alzheimer's disease (AD) is a neurodegenerative disorder, whose histological hallmarks are amyloid deposition and tau hyper-phosphorylation. The clinical progression is however correlated to an unbalance of multiple transmitter systems (i.e. acetylcholine, noradrenaline, 5-HT). So far, symptomatic treatment are mostly focused on cholinomimetics but several evidences indicate the potential use of aminergic compounds.

In this view, we studied the effects of L-dopa administration on an electrophysiological parameter impaired in demented patients with a neuropsychological profile suggestive of AD patients (Pierantozzi et al., 2004): we have investigated the motor threshold and intra-cortical inhibition (ICI) and facilitation (ICF), after paired-pulse TMS, in AD patients not treated with AchE-I (n = 18). Aged-matched healthy subjects (n = 11) were the control group.

Moderate AD (n = 9) patients showed a significant reduction of ICI compared to control, and assumed L-dopa 200 mg p.o. No significant changes in ICI were found in healthy subjects. The acute administration of L-dopa reversed the modified ICI in AD group. These results, though preliminary, confirm the functional importance of dopamine in the modulation of ICI. In addition, they suggest that the well known impact of "cholinergic agents" on ICI-ICF, as previously described (Liepert et al., 2001) is not specific. It is under evaluation a) to what extent dopamine and acetylcholine play similar or synergistic role and b) the impact of chronic DOPA therapy on cognitive domains.

The modulation of ICI pattern by dopamine in AD patients represents a reliable tool to study new therapeutic strategies for AD.

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Rotigotine Exhibits Antidepressant Properties in the Rat Model of Learned Helplessness

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Background and Aim: Rotigotine (Neupro ®) is a non-ergolinic D3, D2, D1 dopamine agonist approved in EU for the treatment of early Parkinson's Disease. As dopamine agonists may have antidepressant properties, the present study was designed to evaluate the effects of rotigotine in the learned helplessness model of depression.

Methods: To induce the helpless state, rats were exposed to mild electric footshocks (15s per min for 1h). Two, three and four days later, rats were tested in a shuttle-box using an active avoidance task. Each session consisted of 30 trials. Conditioned responses, unconditioned responses, escape failures and intertrial crossings were counted. Rotigotine slow release formulation was administered s.c. once daily for 5 days at 0.5, 1.0 and 5.0 mg/kg; controls received vehicle; imipramine 32 mg/kg/d i.p. was used as a reference compound.

Results: At all doses, subcutaneous treatment with rotigotine significantly reversed the elevation in escape failures caused by uncontrollable electric shocks; the escape failure rate of rotigotine-treated rats was similar to escape failure rate of nonhelpless controls. At 5 mg/kg/d, however, rotigotine induced hyperactivity during shuttle-box sessions and when tested separately in an activity box. Imipramine (32 mg/kg/d, i.p.) significantly improved escape performance in the avoidance task.

Conclusion: The data show that subchronic treatment with the lower doses of rotigotine has antidepressant-like activity in the rat learned helplessness test. At present, it cannot be determined whether the 5mg/kg dose induced an unspecific stimulation or true antidepressant activity.

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Rotigotine Transdermal Patch: Effects on Early Morning Motor Function, Sleep Quality, and Daytime Sleepiness in Patients With Idiopathic Parkinson's Disease

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Background and aims: Given the stable 24-hour plasma levels achieved with rotigotine transdermal patch (Neupro®), control of early morning motor impairment and sleep disorders were assessed in patients with idiopathic Parkinson's disease.

Methods: In this open-label, multinational, single-arm trial, subjects received once-daily rotigotine doses up to 16mg/24h (80 cm2 patch). After baseline assessment (as in-patients), 8 weeks of titration and 4 weeks of maintenance, patients were rehospitalized and assessed as in baseline. Primary outcome was early morning (12 hours off medication) UPDRS Part III (motor score). Sleep quality and daytime sleepiness were assessed as well.

Results: A total of 54 patients (52 using levodopa concomitantly) were treated in this trial. Compared to baseline, there was a mean improvement of 11.5 points (Per Protocol Set) in the early morning UPDRS motor score at the end of maintenance. In addition, there was a marked improvement in sleep quality in almost all items of the Parkinson's Disease Sleep Scale (PDSS; total score increase from 95.3 to 105.9 points), in nighttime akinesia scores (reduction from 1.8 to 0.8 points), with no worsening in daytime sleepiness as assessed by the Epworth Sleepiness Scale (ESS, reduction from 7.3 to 6.1 points). The most common adverse events were application site reactions and nausea (20% and 19% of subjects, respectively).

Conclusions: The rotigotine transdermal patch at doses up to 16mg/24h led to clinically relevant improvement in early morning motor function, sleep quality, and daytime somnolence in patients with Parkinson's disease in this open-label trial.

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Dopamnergic Regulation of Adenylyl Cyclase Activity; Relevance to Parkinson's Disease

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It has been suggested that fluctuations in L-DOPA (LD) blood concentrations that lead to intermittent stimulation of striatal dopaminergic receptros (SDR), contribute to the development of late motor complications in Parkinson's disease. The mechanism underlying this deleterious effect is

unknown. In this study we investigated the effect of continuous and intermittent dopaminergic stimulation on adenylyl cyclase (AC), the effector enzyme of the SDR. COS7 cells, expressing D2 dopaminergic receptors (D2R) and AC, were stimulated by quinpirole or dopamine for 18 h continuously, or intermittently by repeatedly removing the agonist and then returning it after 15 min. AC activity was assessed by measuring intracellular cAMP levels and features of the D2R were assessed by a binding assay with [3H]spiperone. Continuous activation of the D2R by quinpirole caused an inhibition of AC activity, whereas continuous stimulation by dopamine caused AC activation. Removal of either agonist after prolonged receptor stimulation resulted in an activation of AC above the basal level (AC-superactivation). Intermittent dopaminergic stimulation enhanced AC inhibition but decreased AC superactivation. Under these conditions, the number of the dopaminergic receptors and the affinity of quinpirole to the D2R were unchanged. We concluded that AC superactivation is not necessarily associated with a prior AC inhibition, but may rather be related to AC regulation by the β subunits of G-proteins. The supersensitivity of the D2R and the dysregulation of AC, following intermittent receptor stimulation, may mediate the development of late motor complications in Parkinson's disease patients, which are on prolonged oral LD treatment.

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Inhibition of Glycogen Synthase Kinase-3β Reduces L-Dopa-Induced Neurotoxicity Against Neuronal Cells

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Background & Objectives: Neurotoxicity of L-3,4-dihydroxyphenylalanine (L-DOPA), used for the treatment of Parkinson's disease, still remains controversial. Although there are much more reports suggesting that long-term treatment of L-DOPA causes neuronal death, recently increasing evidences have proposes that L-DOPA might be neuroprotective rather than neurotoxic. We investigated the effect of L-DOPA on SH-SY5Y human neuroblastoma cells depending on its concentration. And, we also studied whether glycogen synthase kinase (GSK)-3β activation is related to L-DOPA-induced neurotoxicity. Method: To evaluate the effect of L-DOPA on SH-SY5Y human neuroblastoma cells, we treated several doses of L-DOPA for 24h. To investigate the role of GSK-3β in L-DOPA-induced neurotoxicity, we simultaneously treated several doses of L-DOPA and GSK-3β inhibitor for 24h Results: MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay, trypan blue staining, cell counting kit-8, and DAPI staining showed that L-DOPA decreased SH-SY5Y cell viability in high concentration. In the study to investigate the neurotoxic mechanisms of L-DOPA, expression and activity of GSK-3β were significantly increased in a concentration-dependent manner and treatment of GSK-3β inhibitor prevented L-DOPA-induced cell death. Conclusion : These results suggest that L-DOPA induces neuronal cell death in high concentration, that the neurotoxic effect of L-DOPA might be partly mediated by GSK-3β activation, and that inhibition of GSK-3β reduces L-DOPA-induced neurotoxicity.

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Levodopa Pharmacokinetics and Catechol-O-Methyltransferase (COMT) Inhibition Following Administration of Nebicapone and Controlled-Release Levodopa/Carbidopa (Sinemet® CR 250)

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Objectives: To investigate the effect of single oral doses (50 mg, 100 mg and 200 mg) of nebicapone, a new COMT inhibitor, on the levodopa pharmacokinetics and erythrocyte soluble COMT activity when administered in combination with a single-dose of controlled release levodopa/carbidopa 200/50 mg (Sinemet® CR 200/50) in healthy subjects (n=16).

Methods: Double-blind, randomised, placebo-controlled, 4-way crossover study. Washout between periods was >5 days.

Results: A dose-dependent and significant increase was found in levodopa extent of exposure (AUC0-inf), without significantly changing peak exposure (Cmax). Taking placebo as "reference", point estimates (PE) and 90% confidence intervals (90%CI) of geometric mean ratios (GMR) following nebicapone 50 mg, 100 mg and 200 mg were respectively 1.130 (0.982;1.299), 1.036 (0.900;1.191) and 1.100 (0.956;1.265) for Cmax, and respectively 1.263 (1.155;1.342), 1.373 (1.274;1.749) and 1.470 (1.418;1.647) for AUC0-inf. Regarding 3-O-methyldopa, PE (90% CI) were respectively 0.607 (0.549;0.674), 0.452 (0.408;0.501) and 0.328 (0.297;0.364) for Cmax, and respectively 0.685 (0.606;0.775), 0.529 (0.408;0.614) and 0.408 (0.370;0.473) for AUC0-inf. Nebicapone dose-dependently and significantly decreased COMT activity. Maximum COMT inhibition (Emax) occurred between 1.5 and 2.4 hours post-dose and ranged from 56% to 73% with nebicapone 50 mg and 200 mg, respectively. There was a good correlation between plasma concentrations of nebicapone and inhibition of S-COMT activity. Treatments were well tolerated.

Conclusion: Following concomitant administration with controlled release levodopa/carbidopa 200/50 mg, single-doses of nebicapone 50 mg, 100 mg and 200 mg inhibited S-COMT activity, increased systemic exposure to levodopa and reduced 3-O-methyldopa formation in a significant and dose-dependent manner.

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Levodopa Pharmacokinetics Following Administration of Nebicapone, a New COMT Inhibitor, and Immediate-Release and Controlled-Release Formulations of Levodopa/Benserazide (Madopar®)

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Objectives: To investigate the effect of single doses (50 mg, 100 mg and 200 mg) of nebicapone on the levodopa pharmacokinetics when administered in combination with a single-dose of levodopa/benserazide 100/25 mg immediate (Madopar® 125) or controlled (Madopar® HBS 125) release formulation, in healthy subjects.

Methods: Double-blind, randomised, placebo-controlled, single-dose, crossover studies. Washout between periods was >5 days.

Results: Mean±SD pharmacokinetic parameters of levodopa following administration of Madopar® 125 or Madopar® HBS 125 concomitantly with placebo or nebicapone are presented in Table 1.

Point estimates (PE) and confidence intervals for geometric mean ratios of levodopa Cmax and AUC following Madopar®+nebicapone versus Madopar®+placebo are presented in Table 2.

Conclusion: Single-doses of nebicapone 50 mg, 100 mg and 200 mg dose-dependently improved levodopa bioavailability following concomitant administration with immediate and controlled release formulations of levodopa/benserazide.

Table 1

Treatment period	Madopar® 125			Madopar® HBS 125		
	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0-\infty}$ (ng.h/mL)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0-\infty}$ (ng.h/mL)
Placebo	1091±874	1.0	1366±588	259±129	3.0	1297±252
50 mg	880±427	1.0	1809±692	325±135	1.75	1196±536
100 mg	1006±419	1.0	2197±931	317±143	3.0	1620±834
200 mg	876±331	1.0	2083±874	347±164	2.5	1637±589

Table 2

Nebicapone dose	Madopar® 125/ placebo PE (95%CI)		Madopar® HBS 125/ placebo PE (90%CI)	
	C_{max}	$AUC_{0-\infty}$	C_{max}	AUC_{0-t}
50 mg	0.91 (0.58;1.44)	1.39 (1.06;1.69)	1.302 (1.061;1.598)	1.369 (0.910;1.801)
100 mg	1.06 (0.69;1.63)	1.60 (1.27;1.98)	1.245 (1.014;1.528)	1.141 (0.834;1.649)
200 mg	0.93 (0.83;1.56)	1.52 (1.22;1.90)	1.338 (1.090;1.642)	1.423 (0.969;1.917)

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Neuroprotective Mechanisms of Acetylcholinesterase Inhibitor, Donepezil, Through Inhibition of GSK-3beta Activity in in Vitro Model of Alzheimer's Disease

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Background & Objectives : Acetylcholinesterase inhibitors are used for the treatment of Alzheimer's disease. Recently, donepezil, an acetylcholinesterase inhibitor, is suggested to have neuroprotective effects. However, the precise protective mechanisms of donepezil have not yet been clearly understood. We investigated the neruoprotective effects and mechanisms of donepezil against β-amyloid (1-42) induced neurotoxicity in rat cortical neurons. Method : To evaluate the effects of donepezil on β-amyloid (1-42) induced neurotoxicity, we treated several doses of donepezil alone for 18h after combined treatment of β-amyloid (1-42) and donepezil for 6h. Results : MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay, trypan blue staining, cell counting kit-8, and DAPI staining showed that donepezil increased neuronal cell viability in a concentration-dependent manner. In the study to investigate the neuroprotective mechanisms of donepezil, the neuroprotective effects of donepezil were completely blocked by LY294002 (10μM), a PI3K-inhibitor, but were partially by mecamylamin (10μM), a blocker of nicotinic acetylcholine receptors (nAChRs). And, they were achieved by activating PI3K, enhancing phosphorylation of Akt and GSK-3β, and reducing phosphorylated tau, as confirmed by western blot analysis. Donepezil decreased GSK-3β activity, as well. Conclusion : These results suggest that donepezil prevents β-amyloid (1-42) induced neurotoxicity in primary cultures of rat cortical neurons through the activation of PI3K and Akt and the inhibition of GSK-3β, as well as the activation of nicotinic acetylcholine receptors, and that PI3K activation and GSK-3β inhibition are more important mechanisms of donepezil than nicotinic receptor activation.

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Does Alpha-Synuclein Inhibit Phospholipase D2 Activity?

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α-Synuclein (αS) is a small cytosolic protein that is both genetically and neuropathologically linked to Parkinson's disease (PD). Reports from several labs suggest that αS inhibits phospholipase D2 (PLD2) activity in cell-free assays and in cell culture. PLD2 is a membrane-associated enzyme that hydrolyzes phosphatidylcholine into phosphatidic acid and choline. If PLD2 regulation is a physiological function of αS, then PLD2 dysregulation could be an early event in the pathogenesis of PD and other synucleinopathies.

Using purified proteins, we tested the ability of αS to inhibit PLD1 and PLD2 activity in cell-free assays. Despite using a range of experimental conditions, including those previously published, we did not observe PLD inhibition by αS. Next, we used several cell lines and transfection conditions to determine whether αS inhibits endogenous and/or overexpressed PLD in cultured cells. Our data to date do not support statistically significant inhibition of PLD activity by αS in any of our cell lines. However, overexpression of PLD2 has failed to produce significant increases in PLD activity in our assays. Experiments to address the latter issue are ongoing, as are additional experiments searching for αS inhibition of PLD. Given the lack of robust PLD inhibition by αS in our cell-free assays and cultured cells, we conclude that dysregulation of PLD2 activity due to αS dysfunction is not likely to play a major role in the pathogenesis of PD.

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A-Synuclein Oligomeric Forms-Toxic Species in Parkinson's Disease

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Parkinson's disease (PD) is pathologically characterized by a loss of dopaminergic neurons from the substantia nigra and aggregated protein deposits called Lewy bodies. The main component of these intracellular deposits is aggregated alpha synuclein (-syn). Besides PD, aggregates of α-syn are also found in several other neurodegenerative disorders, summarized as synucleinopathies.

A growing body of evidence suggests that prefibrillar oligomers and protofibrils, rather than fibrils of α-syn, are the pathogenic species in PD.

Therefore the aims of this project were to identify the pathologically relevant α-syn species and to investigate their resulting toxic effects on neuronal cells.

We first developed three novel protocols for oligomer generation based on published observations. For the first time we compared the oligomers directly in shape, morphology and size using two single particle analysis methods. Finally we investigated their bioactivity on human cells.

We found that our three aggregation protocols resulted in a heterogeneous population of oligomers, which were disruptive to different cellular mechanisms. On one hand oligomer types induced apoptosis via breakdown of calcium homeostasis of cells with a pore-forming mechanism. On the other hand other oligomer forms directly entered the cell resulting in increased protein aggregation. Based on our results we propose that under various physiological conditions heterogeneous populations of oligomeric forms will be formed, which can directly or indirectly result in cell damage.

Blocking the early events in oligomer formation would consequently prevent all oligomer related toxicities and has therefore promise for the development of effective drugs against PD and other synucleinopathies.

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Subcellular Localization and Trafficking of The Gamma-Secretase Complex Components

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Gamma-secretase is a multiprotein complex consisting of PSEN1 or 2, NCT, PEN-2 and APH-1. To find out in which subcellular compartment(s) the gamma-secretase complexes are assembled, where the respective active complexes reside and when the different substrates associate with them, we optimized different subcellular fractionation procedures aimed at highly enriching different membrane compartments, including ER, IC and endosomal fractions. Subsequently, these enriched compartments were examined for gamma-secretase activity using a cell-free assay, and for the presence of (sub)complexes using blue native PAGE.

In 0.5% DDM extracts, the relative amount of mature 440 kDa complexes was the lowest in ER, but was significantly increased in IC-enriched fractions. As expected, essentially only mature complexes were recovered in endosomal fractions, where these complexes coincided with gamma-secretase activity. However it should be noted that these complexes are most likely localized only in a subset of endosomes.

Since our data indicate that gamma-secretase complex formation likely occurs in pre-Golgi compartments, we next explored whether assembly occurs prior to or after exit from the ER, using an in vitro ER-budding assay. This assay was performed on wild-type fibroblasts and cell lines deficient for one of the gamma-secretase complex members, allowing us to dissect the requirements for ER exit. The first data, based on 0.5% DDM extracts, indicate that mainly subcomplexes and monomeric components exit the ER in COPII transport organelles.

A further detailed knowledge about the subcellular localization and trafficking of gamma-secretase complex members may open future avenues for interfering with APP processing and Abeta production.

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Rer1p Competes With APH-1 for Binding to Nicastrin and Regulates Gamma-Secretase Complex Assembly in the Early Secretory Pathway

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The γ-secretase complex, consisting of presenilin, nicastrin, PEN-2 and an APH-1 cleaves type I integral membrane proteins like APP and Notch in a process of regulated intramembrane proteolysis. The regulatory mechanisms governing the multistep assembly of this 'proteasome of the membrane' are unknown. Here we characterize a new interaction partner of nicastrin, the retrieval receptor Rer1p. Rer1p binds preferentially immature nicastrin via polar residues within its transmembrane domain that are also critical for interaction with APH-1. Absence of APH-1 significantly increased binding of nicastrin to Rer1p demonstrating the competitive nature of these interactions. Moreover, Rer1p expression levels control the formation of γ-secretase (sub)complexes and concomitantly total cellular γ-secretase activity. We identify Rer1p as a novel limiting factor that negatively regulates γ-secretase complex assembly by competing with APH-1 during active recycling between the endoplasmic reticulum and Golgi. We conclude that total cellular γ-secretase activity is restrained by a secondary endoplasmic reticulum control system with a potential therapeutic value.

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Therapy Follow Up in Mild Dementia Subjects

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Although there is no cure to Alzheimer's disease, some medications seem to delay the progress of the disease. We tested the using.

Cognitive assessment are usually used to test effect of these medications, but are limited either by low sensitivity (i.e. MMSE) or by high complexity and cost (i.e. neuropsychologist's evaluation). Affordable computerized neuro-cognitive tests are not useful for computer-naive elderly patients.

Thirteen elderly subjects , 64-87 years old were recruited from the memory disorders clinic in the Sourasky medical center. MMSE scores range was 21-27. Seven subjects were computer naive. NexAde, a self-administered computerized neuropsychological battery was used for cognitive testing. Subjects received Rivastigmine tartrate (n=6) or Donepezil HCl (n=7) for 21 - 189 days after baseline and before retest.

Age ($F=.457$, $p=.833$), gender ($F=.457$, $p=.833$), and computer skill ($F=2.250$, $p=.208$) of the subjects did not influenced their performance in neither NexAde subtest. Performance in NexAde subtests was correlated to MMSE scores at baseline ($F=15.323$, $p=.017$). MMSE scores did not change with treatment ($F=.017$, $p=.902$). Nexde results improved with treatment ($F=14.885$, $p=.018$). Improvement was largest in visual identification, visual recall, and digit span backward subtests. There was no difference in performance of patients taking different medications ($F=.801$, $p=.510$).

Our study confirmed previous results on modest cognitive improvement in mild to moderate dementia patients. While the computerized battery used in the current study was sensitive enough to measure the improvement, MMSE failed to do so. Different cognitive functions were influenced differently by the treatment implying high variance in treatment response.

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Screening for Dementia in the Elderly Community

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One of the challenges in elderly health care is prediction of cognitive decline. This study investigated which individuals in a group of community-dwelling, healthy elderly volunteers in Tel Aviv-Yaffo, Israel, have cognitive impairment. We also compared the cognitive performance of those subjects with subjects who were referred to memory clinics, complaining of memory impairment.

278 elderly subjects were screened in 12 elderly clubs in Tel Aviv-Yaffo municipality. 49 subjects were recruited from the memory disorders clinic of Sourasky medical center and 50 age-matched volunteers were controls. All subjects were 55-89 years old, had good or corrected vision and hearing and could understand Hebrew, English or Russian. Approximately 75% of the subjects had no computer experience.

Subjects went through an interview, MMSE and a self-administered computerized neuropsychological tests battery, NexAde.

359 of 377 subjects completed the NexAde battery and reported pleasant user experience. 16% of the subjects referred to the memory clinic and 7% of the home dwelling subjects had MMSE scores ≤ 23 and all had also the lowest NexAde scores. From those who had MMSE scores 24 the elderly clubs population had significantly higher ($p<0.005$) NexAde scores than the memory clinic subject, but not significant difference in the MMSE scores. Two clusters containing 22% and 78% of the subjects were detected in the clubs population, the first similar in NexAde scores to the memory clinic subjects while the latter significantly different. Computer skills did not affect NexAde scores. NexAde's computerized tests thus enabled population screening efficiently and at low costs.

Neuro–degenerative Diseases

Neurodegenerative Dis 2007; 4(suppl 1):1-350

Friday, March 16, 2007

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Regulated Intramembrane Proteolysis and Its Relevance for Alzheimer's Disease

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Amyloid β-peptide (Aβ) generation has turned out to be just one example of a general physiological mechanism now known as regulated intramembrane proteolyis (RIP). In a principal version of this process, membrane proteins first undergo a regulated shedding of their ectodomains by membrane-anchored proteases referred to as secretases or sheddases, releasing the large luminal domains into extracellular fluids. The membrane-retained stubs can then be cleaved within their transmembrane domains (TMDs) to release small hydrophobic peptides (e.g., Aβ in the case of APP) into the extracellular space and the intracellular domains (ICDs) into the cytoplasm. The free ICDs may have specific functions in the cytosol, including the activation of nuclear signaling pathways, as in the case of the Notch ICD. In the case of APP and certain other RIP substrates, ectodomain shedding can be effected by either of two distinct membrane-anchored proteases, α-secretase (believed to be one or more members of the ADAM family of metalloproteases) or β-secretase (also called β-site APP cleaving enzyme or BACE). The membrane associated stub created by BACE cleavage can then undergo an intramembrane scission mediated by a unique protease complex, γ-secretase, that is composed of four proteins. These are presenilin-1 or 2 (PS1/PS2), Nicastrin, APH-1, and PEN-2. All four proteins are both necessary and sufficient to reconstitute γ-secretase activity.

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M1 Muscarinic Agonists as Treatment Against Major Hallmarks of Alzheimer's Disease (AD) and the Pivotal Role of Brain M1 Receptors

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M1 muscarinic receptors (M1mAChR), preserved in AD, are a pivotal therapeutic target. The M1 selective muscarinic agonists AF102B [Cevimeline], AF150(S) and AF267B - i) elevated αAPPs, decreased Aβ levels and tau hyperphosphorylation; ii) blocked Aβ -induced neurotoxicity via M1 mAChR-modulation of PKC and GSK3β and a cross-talk with Wnt signaling; and iii) restored cognitive deficits, cholinergic markers, and decreased tau hyperphosphorylation in several models with a wide safety margin [Fisher et al, J Neural Transm, 2002; Farias et al, Neurobiol Disease, 2004]. AF267B decreased brain Aβ levels in hypercholesterolemic rabbits [Sparks et al, ADPD2003] and decreased CSF Aβ42 in rabbits and removed vascular Aβ42 deposition from cortex in cholinotoxin-treated rabbits [Beach et al, Curr Med Chem, 2003]. In 3xTg-AD mice chronic AF267B treatment via M1mAChR-activation - rescued behavioral deficits and decreased Aβ42 and tau pathologies in the cortex and hippocampus (not amygdala), activated PKC/MAPK and ADAM17/TACE, decreased BACE1 levels and inhibited GSK3β activity. The M1 antagonist dicyclomine had opposing effects to AF267B, demonstrating the central role of the M1 mAChR in this model [Caccamo et al, Neuron, 2006]. PKC and α-secretase are decreased, while BACE1 and GSK3β are elevated in AD brains. Unlike direct modulation of these therapeutic targets, AF267B - M1mAChR -mediated signaling via these enzymes could be safer as these pathways mimic acetylcholine-mediated M1 physiology. A broad therapy should target all AD hallmarks. AF267B is the 1st reported low MW monotherapy that meets this challenge, and clinical trials will have to determine its value in AD, CAA and other CNS diseases.

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Muscarinic Receptor Regulation of Amyloid Precursor Protein Processing in Primary Neuronal Culture

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Amyloid precursor protein (APP) is a transmembrane protein that is cleaved to produce the Aβ peptide found in neuritic plaques, hallmark neuropathologic lesions of Alzheimer's disease (AD). Two enzymes, termed α and β secretase, mediate the proteolysis of APP, with α-secretase preventing A? production and β-secretase promoting it. Muscarinic acetylcholine receptors (mAChRs) are important regulators of APP processing, but less is known about the precise contributions of distinct mAChR subtypes to the regulation of APP cleavage. Using primary neuronal cultures from mice deficient in one or more mAChR subtypes, we have begun to dissect the details of muscarinic receptor regulation of APP processing at the genetic level. Our results indicate that cultures from M1 knockout (KO) mice exhibit a shift towards amyloidogenic APP processing, evidenced by increased accumulation of Aβ and decreased production of the non-amyloidogenic derivative APPsα. Conversely, M2/M4 double knockout cultures display the opposite pattern, with increased secretion of APPsα and decreased production of a β-secretase-generated carboxy-terminal fragment. Collectively, these data suggest that specific mAChR subtypes differentially

regulate cleavage of APP. Furthermore, our preliminary studies suggest that mAChR subtypes may also affect ?-secretase cleavage, resulting in a shift in the ratio of Aβ42: Aβ40. Taken together, these data support the hypothesis that altered cholinergic signaling is a pivotal event in the development and progression of AD, and suggest that therapies designed to modulate cholinergic function may not only treat AD symptoms, but may also alter the course of the disease.

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Amyloid Beta Oligomers Alter the Structure and Function of the Post-Synaptic Region. the Role of Wnt Signaling Pathway

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Synaptic dysfunction is an early manifestation of Alzheimer's disease. The cellular mechanism by which Abeta affects synapses remains unclear. Abeta oligomers target synapses in cultured rat hippocampal neurons suggesting that they play a key role in the regulation of synapses. We report here: (1) the molecular players involved in the anchorage of Abeta oligomers at the synapse and (2) the specific synaptic changees of Abeta oligomers.

Neuroligin is a post-synaptic cell adhesion protein. Its extracellular domain is mainly formed by an acetylcholinesterase-like domain. We propose that neuroligin could target of Abeta oligomers at the synapse. We have also studied the glutamatergic synaptic transmission of the Schaffer collateral excitatory synapses onto CA1 pyramidal cells in rat hippocampal slices. Abeta oligomers show a decrease in the evoked response over 50% in field recording and over 60% in intracellular recording. The analysis of the facilitation ratios suggest that Abeta oligomers effect depends on the postsynaptic site. This effect of Abeta oligomers correlates with a reduction of PSD-95. In addition Wnt-5a, a non-canonical Wnt ligand, prevents the decrease triggered by Abeta oligomers in the glutamate receptor and PSD-95. Interestingly, Abeta oligomers affect the maintenance of the cadherin - catenin complex which mediates synaptic plasticity. Altogether, our results suggest that Abeta oligomers decrease the amplitude of synaptic responses by affecting the postsynaptic region at different levels. The Wnt signaling activation opens a therapeutic intervention in the prevention of the synaptic changes in Alzheimer´s disease.

Supported by: FONDAP Nº13980001, MIFAB.

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First-in Man Experience With NGX267, a Selective M1 Agonist for Disease Modification and Symptomatic Treatment of Alzheimer's Disease

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Clinical development programs for Alzheimer's disease (AD) therapeutics utilize small, time-lagged studies in young and older normal volunteers to provide safety, tolerance, pharmacokinetic (PK) and occasionally pharmacodynamic (PD) insights that inform subsequent efficacy paradigms. NGX267, a rigid analogue of acetylcholine, preserves a selective M1 muscarinic pharmacophore at M1 receptors and along signal transduction pathways. Pharmacological properties compatible with disease modification and symptomatic improvement in AD have been suggested (Caccamo et al. Neuron 2006 Mar 2; 49(5): 671-82). Innovative dose-escalation studies completed in normal young (n=34; 24 NGX267; 10 placebo) and elderly (n=26; 20 NGX267; 6 placebo) volunteers using sequential cohort, adaptive dosing designs have been completed that provide correlated observations across safety, tolerance, PK and PD measures. Collectively these data argue for a) predictable, dose-related increases in exposure following oral doses in young and elderly subjects; b) dose-limiting adverse events consistent with M1 activation in the absence of clinically important changes in vital signs or laboratory parameters; and c) insights based upon PD/PK relationships in man vs. animals that suggest a therapeutic index as disease modification therapy.

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Novel Approaches for the Treatment of AD – Is There Still Hope for Improved Symptomatic Therapies?

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Introduction: Alzheimer's disease (AD) is a progressive neurodegenerative disorder with increasing evidence of neuronal dysfunction prior to the accumulation of Ab containing plaques. Present treatment standards for AD are primarily founded on the replenishment of the neurotransmitter acetylcholine and are poorly effective at best.

Methods: Cognitive function has been assessed using a variety of standard behavioral assays including novel object recognition, contextual fear conditioning and Morris water maze assays in both wild type rodents and transgenic animals models of AD (tg2576 animals). Compounds were dosed orally and activity determined as a dose responsive and significant improvement in cognitive performance in each particular behavioral assay. In vivo neurochemical analysis using microdialysis was also used to assess the mechanism by which compounds may be improving cognitive performance.

Results: Orally dosed 5-HT1a and 5-HT6 antagonists significantly improved performance in a variety of rat and mouse models of cognition. Neurochemical analysis following compound administration also demonstrated acute increases in both acetylcholine and glutamate levels in the hippocampus of animals. Subsequent experiments comparing current treatment standards, such as Aricept and Memantine, suggest that 5-HT1a and 5-HT6 antagonists may be more effective and better tolerated.

Conclusions: Novel symptomatic approaches for the treatment of AD may offer patients improvements in both efficacy and tolerability, by targeting CNS specific receptors able to modulate multiple neurotransmitter systems in the brain.

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When Does Parkinson's Disease Begin ?

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Neuroprotective therapies have been defined as interventions that produce enduring benefits by influencing underlying pathogenesis and thereby preventing or delaying onset of the disease or its progression. Within this definition the best neuroprotective trials in Parkinson's disease would be those that could target at-risk populations or those patients harbouring the disease without yet having expressed the full motor phenotype. Neuropathological studies in PD suggest that there is a 40 % nigral cell loss before motor dysfunction becomes apparent and that there may be a "pre-clinical window" of about 6 years. A similar duration of pre-clinical nigrostriatal terminal dysfunction has also been suggested by neuroimaging studies. More recently it has become clear that a variety of non-motor symptoms might potentially herald the onset of classical parkinsonism – these include mood disorders like depression and panic attacks, sensory dysfunction like hyposmia, gastrointestinal symptoms like constipation as well as REM sleep behaviour disorder (RBD). All these "pre-clinical" syndromes should probably be viewed as the earliest manifestations of Parkinson's disease – consistent with current neuropathological staging hypotheses. Identifying at-risk populations by these clinical signs and symptoms – as well as by genetic, proteomic or imaging markers will eventually lead to a shift from concepts of neuroprotection to neuroprevention.

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When Should We Begin Treatment for Parkinson's Disease?

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The period from onset of neuronal cell dysfunction to death and then the emergence of clinical symptoms in PD is not known. In the monogenic forms of familial PD this may be over several decades. Recent studies using serial imaging in sporadic PD have suggested that the pre-symptomatic latent period from onset of dopaminergic cell loss to diagnosis is approximately six to seven years. Clinical progression in early PD is relatively rapid; the united Parkinson's disease rating scale (UPDRS) deteriorates by 8-10 points in the first year and this is associated with a significant decline in quality of life.

Traditionally, treatment for PD has been withheld until symptoms have sufficient impact upon function in the workplace, social or domestic life. This view arose and developed during the levodopa era and became established teaching, but perhaps deserves re-evaluation given the range of new treatments now available. Some have proposed that in the appropriate patient, early symptomatic treatment is beneficial in the short and long term to improve motor control and quality of life, and can be achieved without an increase in the frequency of motor complications. Others consider that it remains better to delay initiation. Furthermore, it has been suggested that early correction of the basal ganglia functional abnormalities caused by dopaminergic cell loss and dopamine deficiency is a means to support the intrinsic physiological compensatory mechanisms and both limit and delay the circuitry changes that evolve as PD progresses. Review of the outcomes of the DATATOP, ELLDOPA and TEMPO studies supports such a proposition.

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New Approaches in Symptomatic Treatment

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Antiparkinsonian medications and functional surgery drastically improve the motor symptoms of Parkinson's disease (PD) patients. Nevertheless, PD patients become progressively more disabled and suffer sooner or later, from 3 types of therapeutic limitations that warrant the search for and the development of novel therapeutic approaches:

-"dopa-resistant" motor symptoms,
-"dopa-resistant" non motor signs,
-drug-related side effects.

A first strategy is to develop "neuroprotective" medications to prevent patients from reaching the advanced stages of the disease. Unfortunately, this is not presently achieved.

A second strategy is to develop new tools to control the remaining symptomatic challenges via dopaminergic or non dopaminergic mechanisms, aiming at a better delivery of dopamine stimulation within the striatum. This is the case with novel dopaminergic agents (reuptake inhibitors, new selective, partial dopamine agonists) or with new delivery systems to achieve more continuous dopamine stimulation at the periphery (dopamine agonists transdermal patches, subcutaneous pumps, intraduodenal infusion) or within the striatum (pumps, cell or gene therapies). Several novel approaches aim at manipulating non dopaminergic systems that are also directly or indirectly involved into pathophysiology of the motor and non motor signs. Based on the demonstration of their symptomatic efficacy in PD animal models or other disorders with similar symptoms, a variety of new compounds are presently in clinical development (cholinesterase inhibitors, adenosine A2 antagonists, glutamate NMDA and AMPA antagonists, serotoninergic agonists and antagonists, noradrenergic alpha-2 antagonists, cannabinoïds, … The true benefit/risk ratio of these agents in PD patients remains to be assessed in adequate clinical trials.

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Dopaminergic Neurons and GDNF-Producing Astrocytes Generated, From Human Bone- Marrow Derived Mesenchymel Stem Cells:, Therapeutic Potential in Parkinson's Disease

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The major pathogenetic substrate of Parkinson's disease (PD) is the progressive degeneration of nigrostriatal dopaminergic neurons. Most treatments currently available may offer symptomatic relief with many potential side-effects but are not curative. There is an urgent need for therapies that would provide more robust clinical benefit and also be of neuroprotective and neurorestorative values. Stem cells-based strategies hold a great promise in the field, but there are many limiting obstacles. For instance, use of embryonal stem cells carries many ethical, political, social and religious problems and also the threat of teratogenicity. We chose therefore to bypass these limitations and focus on adult mesenchymal stem cells isolated and expanded from human bone marrow, as a preferable source for cell replacement therapy in PD. We have shown that after exposure to various combinations of external stimulations (e.g., by trophic factors, cytokines, etc.), such cells can be induced to become TH-positive dopamine-producing and-releasing neurons. When exposed to other external "cocktails", these stem cells can be transformed to astrocyte-like cells that carry specific biological markers and generate and release several neurotophic factors including GDNF, BDNF and NGF. Such human bone-marrow derived dopaminergic neurons or trophic factors-producing astrocytes were engrafted into the deafferented striatum in cyclosporine-treated rodents with unilateral nigrostriatal 6-OH-DA lesions. In these animals, apomorphine-induced rotational behavior (number of rotations is a correlate of severity of nigrostriatal dopaminergic neuronal destruction) progressively decreased over a period of three months as compared with control animals. Immunohistochemical analysis of striata indicated that the human originating cells survived in the rodent brain for the duration of the experiment. We suggest that autotransplantation into basal ganglia of dopamine-producing neurons or neurotrophic factor-generating astrocytes harvested from the patients own bone marrow, may become a novel therapeutic strategy for symptomatic relief and neuroregeneration in PD patients.

Supported, in part, by Brainstorm-Cell Therapeutics, Ltd., and the Norma and Alan Aufzien Chair for Research in Parkinson's Disease, Tel Aviv University.

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Interaction of Cholinergic Compounds With Aß Processes in Different Transgenic Mice Models

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Accumulation of beta amyloid (Aβ) is an early event but probably not the only driving force in the cascade of events leading to AD pathology. Inflammatory and oxidative stress mechanisms, tau hyperphosphorylation cause disturbances in neurotransmitter function and subsequent cognitive deficits. Whether these pathological processes are a direct effect of amyloid-induced neurotoxicity remains to be confirmed. A direct interaction between Aß and acetylcholinestesterase (AChE) may lead to an increased Aß toxicity. Different AChE inhibitors show various effects on APP and sAPP in cell lines and transgenic mice models suggesting novel mechanisms by which AChE inhibitors may alter Aß pathology. Transgenic mice APP swe mice over expressing AChE show higher amount of Aß than APPswe mice. In both transgenic mice models Aß is measurable in brain at early age. Long-term or short-term nicotine treatment markedly reduces the amyloid load in the brains of APP transgenic mice. This effect is opposite what is seen in APPswe/AChE mice where nicotine treatment causes an elevation of soluble Aß. In both transgenic mice models nicotine treatment lower the GFAP innunoreactive atrocytes and increase the levels of synaptophysin. Galantamine and memantine treatment to APP swe mice increase the levels of synapthophysin and APP respectively. The a7 nicotinic receptors probably play an important role in the interaction with Aß. A deeper understanding will be provided with studies of signal transduction mechanisms

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Butyrylcholinesterase as a Natural Protector From Amyloid Pathologies: From Structure to Function

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In Alzheimer's disease, both acetyl- and butyrylcholinesterase (AChE, BChE) co-localize with brain fibrils of amyloid-β peptides (Aβ). Brain AChE levels decline while BChE levels increase with age, suggesting greater impact of BChE on aged brain's physiology. Others have shown that synaptic AChE-S facilitates fibril formation by association with insoluble Aβ fibrils. We recently demonstrated that human BChE and BSP41, a synthetic peptide derived from the BChE C-terminus, inversely associate with the soluble Aβ conformers, and that they delay the onset and decrease the rate of Aβ fibril formation in vitro, at a 1:100 BChE/Aβ molar ratio and in a dose-dependent manner (Diamant et al., 2006, PNAS 103: 8628-8633). Subsequent cross-linking experiments demonstrated that both BChE and BSP41 prolonged the half-life in solution of small amyloid multimers, while slowing down the formation of insoluble large Aβ multimers. Circular dichroism and molecular modeling confirmed that BSP41 is an amphipathic α-helix with a protruding aromatic tryptophan residue in the polar side of the C-terminus. That this aromatic residue is causally involved in the attenuating effect of BChE was further supported by mutagenesis experiments, where W8R BSP41 showed suppressed capacity to attenuate fibril formation. In Alzheimer's disease, BChE may have thus acquired an inverse protective role to that of synaptic AChE by adopting imperfect amphipathic characteristics of its C-terminus.

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Potent Beta-Amyloid Modulators

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Currently, the potential to interfere with the pathology of b-amyloid targeting a well-known drugable enzyme, the acetylcholinesterase (AChE), is open. Peripheral or dual binding site inhibitors of AChE may simultaneously alleviate the cognitive and behavioural deficits in Alzheimer´s disease

(AD) patients and, more importantly, act as disease-modifying agents delaying amyloid plaque formation. Furthermore, the butyrylcholinesterase (BuChE) emerges as a new target for AD playing important roles in the b-amyloid pathology.

As part of a rational drug design program directed to find dual binding site AChE inhibitors, several families of compounds have been synthesized as potent AChE inhibitors. From these series, several drug candidates were selected based on their potent and selective inhibition of AChE (sub-nano molar activity) and their interference with the b-amyloid aggregation in vitro (IC50 in the low micro molar range). We have also synthesized potent and selective BuChE inhibitors (sub-nano molar activity).

Both families of compounds are able to reduce efficiently the production of beta-amyloid in different transfected cell lines, showing a strong interference with the beta-amyloid pathology.

First in vivo data confirm our initial hypothesis. Oral treatment with NP-61 for 3 months is able to reverse the cognitive impairment (Morris Water Maze) and to reduce plaque load in the brains of hAPP transgenic mice (Swedish and London mutation).

These results suggest that NP-61, a potent beta-amyloid modulator, is able to reverse the AD-like neurodegenerative phenotype in transgenic mice, indicating a promising disease-modifying agent for clinical application.

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Cholinesterase Inhibitors Future Role in the Therapy of Dementia

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Twenty years after the first report of Summers in 1986, several million Alzheimer Disease (AD) patients, have been treated with cholinesterase inhibitors (ChEI) with no evidence of severe side effects. There is no evidence that treatment is not cost effective. The time limit in long-term clinical effects is at least 3 yrs, this term has now been extended to severe patients (MMSE less than 10). The recent discovery of the role of butyrylcholinesterase (BuChE) in brain points to this enzyme as a new target for AD treatment in advanced AD cases. Selective BuChEI should be tested in more severe cases. Based on the functional role of the cholinergic system, indication for ChEI treatment should be extended to those diseases or syndromes showing a cholinergic deficit such as Lewy Body Disease, Vascular Dementia, Parkinson Dementia, Delirium, Brain injury, attention deficits and HIV dementia. Bifunctional ChEI are being developed to add non-cholinergic to cholinergic effects such as APP synthesis inhibition, anti-oxidation, MAO inhibition, A-beta anti-aggregation, 5HT uptake inhibition.ChEI will continue to play an important role in AD therapy for many years to come.

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Alzheimer's Disease and Vascular Dementia

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Evidence is accumulating that Alzheimer's disease (AD) is associated with vascular changes, whereas patients with vascular dementia frequently have Alzheimer's type pathology. There is therefore a need for new thinking about these nosological entities and criteria to diagnose them.

Clinical course: Insidious onset and relentless evolution are not the unique hallmarks of AD, but can occur in patients whose dementia appears following a stroke. Also, patients with pure AD may have a fluctuating course, for example, episodes of delirium.

Clinical criteria: Aphasia, apraxia, etc., may occur in AD as well as with cortical strokes and even white matter lesions. Impairment of short term memory may not occur in spite of dysfunction in multiple areas of cognitive dysfunction due to focal cortical damage, like strokes.

Pathological criteria: Autopsy findings have traditionally been accepted as the hallmark of accurate diagnosis. However, at present neither anatomical nor neurochemical findings can diagnose dementia. Moreover, since dementia is a syndrome, it is hard to envisage that such pathological criteria will ever be found.

Neuroimaging: The resolution of CT and MRI is much lower than neuropathology. Therefore, these methods cannot be seen to distinguish the two disorders. The utility of functional imaging of the brain remains to be established but to the extent that these reflect and correlate with the anatomical changes and cognitive state, these too are unlikely to be reliable tools for the positive diagnosis of the two entities.

Therapy: Treatment of demented individuals involves non-specific measures, such as the creation of a safe and supportive environment, drugs to alleviate anxiety, depression, insomnia and psychosis.

More specific therapies depend on the diagnosis. Most cases of dementia are due to Alzheimer changes, frequently with a vascular contribution. The main treatment is with cholinesterase inhibitors (ChEI's), including donepezil, rivastigmine and galantamine. These drugs have all been tested in AD and their efficacy is proven in improving cognition, particularly attention and concentration. It is speculated that ChEI's can, in addition, prevent the further cognitive deterioration, but there is only meager data supporting this view.

There is impressive accumulating data suggesting that ChEI's are also helpful in the treatment of related dementias, such as mixed dementia and VaD. New evidence about the usefulness of memantine in AD and VaD show that there are several lines which can be used for comprehensive therapy.

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Treatment Options for Alzheimer's Disease

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Currently the treatment of AD through its different stages takes into account the best available evidence from randomized clinical trials (RCT). Cholinesterase inhibitors and the NMDA receptor antagonist memantine are the standard symptomatic drugs for AD. Combination of these drugs acting in complementary ways may be desirable. Efficacy assessment has not easily translated from RCT to clinical practice. Treatments that could modify disease progression through reduction of amyloid deposition, enhancement of brain repair systems, or modification of cholesterol or glucose transport, are being evaluated in international RCT. There are stages of AD where such treatments may be most effective: anti-amyloid therapies may prove to be more effective prior to symptom onset or in the amnestic MCI stage and in younger patients, whereas statins may help older subjects. Stage-specific benefits of current drugs have also been demonstrated: cholinesterase inhibitors are more effective in mild to moderate stage than amnestic MCI, memantine is more effective in moderate to severe stages. Pharmacogenomics may allow for the selection of the most appropriate drug class and drug within a class, for individual patients. Prevention of AD through delaying emergence of symptoms may be achievable using our knowledge of protective and risk factors. Risks towards AD could be assessed for asymptomatic individuals based on family history, cognitive performance, genotype, brain imaging and other biological markers, leading to evidence-based long term changes in life-style and, when appropriate, long term treatment with disease-modifying agents.

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Neuroprotective Strategies in An Amyloid Precursor Protein Transgenic Animal Model of Alzheimer's Disease

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Background and Aims: It is proposed that neurodegenerative alterations in Alzheimer's Disease(AD) might be related to abnormal accumulation of Abeta oligomers at the synaptic sites, leading to signaling abnormalities and tau phosphorylation. Development of therapies aimed at protecting synapses by regulating signaling pathways or preventing Abeta aggregation holds significant promise. Development of AD treatments includes anti-aggregation or pro-degradation compounds, neuroprotective compounds, neurotrophic agents, vaccination, and stem cell and fetal cell grafting.

Methods: We tested gene therapy approaches using lentiviral vectors and neurotrophic agents such as BDNF, lithium, and valproic acid. Among them, Cerebrolysin(CBL) (peptide-mixture w/neurotrophic effects) has the ability to ameliorate the neurodegenerative alterations in AD. The neuroprotective effects of CBL might be related to its ability to regulate APP metabolism and promote neurogenesis in the hippocampal dentate gyrus(DG). To study this, tg mice expressing mutant APP under the thy-1 promoter were treated with CBL for 1 and 3 months.

Results: CBL rescues the synaptic pathology and behavioral deficits in the mice by regulating GSK3b activity. This results in decreased phosphorylation of APP and decreased Abeta production. Moreover, in APP tg mice treated with CBL the numbers of neuronal precursor cells (NPC) in the hippocampus increased. There was a significant increase in BrDu(+) cells, doublecortin(+) neuroblasts and a decrease in TUNEL(+) NSC.

Conclusions: CBL might rescue the alterations in neurogenesis in APP tg mice by preserving the NSC and decreasing the rate of apoptosis. Such an anti-amyloidogenic property might also provide a novel strategy for treatment of other neurodegenerative disorders.

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Alzheimer's Disease Treatment: Slowing Down the Progress

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The development of disease-modifying treatments able to slow down the progression of Alzheimer's disease represents a major challenge in Neuropharmacology research nowadays. This kind of compounds must interfere with Alzheimer's pathogenic events like neuronal degeneration, synaptic loss, amyloid deposition, immune activation and/or the decrease of brain neurotrophic activity. In addition, disease-modifying medications have to improve symptoms and to reduce the rate of decline in AD patients.

Approved anti-dementia drugs, mainly cholinergic agents, provide some symptomatic relieve to the patients, but it is no clear that these compounds can exert a disease-modifying activity. Studies on this topic with cholinergic drugs will be reviewed.

Compounds able to enhance brain neurotrophic activity constitute promising candidates for the disease-modifying treatment of Alzheimer's disease. Cere, a peptide preparation with a neurotrophic-like mode of action, is one of these agents. In experimental studies it has been found that Cere: enhances neuronal survival and sprouting in culture; exerts a NGF-like effect on dorsal root ganglia neurons; rescues medial septal cholinergic neurons from degeneration after fimbria fornix trans-section; reduces neuronal loss, apoptosis and microglia activation induced by amyloid-beta; preserves synaptic integrity and reduces amyloid deposition in APP transgenic mice; and induces neurogenesis in the rat hippocampus. According to these preclinical data indicating that Cere influences the main etiopathogenic events occurring in AD, it is postulated that this compound might slow down the progression of Alzheimer's disease. Results of the last clinical trial with Cere in AD patients will be presented.

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of Paperclips and Beta-Ropes: Structural Approaches to the Pathological Conformation and Aggregation of Tau

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The microtubule-associated protein Tau forms aberrant aggregates ("paired helical filaments") in Alzheimers disease and other brain diseases ("tauopathies"). Tau is highly soluble so that its aggregation is difficult to rationalize. Moreover, tau is unique because it is the only MAP which aggregates in neurons. To understand the pathway of aggregation, and to direct our search for aggregation inhibitors we are investigating the properties of soluble tau and tau aggregates. Important features of tau are the hexapeptide motifs in the repeat domain which can nucleate filaments with cross-beta structure. We started a search for the conformations of tau in solution, using FRET between labeled residues. This showed that the N-terminal and C-terminal ends of the tau molecule can approach each other in a "paperclip-like" arrangement, in spite of the "natively unfolded" character of the protein. New insights into the packing of paired helical filaments came from a combination of STEM and limited proteolysis which showed that the repeat domain at the core of PHFs is generally not accessible to proteases, but there are sensitive regions which probably represent exposed loops, and which occur in a repetitive fashion, commensurate with the repeats in the sequence. Finally, to identify low-MW compounds capable of disrupting the filament structure (i.e. potential inhibitors of tauopathy) we screened banks of compounds for their interactions with tau and PHFs. Several promising compounds emerged, including some that disrupt PHF structure in vitro and in inducible cell models of tauopathy. - Research supported by DFG and ISOA.

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Lithium, a Potential Protective Drug in Alzheimer's Disease: a Study Carried Out in Transgenic Mice Overexpressing GSK-3beta and FTDP-17 Tau

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The neuropathological hallmarks of Alzheimer's disease (AD) are senile plaques and neurofibrillary tangles (NFTs). NFTs are intraneuronally generated aggregates of paired helical filaments (PHFs), which are assembled from hyperphosphorylated forms of the microtubule-associated protein tau. Glycogen synthase kinase-3 (GSK-3), has been proposed as the main kinase able to aberrantly phosphorylate tau in AD and in related tauopathies. This fact raises the possibility of designing novel therapeutic interventions in AD based in GSK-3 inhibition. Lithium, a drug widely used in patients suffering from affective disorders, inhibits GSK-3 at therapeutically relevant concentrations and therefore its study as a potential drug in AD should be of great interest. We have previously generated a double transgenic mouse model overexpressing GSK-3beta and the human tau protein carrying FTDP-17 mutations (Neurobiol Aging. 2006;27:1258-68). These transgenic mice present in hippocampal neurons, area relevant for AD, tau hyperphosphorylation, NFTs and the formation of PHFs with a width similar of those found in tauopathies. This animal model will be used to test two intriguing questions. First, if chronic lithium treatment is able to prevent AD tau hyperphosphorylation and the formation of aberrant tau aggregates and, second, if lithium is able to revert tauopathy symptoms present in aged animals. Our data (J Neurochem 2006 Oct 24; [Epub ahead of print]) demonstrate that the progression of AD can be prevented by the administration of lithium at early disease stages and still partially revert tau pathology at late stages of the disease, although already formed NFTs-like structures do not revert.

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The Effect of BDNF on Tau Phosphorylation

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The two hallmarks of Alzheimer disease that are histochemically characterized include the paired helical filaments (PHFs) composed of hyperphosphorylated tau protein in neurofibrillay tangles (NFTs), and amyloid beta protein in senile plaques. We are interested in the molecular control of tau expression, and in the signal transduction pathways controlling its phosphrylation.

Previous studies using P19 EC stable cell lines expressing GFP-Tau mutated constructs in two phosphorylation sites showed inhibition of neuronal differentiation, due to the observed formation of PHFs. In a recent study, we have used Brain Derived Neurotrophic Factor (BDNF) to study its effect on tau phosphorylation. A decrease in levels of BDNF mRNA and protein has been shown in brains of Alzheimers disease patients. So far, no known link between the decrease in BDNF and Alzheimers disease pathology has been found. Our results demonstrate that BDNF stimulation of neuronally differentiated P19 cells resulted in a rapid, 60% decrease in tau phosphorylation, at a phospho-tau epitope recognized by the AT8 and Tau1 antibodies. K252a, a Tyrosine Receptor Kinase (Trk) inhibitor, attenuated this dephosphorylation event, suggesting that BNDF activation of TrkB is responsible for the tau dephosphorylation. In addition, BDNF had no affect on tau phosphorylation in the presence of wortmannin, a PI-3Kinase inhibitor, or lithium, a GSK3beta inhibitor, suggesting that these two kinases are part of the signaling transduction cascade leading from TrkB receptor activation to tau dephosphorylation. These results suggest a link between the decrease in BDNF levels in AD, and tau hyperphosphorylation.

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Choking of Synapses by Tau

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Among the numerous microtubule-associated proteins, the neuronal tau-protein is unique because it is linked to neurodegeneration in a number of brain diseases, collectively termed "tauopathies". Its pathological alterations are observed prominently not only in Alzheimer's disease but also in Parkinson's disease, frontotemporal dementias and others. We are interested in the cell and animal models that reveal the physiological functions of tau and pathways of degeneration. Apart from the well-known function of stabilizing microtubules and thus promoting neurite outgrowth, tau has the potential of clogging the microtubule tracks and to retard the transport along cell processes. This affects many cargoes of microtubule motors, in particular mitochondria. One consequence is that synapses loose their energy supply and begin to degenerate. This could correspond to the early loss of synapses that is characteristic of incipient stages of Alzheimer's disease. The energetic "choking" of synapses can be relieved by protein kinases that detach tau from the tracks and facilitate transport, for example the kinase MARK. A second case of traffic defect concerns vesicles carrying APP, which is of interest since this effect links the two proteins most prominently involved in AD. The interaction of APP with motors and microtubules has been a matter of debate. Our experiments suggest that trafficking of APP and the secretases of APP are distinct events, occur on different carriers and at different rates. As a consequence, inhibition of APP traffic by tau has no direct influence on the intracellular processing of APP. - Research supported by DFG.

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Novel Tau Transgenic Mouse and Tissue Culture Models

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Tau dysfunction characterizes a range of human disorders including Alzheimer's disease (AD) and frontotemporal dementia (FTD). The former is characterized by both Abeta plaques and tau-containing neurofibrillary tangles (NFTs), and the latter by NFTs and scant Abeta plaques. We are interested in how tau dysfunction causes the various facets of human disease and how tau and Abeta interact. Therefore, we have previously generated transgenic mice which express human tau together with the mutation P301L which is associated with FTD with Parkinsonism linked to chromosome 17 (FTDP-17). These mice develop tau filaments and NFTs and reveal a behavioral impairment. Based on our findings both in vivo and in vitro, that Abeta induces a significant increase in tau filaments and NFTs, we applied proteomic and transcriptomic approaches to Abeta-injected P301L mice and to P301L tau-expressing human SH-SY5Y neuroblastoma cells incubated with Abeta, as compared to controls. Our proteomic approach identified proteins in categories such as stress response and cytoskeletal organization. Our transcriptomic approach identified a role for aberant cell cycle reentry in AD. Validation of the proteomics and transcriptomics findings including immunocytochemistry and Western blot analyses of human brain samples will be presented. Furthermore, we generated a second mutant tau transgenic mouse strain modeling Parkinsonism as a defining characteristic feature of FTDP-17. We were able to dissect the underlying pathomechanism. Together, our results suggest that tau and Abeta cause disease by functional impairment of overlapping, yet distinct cellular processes.

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Involvement of More Than One Signaling Pathway in Alzheimer Neurofibrillary Degeneration

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Background and Aims: Neurofibrillary degeneration of abnormally hyperphosphorylated tau is a hallmark of Alzheimer disease and related tauopathies. The number of this lesion directly correlates with dementia, and certain mutations in tau gene are known to cause frontotemporal dementia. As yet the exact nature of the signaling pathway(s) involved in this pathology is not understood.

Methods: We investigated protein kinase and phosphatase activities involved in converting tau into Alzheimer abnormally hyperphosphorylated tau.

Results: We have found that dephosphorylation of the cytosolic abnormally hyperphosphorylated tau (AD P-tau) by protein phosphatase-2A (PP-2A) inhibits its self-assembly into paired helical filaments (PHF)/straight filaments (SF). Sequential rephosphorylation of the PP-2A dephosphorylated AD P-tau with protein kinase A (PKA), calcium, calmodulin protein kinase II (CaMKII), and glycogen synthase kinase-3Beta (GSK-3Beta) or cyclin-dependent protein kinase 5 (cdk5), or by cdk5 and GSK-3Beta, promotes its assembly into bundles of PHF/SF. In contrast, rephosphorylation of the PP-2A–AD P-tau with none of these kinases individually promotes tau's assembly into PHF. The bundles of PHF formed on rephosphorylation by the combined actions of PKA, CaMKII, and GSK-3Beta or cdk5, and by cdk5 and GSK-3Beta, are congophilic, reminiscent of neurofibrillary tangles from AD brain.

Conclusions: These studies demonstrate that neurofibrillary tangles of PHF can be formed by the activation of more than kinase signaling and that this pathology can be inhibited by PP-2A.

(Supported in part by the New York State Office of Mental Retardation and Developmental Disabilities and NIH grant AG019158

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Bidirectional Link Between Cholesterol and Aß Generation

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Lipid homeostasis is controlled by several functionally overlapping regulatory cycles. Especially well understood is cholesterol homeostasis. Detailed analysis of cholesterol homeostasis revealed a critical role for Aß peptides, which works in two ways. Aß down regulates the activity of the key enzyme of cholesterol and isoprenoid synthesis. Feedback regulation is then provided by cholesterol which increases secretase activity leading to increased Aß production. However, this regulation is further dependent on the co-regulation by sphingomyelins. Sphingomyelin binds to cholesterol, which changes its biological properties. Like with cholesterol, sphingomyelin levels are down regulated by Aß. Independent regulation is possible, because Aß42 exclusively activates sphingomyelin degradation whereas Aß40 is specific for cholesterol. Moreover, the list of lipids involved in Aß modulation and respective feed-back cycles is rapidly increasing. Ganglioside GM1 strongly increases Aß production. All of these lipids are major components of rafts. We have also studied plasmalogens – phospholipids which are decreased in AD, enriched in rafts and contain especially high levels of long polyunsaturated fatty acids like DHA. In line with the other raft lipids plasmalogens increase Aß generation. Moreover, expression of PS-FAD mutations change the relative ratio of these lipids to each other. These changes are in direct correlation to the mutation induced change in Aß40/Aß42 ratio and age of disease onset.

Taken together these findings show that regulating neuronal lipid metabolism is a fundamental function of the APP processing system, which provides an excellent platform for identification of therapeutic targets.

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ACAT Inhibition Regulates APP Metabolism

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Genetic and epidemiological data support a role for altered cholesterol metabolism in the pathogenesis of AD. At the molecular level, intracellular cholesterol regulates APP processing and Aβ production. Therapies already developed for dyslipidemia and atherosclerosis are becoming attractive as potential strategies for reducing AD-related amyloid pathology. Acyl-coenzyme A: cholesterol acyltransferase (ACAT) inhibitors, which prevent conversion of cholesterol and fatty acids into cholesteryl-esters, are not marketed but are being actively developed for treatment of cardiovascular disease. We have previously shown that a well-characterized ACAT inhibitor, CP-113,818, reduces secreted Aβ levels in neuronal and non-neuronal cells, dramatically improves AD-like pathology in the brains of hAPP transgenic mice, and also regulates processing of endogenous mouse brain APP. A different ACAT inhibitor, CI-1011 (avasimibe) also reduces Aβ generation in cell-based assays, while decreasing APP-CTF levels. CI-1011 has previously reached phase III trials for prevention of atherosclerosis. Recently, we have been studying APP-interacting proteins with altered affinity for APP upon ACAT inhibitor treatment. CHO cells expressing APP751 were treated with CP-113,818 or DMSO. To identify proteins whose interaction with APP was changed by ACAT inhibition, we performed MS analysis of proteins coimmunoprecipitating with APP in the absence or presence of CP-113,818. We identified at least four protein with altered affinity for APP in CP-113,818-treated cells. These newly identified proteins can lead to novel pathways involved in APP processing, regulated by ACAT inhibition. ACAT inhibition offers hope as a novel strategy for the treatment and prevention of Alzheimer's disease.

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Cholesterol, Copper and Aß Clearance

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Mounting evidence suggests that copper may influence the progression of Alzheimer's disease by reducing clearance of the amyloid beta protein (Aß) from the brain. We propose that Aß is cleared from the brain by tagging along with cholesterol/ApoE in traversing the BBB, with subsequent incorporation into HDL for delivery of the toxin to the liver. It is suggested that either ABC-A1 or LRP, or both are involved in Aß transport across the BBB, as well as normal cholesterol efflux. An animated view of normal and cholesterol-induced abnormal APP metabolism leading to Aß production is presented. The cascade of cholesterol influence is supported by evidence identified in the brains of cholesterol-fed rabbits. We have previously shown that addition of only 0.12 PPM copper (one-tenth the Environmental Protection Agency Human consumption limits) to distilled water was sufficient to precipitate the accumulation of Aß in the brains of cholesterol-fed rabbits. Here we show that in a setting of elevated cholesterol levels, overproduced Aß is cleared to the blood and can simultaneously be identified in the liver if copper ion is absent from the animal's drinking water, but if trace levels copper (0.12 PPM) are added to the drinking water Aß accumulates in the brain, while the levels in the liver are greatly reduced.

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Screening of Cholesterol-Related Genes for Association With Alzheimer's Disease

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Several genes related to cholesterol metabolism have been associated with the risk for sporadic Alzheimer's disease (AD). We have systematically investigated genes related to cholesterol metabolism in chromosomal regions linked to AD in full genome scans for association with disease risk. In a screening sample of n=306 individuals we analyzed 84 single nucleotide polymorphisms (SNPs) in 28 genes for association with the diagnosis of AD. HMGCS2, FDPS, RAFTLIN, ACAD8, NPC2, and ABCG1 were associated with AD at a significance level of $P<0.05$. Association with AD was confirmed for HMGCS2, FDPS, NPC2, and ABCG1 in at least one of five replication samples ($P=0.05$ to $P=0.001$) but none of the investigated markers was associated with AD in a pooled sample (n=2864). Stratification of this sample revealed an APOE-dependent association of HMGCS2 with AD ($P=0.001$). We conclude that, if at all, the investigated genes have only weak effects on the risk for AD.

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Serum Cholesterol and the Risk of Parkinson's Disease

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Introduction: No previous prospective studies on the association between serum total cholesterol and the risk of Parkinson's disease have been reported. Our aim was to examine the association between serum total cholesterol and the risk of Parkinson disease.

Methods: Study cohorts comprised 24,773 Finnish men and 26,153 women aged 25 to 74 years at baseline without a history of Parkinson's disease and stroke at baseline. Hazards ratios of incident Parkinson's disease were estimated for different levels of serum total cholesterol at baseline.

Results: During a mean follow up period of 18 years, 321 men and 304 women developed an incident Parkinson's disease. After adjustment for confounding factors (age, study years, body mass index, systolic blood pressure, education, leisure-time physical activity, smoking, alcohol consumption, coffee and tea consumption, and history of diabetes), the hazard ratios of Parkinson's disease at different levels of total cholesterol (<5, 5-5.9, 6-6.9, and ≥7 mmol/l) were 1.00, 1.33, 1.53, and 1.84 (P for trend =0.035), respectively, in men and 1.00, 1.55, 1.57, and 1.86 (P for trend =0.113), respectively in women. In both sexes combined, the multivariate-adjusted direct association between total cholesterol and the risk of Parkinson's disease was present both in subjects aged 25-44 years and 45-54 years, and in never smoker and smokers.

Conclusion: High serum total cholesterol is associated with an increased risk of Parkinson's disease, similarly in men and women. The effect is graded and independent of other risk factors.

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APP Processing, Cholesterol and Secretases as Therapeutic Targets

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We have shown that inhibition of cholesterol biosynthesis down-regulates the release of the ß amyloid peptide (Aß) by beta- and gamma-cleavage of APP. A role of cholesterol in Alzheimer's disease (AD) has also been supported by genetics and epidemiology. The e4 allele of apolipoprotein E is a major risk factor for AD, and elevated cholesterol levels during mid-life increase the risk of developing AD. A reduction of Aß40 and Aß42 production could also be achieved in the brains of patients with AD that were treated with statins. This reduction turned out to be most effective in patients with mild to moderate AD.

There is a drastic reduction of the production of ß-stumps by beta-secretase (BACE) when cholesterol biosynthesis or transport is inhibited. Cholesterol synthesis inhibition resulted also in a reduction of Aß production from ß-stumps, whereas cholesterol loading experiments and inhibition of cholesterol transport resulted in enhanced gamma-secretase cleavage. This shows that gamma-secretase is also regulated by cholesterol and/or intermediates of cholesterol biosynthesis. Regarding the mechanism of the regulation of Aß production by cholesterol, alterations in cholesterol transport affect the intracellular transport of APP, BACE and the presenilins. Exposure of neuronal cells to inhibitors of cholesterol-transport resulted in a marked decrease in beta-secretase cleavage products of full-length APP. Alpha-secretase activity was not increased because the corresponding APP fragments (alpha-stumps) were not elevated. The latter obersevation may be important in regard to not interfering with APP function since our recent studies suggest a function of uncleaved, full-lenght APP in brain plasticity.

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Imaging of Amyloid-Plaques and Cerebral Glucose Metabolism in Semantic Dementia and Alzheimer's Disease

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Background: Semantic dementia (SD) is a rare clinical syndrome, assigned to the group of frontotemporal lobar degenerations (FTLD). Some parallels to Alzheimer's disease (AD) have been shown. In contrast to AD, the deposition of amyloid plaques has not been consistently described in the histopathological analysis of SD.

Aims: To determine whether differences between AD and SD can be found by means of in vivo amyloid plaque imaging, providing complementary information to results obtained by measurement of cerebral glucose metabolism.

Methods: AD and SD patients were recruited using established clinical criteria, matched for gender, age and severity of dementia. Cerebral glucose metabolism was examined with [F18]FDG-PET, cerebral amyloid plaque density was assessed using [C11]PIB-PET. Voxel based statistical group comparisons were carried out between the patient groups (FDG- and PIB-PET data) and a group of healthy controls (FDG-PET data). A volume of interest analysis of temporal/cerebellar cortical PIB retention ratios was conducted.

Results: Characteristic patterns of hypometabolism were detected in AD. Also in SD typical temporal and minor frontal mesial hypometabolism was found. Significantly stronger cortical retention of PIB was observed in all patients with AD (bilateral temporoparietal, frontal, posterior cingulate cortex and precuneus) than in SD extending the differences found in FDG-PET between the groups. Only subtle, subcortical PIB retention was found in SD.

Conclusions: These findings point to a possible association of amyloid plaque pathology with neuronal dysfunction in AD and support the idea of a different pathology underlying SD.

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Amyloid Load Correlates With Memory Dysfunction in Mild Cognitive Impairment But Not in Alzheimers Disease

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Background and Aims: Mild cognitive impairment (MCI) is considered a transitional stage between normal aging and Alzheimer's disease (AD) and carries a high risk of conversion to AD. Deposition of beta-amyloid is a ubiquitous neuropathological feature of AD, which presents with progressive impairment in memory and cognition. The relationship between amyloid burden and cognition, however, is not well established in AD and has not been explored at all in MCI. This study examined the relationship between amyloid burden and cognitive function in healthy controls (HC), AD, and MCI.

Methods: Participants completed the Mini-Mental State Examination (MMSE), California Verbal Learning Test-Second Edition, Rey Complex Figure Test (RCFT), Digit Span, Clock drawing, Verbal fluency, Boston Naming Test, and Digit Symbol-Coding. Neocortical amyloid burden was quantified using standardised uptake volume normalised to cerebellar cortex (SUVR).

Results: Two-thirds of the participants with MCI were amyloid positive. Amyloid positive MCI participants performed worse than amyloid negative participants on the MMSE and delayed recall of the RCFT. Neocortical SUVR also correlated negatively with MMSE and memory measures for MCI participants. There were no correlations, however, between amyloid load and impaired cognitive performance in AD or in HC.

Conclusions: Increased amyloid burden is related to decreased memory in MCI. This suggests that amyloid deposition is an early event in the pathogenesis of AD.

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Early Detection of Alzheimer Disease Beta-Amyloid Molecular Pathology by Non-Invasive Quasi-Elastic Light Scattering in Vivo

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Background: Alzheimer disease (AD) is the most common form of age-related dementia. AD is characterized by excessive accumulation of beta-amyloid (Abeta) in the brain that begins long before the onset of cognitive symptoms. Presymptomatic detection of the underlying disease process will facilitate early therapeutic intervention when emerging treatments are expected to be most effective. We previously reported the presence of Abeta and distinctive AD-linked amyloid pathology in the lenses of AD patients. Aims: To develop and test novel laser technology for early quantitative detection/monitoring of AD-associated amyloid lens pathology in vivo. Methods: Non-invasive infrared quasi-elastic light scattering (QLS), stereophotomicroscopy, quantitative western blot, ELISA. Results: We utilized QLS to quantitatively detect Abeta in the lenses of Tg2576 transgenic (Tg) mice as a marker for cerebral Abeta accumulation. Human Abeta was generated from amyloid precursor protein in Tg mouse lenses where it accumulated in the cytoplasm of lens fiber cells as electron-dense microaggregates that scatter light. In vivo, QLS was able to accurately discriminate Tg mice with clear lenses from age-matched wild-type controls by 10 months of age before beta-amyloid pathology was detectable in the brain. Human Abeta promoted aggregation of mouse lens protein and corresponding light scattering signal changes in vitro that were consistent with the QLS changes we detected in the Tg mice in vivo. Conclusions: Our data support AD-linked Abeta aggregation in the lens as an early disease biomarker candidate, and underscores the potential of non-invasive QLS as technology for AD screening, diagnosis, and monitoring.

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Longitudinal Monitoring of Amyloidogenesis, Glial Activation and Anti-Amyloid Treatment Strategies in Mouse Models of Alzheimer's Disease by in-Vivo Imaging Technologies

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Compelling evidence that piling-up of amyloid filaments, composed of amyloid peptide and tau protein, is mechanistically implicated in the pathogenesis of Alzheimer's disease has led us to investigate fibrillization of these amyloidogenic molecules in living brains, in order to gain profound insights into possible strategies for radical cure of the disease. Our recent efforts to capture amyloid plaques in amyloid precursor protein transgenic mice by positron emission tomography (PET) have offered quantitative information on the progressive deposition of A aggregates with aging and elimination of amyloid in the course of A immunization. Such technology also facilitates comparative selection of imaging agents suitable for specific purposes, such as detection of either initial or mature plaque lesions and assessments of emerging anti-amyloid treatments. Roles of microglia in anti-amyloid therapies are pursued by more direct modification of microglial activity, and these cells indeed ameliorate and deteriorate neuronal integrity, depending on the extent of their activation. Our work on mouse models has indicated that PET imaging of peripheral benzodiazepine receptor, which is selectively upregulated in activated glial cells, provides a quantitative measure in regulating microglial activity at a desirable level. This experimental system to preclinically test diagnostic and therapeutic approaches is also being applied to mouse models of tau pathology.

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in Vivo 1h Mrs Study of Stalevo-Treated and Untreated Patients With Parkinson's Disease (PD)

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Background and aims: We propose the quantitative indicators for the characteristics of the functional activity of the brain in patients with PD after single dose of STALEVO (Levodopa/Carbidopa/Entacapone (150/50/200)) treatment.

Methods: Three groups of patients are studied by 1H MRS with 1.5T Magnetom Vision (SIEMENS). The 1st group (TPG) includes 10 STALEVO-treated subjects with PD. The 2nd group (PG) includes 10 untreated subjects with PD. The 3nd group (VG) consists of 20 healthy volunteers. For all subjects spectra are recorded in the putamen with STEAM method: TR/TE=1500/135,155,175,200,270 ms. For subjects of TPG and PG the spectra are obtain in the putamen both ipsilateral and contralateral to the worst affected side.

Results:We found a significant reduction in NAA/Cho ratios from the putamen contralateral to the most affected side in the PG, but not the STALEVO-treated TPG grpups compared with VG. There were no significant differences in NAA/Cr or Cho/Cr ratios. In untreated patients of PG reduced putaminal NAA/Cho ratios may reflect loss of nigrostriatal dopamine terminals or alternatively indicate a functional abnormality of striatal putaminal neurons, such as membrane dysfunction due to striatal deafferentation.

Conclusions: This study suggests that NAA/Cho ratios may be affected by STALEVO-therapy and NAA/Cho values may provide an indicator (a reversible marker) of neuronal dysfunction in the striatum. This study gives a new insight into brain biochemistry in patients with PD.

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Metabolic Correlates of Cognitive Reserve in Dementia With Lewy Bodies: and FDG PET Study

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Background: Neuropathological, clinical, epidemiological, and imaging studies suggest that cognitive reserve allows patients with more years of schooling to cope better with brain damage. Research has been mainly focussed on Alzheimer's disease and no studies exist on patients with dementia with Lewy bodies (DLB). The aim of this study was to provide initial evidence for cognitive reserve in DLB. Methods: 21 consecutive patients with DLB and 16 age-matched healthy controls were included in the study. All study participants underwent cerebral 18F-FDG PET imaging at rest. First, a group comparison was conducted in SPM2 between the patient and control groups. Second, a linear regression analysis with relative metabolic rate of glucose as dependent and years of schooling as independent variable was performed. To control for demographical differences and cognitive status, age, gender, and a total score of the CERAD neuropsychological battery were included as covariates of no interest into the analysis. Results: Patients showed a significant metabolic reduction in the frontal and posterior association cortices, the basal ganglia, and the pulvinar of the thalami. Metabolic rate of glucose consumption and education showed an inverse relationship in an extensive cluster in the left temporo-parieto-occipital cortex. Conclusion: Similar findings were previously reported in Alzheimer's disease and are regarded as evidence for cognitive reserve. Therefore, we suggest that cognitive reserve is also present in DLB, allowing patients to cope better with neurodegenerative brain damage.

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In Vivo Measurement of Glutathione in the Human Brain in Aging and Alzheimer's Disease Using Magnetic Resonance Spectroscopy at 3T

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Glutathione (GSH) is the major antioxidant and plays a central role in the antioxidant defense mechanism to protect cells against oxidative damage caused by ROS in aging and neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). However, in vivo measurement of cerebral GSH in aging and AD has not been described to date. In this study, we measured GSH to investigate the role of GSH as a sensitive indicator of increased susceptibility to oxidative damage and as an in vivo biomarker to assess progression of aging and the neurodegenerative diseases in the living human brain.

GSH was measured in the fronto-parietal region of the brain (9 young controls; 13 elderly; 1 AD patients) using a novel MR CSI technique for GSH (8x8 matrix, FOV=20 cm, slice thickness 3-3.5 cm) at 3T.

The cerebral GSH concentrations in the parietal region were 0.8±0.1 µmol/g in the young controls and 0.6±0.1 µmol/g in the elderly. The GSH content was lower in the elderly by 25% (p=0.05) than in the young controls and was further decreased in the AD by ~48% compared to the young controls. The results suggest increased oxidative stress and/or lowered antioxidant defence system in aging and neurodegenerative diseases.

The capability of noninvasive measurement of GSH in the human brain should allow us to monitor the progression of neurodegenerative diseases (AD and PD), and the efficacy of pharmaceutical interventions and treatments directed at the antioxidant treatments.

This work is supported by American Health Assistance Foundation.

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Insight in FTD and AD: Conceptual Analysis and Empirical Evaluation of the Consensus Criterion

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Background: Loss of insight is a core criterion of FTD. However, according to clinical experience, FTD patients may be aware of their disease, and insight might be lost in AD. Objectives: To investigate the presence and absence of insight in the diseases, the relation between cognition and insight and to suggest a new formulation of the diagnostic criterion. Methods: Eight FTD and ten AD patients diagnosed by medical and neuropsychological examination, CT and PET participated. Insight was defined as: State awareness: Insight into oneâ€™s own states, e.g. consciousness of the alteration of oneâ€™s condition. In its absence, one is unaware and in a state of denial of alterations and fails to see a problem. Illness awareness: Knowledge that the alterations are symptoms of illness. In its absence, the patient is aware of a problem, but does not interpret it as a sign of illness. Medical awareness: Insight into the fact that the altered condition is an illness caused by given factors. Cognition was assessed by neuropsychological tests and insight by semi-structured, quantified interviews. Data were analysed with non-parametric methods. Conclusions: Insight were present in three FTD and six AD patients. Two types of insight emerged: emotional insight associated with frontotemporal functions, and cognitive insight, related to posterior cognitive functions. These results suggest that loss of insight should not serve as a core criterion of FTD, but serves well as a supportive criterion of the disease. The results also suggest that insight does not necessarily imply that inappropriate behaviour is controlled.

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Biomarkers of Neurodegenerative Disease: Use in Diagnostics and Treatment Evaluation

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Biomarkers are genetic, biochemical, molecular and imaging measures that seek to capture in the living person distinctive features of a disease. Biomarkers have the potential to improve clinical diagnosis, to track the course of disease and to detect the effects of therapy.

In AD, low levels of A?1-42 and high levels of tau in CSF are ~85% sensitive and specific for diagnosis; a similar pattern denotes individuals with amnestic MCI who are destined to develop AD. Plasma levels of A? do not aid in the diagnosis of non-familial sporadic AD, but high levels of A?1-40 (and, independently, high homocysteine levels) are linked to lacunes and white matter hyperintensities on MR brain scans in subjects with MCI, AD and cerebral amyloid angiopathy. Low levels of plasma A?1-40 and 1-42 are associated with a rapid rate of decline in AD. From an imaging perspective, PET brain scans with the radioligand PiB show promise for monitoring the effects of anti-A? therapies in AD. Increased levels of F2-isoprostanes in CSF have been reported in AD, but plasma levels do not distinguish AD.

In PD, levels of ?-synuclein are decreased in CSF and show promise as a diagnostic biomarker. In epidemiological studies, high concentrations of blood uric acid are associated with reduced risk of developing PD. Urates show promise as biomarkers: higher baseline serum and CSF levels are associated with slower progression of PD.

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AD Biomarkers and Neuroimaging in Treatment Evaluation

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Abeta lowering therapies are currently being tested for safety and efficacy in Alzheimer's disease (AD). The clinical evaluation of therapeutic efficacy by cognitive measures requires observation periods of at least 12 or 18 months. The use of biomarkers and neuroimaging may provide biological evidence of mechanistic proof of concept in addition to the clinical outcome over time. We observed restoration of hippocampal volumes measured by volumetric MRI over a period of three years in response to beta amlyoid immunization. Higher hippocampal volumes were correlated with better cognitive outcome. To better characterize clincal outcome in such studies in the future, we explored the use of PET-ligands for brain beta-amyloid in patients with AD and age-matched control subjects and for the glutamatergic system in healthy subjects.

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Cerebrospinal Fluid Biomarkers of Early Stage Alzheimer Disease

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Background and Aims: Identification of "preclinical" Alzheimer's disease (AD), prior to substantial neuronal, synaptic and cognitive loss that is associated with AD, may permit new therapies to have optimal benefit. We assessed the ability of candidate cerebrospinal fluid (CSF) and plasma measures combined with amyloid imaging to discriminate early-stage AD from nondemented aging, and whether these biomarkers can predict future dementia in cognitively normal individuals.

Methods: Healthy volunteers (n=139), 60-91 years of age, clinically judged as cognitively normal (Clinical Dementia Rating, or CDR, 0) or having very mild (CDR 0.5) or mild (CDR 1) AD dementia were recruited from a longitudinal study of healthy aging and dementia. Levels of CSF Abeta40, Abeta42, tau, ptau181 and plasma Abeta40 and Abeta42 were assayed by ELISA.

Results: Individuals with very mild and mild AD have reduced levels of CSF Abeta42 and increased CSF tau and ptau181. There is complete correspondence of CSF Abeta42 level with the presence or absence of brain amyloid as imaged in vivo with the amyloid-binding agent, Pittsburgh Compound B (PIB), in demented and nondemented individuals. CSF tau/Abeta42 (adjusted HR=5.21) and ptau181/Abeta42 (adjusted HR=4.39) predict conversion from CDR 0 to CDR>0.

Conclusions: The very mildest symptomatic stage of AD exhibits the same CSF biomarker phenotype as more advanced AD. Levels of CSF Abeta42, when combined with amyloid imaging, identify individuals with brain amyloid deposits, whether or not dementia is present. Importantly, CSF tau/Abeta42 measures show strong promise as antecedent (preclinical) biomarkers that predict future dementia in cognitively normal older adults.

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Conformationally Altered P53: A Putative Marker for Alzheimer's Disease

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The identification of biological markers of AD can be extremely useful to improve diagnostic accurancy and/or to monitor the efficacy of putative therapies, in addition to increase our understanding of the pathogenesis of the disease. In this regard, peripheral cells may be of great importance, because of their easy accessibility. Recently our group demonstrated an impairment of p53 signalling pathway following an oxidative injury that resulted in a lower vulnerability of AD fibroblasts: fibroblasts from sporadic AD patients specifically express an anomalous and detectable conformational state of p53 (named as mutant p53) that makes these cells distinct from fibroblasts of age-matched non-AD subjects. On the basis of these evidence, data will be presented about the different expression of mutant p53 between AD and non-AD subjects, using peripheral blood cells.

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The Early Detection in Parkinson's Disease: Unmet Needs

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Treatment starting at late stages of Parkinson's disease (PD) comes too late to halt or even to delay the disorder.

We are currently working on projects for the early (preclinical) detection.

1) Neuromelanin (NM) is released from dying dopaminergic cells into the surrounding tissue where it is phagozytised and transported to the blood. We have thus developed an novel assay to detect antibodies to NM in blood samples.

2) Gene chip analyses of four brain regions from controls and PD patients post-mortem indicate significant alterations in the expression of various proteins. Significantly changed expression of certain proteins will be tested by screening

healthy persons for genetic alterations which may provide early markers for this devastating disorder.

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Role of Biomarkers and Neuroimaging for the Early Diagnosis and Evaluation of PD

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A biomarker is a characteristic that is objectively measured and evalueated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention. (Lesko LJ and Atkinson AJ Jr, Annu Rev Pharmacol Toxicol. 2001;41:347-66) Biomarkers in PD should offer the potential to identify individuals at risk for PD, screen new therapies, assist in the diagnosis and help optimize management of PD. In recent years a number of potential candidate-biomarker for the early diagnosis and evaluation of PD have been identified including the use of neuroimaging techniques that reflect function of the dopaminergic system, transcranial sonography of the substantia nigra, clinical tests designed to detect subtle (non)motor manifestations of PD such as the assessment of olfaction by smell tests or genetic testing that however is limited to a small proportion of early-onset PD individuals. Recent findings on the role of biomarkers and neuroimaging for the early diagnosis and evaluation of PD will be discussed.

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Serotonin 5-HT4 Receptors as Targets for the Treatment of Alzheimer's Disease

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The serotonin 5-HT4 receptor is a member of the seven transmembrane-spanning G-protein-coupled receptor (GPCR) family and constitutes an important subtype of the class of serotonin receptors. The 5-HT4 receptor was characterized in mouse colliculi neurons almost 20 years ago and it was shown to be positively coupled to adenylate cyclase leading to cyclic AMP production. Since then, it has received a considerable attention for its physiological effect in the central nervous system (CNS). Indeed, with the availability of selective 5-HT4 ligands and the cloning of 5-HT4 receptor C-terminal splice variants, many progresses have been made in their pharmacological characterisation, tissue distribution and the determination of their functional roles in the CNS. In particular, behavioral and neurochemical studies have shown the involvement of the 5-HT4 receptor in cognitive processes making this GPCR a possible therapeutic target for symptomatic treatment of memory disorders such as Alzheimer's disease. The use of partial 5-HT4 agonists should limit adverse peripheral actions in the gastrointestinal and cardiovascular systems. In addition, recent findings support a link between 5-HT4 receptor and the amyloid precursor protein processing. Here we will present recent advances that highlight the therapeutic potential of the 5-HT4 receptor and its associated signalling pathways in AD.

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Alpha7 Nicotinic Acetylcholine Receptors: Cellular Mechanisms and Therapeutic Opportunities

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Nicotinic receptors (nAChRs) in the brain are predominantly modulatory. The alpha7 subtype of nAChR has attracted particular attention because of its high calcium permeability (comparable to that of NMDA receptors), coupled with rapid desensitisation that would limit calcium influx and avoid excitotoxic consequences. Activation of alpha7 nAChRs can improve cognitive performance and recent evidence from alpha7 knockout mice supports a role in memory and attentional processes. These receptors are relatively abundant in hippocampus and cortex, on GABA and glutamate neurones. Presynaptic alpha7 nAChRs facilitate glutamate release and contribute to changes in synaptic efficacy; alpha7 nAChRs also promote longer term changes in hippocampal neurones by calcium-dependent activation of the ERK/MAP kinase pathway and the transcription factor CREB. Hence alpha7 nAChRs can influence cellular mechanisms of learning and memory, and could provide a rational target for symptomatic improvement in AD to counter cognitive decline. In addition, these receptors are intimately linked with beta-amyloid (1-42): they have been found in amyloid plaques and beta-amyloid has been variously reported to bind to, block, activate and be internalised via alpha7 nAChRs. One view is that alpha7 nAChRs might provide a conduit for beta-amyloid toxicity, although activation of alpha7 nAChRs is neuroprotective in various other models of cell death.

These features have encouraged the development of selective drugs to target alpha7 nAChRs. The partial agonists GTS21 and SSR180711, full agonist Compound A, and positive allosteric modulator PNU-120596 are novel tools for clarifyling the roles of alpha7 nAChRs and evaluating their potential for therapeutic intervention.

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Histamine H3 Receptor Antagonism - A Novel Symptomatic Approach to the Treatment of Alzheimer's Disease

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Progressive decline in cognitive performance is a key characteristic of Alzheimer's Disease. Current therapies such as cholinesterase inhibitors provide only minimal benefit to a subset of patients and for a limited period of time, so a number of alternative symptomatic strategies are being pursued, including the development of selective histamine H3 receptor antagonists. H3 receptors are widely expressed in the mammalian brain, particularly in areas involved in cognitive

processes and arousal, such as the cerebral cortex, hippocampus, basal ganglia and hypothalamus. Activation of these receptors inhibits the release of several neurotransmitters including acetylcholine, histamine, noradrenaline and dopamine. Conversely, blockade of H3 receptors with selective antagonists can increase the release of these neurotransmitters. Selective H3 antagonists have been shown to improve performance in a diverse range of rodent cognition paradigms and can also increase wakefulness. H3 antagonists are currently in clinical trials for a number of disease indications but to date no efficacy data has been reported in patient populations. Various aspects of H3 receptor antagonism as a potential novel approach to the symptomatic treatment of Alzheimer's Disease will be discussed.

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Insulin Secretion and Risk of Dementia

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Background and aims: People with diabetes may have an increased risk of dementia. However, there are few detailed epidemiological data on which mechanisms mediate this relation. We investigated the longitudinal association between insulin secretion, insulin sensitivity, diabetes and the subsequent development of Alzheimer's disease (AD) and dementia.

Methods: The population-based Uppsala Longitudinal Study of Adult Men started at age 50 in 1970. Of total 2322 participants 102 persons were diagnosed with AD and 386 with any dementia or cognitive impairment during a median follow-up of 31.6 years. The insulin response was measured with intravenous glucose tolerance test (IVGTT) at age 50 and with oral glucose tolerance test (OGTT) and euglycaemic insulin clamp at age 70. Associations were analysed using Cox proportional hazard models.

Results: High insulin response was associated with lower risk of AD [insulin increment: HR 0.76 (95%CI 0.63-0.91), early insulin response: HR 0.79 (0.63-0-98)] also after adjustment for APOE ε4, blood pressure, BMI, cholesterol and education level. Similar associations were found when we measured insulin response with IVGTT at age 50 or OGTT at age 70.

Conclusions: In this longitudinal study, impaired insulin secretion was associated with increased risk of AD later in life. We suggest that diabetes contributes to the pathogenesis of AD through changes in insulin metabolism.

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The Caide Dementia Risk Score: A Practical Tool to Predict Dementia Risk in 20 Years Among Middle Aged Persons

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Background: Several vascular risk factors are linked to dementia. Risk scores have been developed to predict cardiovascular events, and could be helpful also in identifying individuals at an increased risk for dementia.

Methods: Population-based CAIDE study includes 1409 survivors who were studied in midlife (mean age 50 years) and re-examined 20 years later to detect dementia. Several midlife vascular risk factors were studied to create the scoring tool. The score values were estimated based on the beta-coefficients, and the Dementia Risk Score was composed as the sum of the individual scores (range 0-15).

Findings: Significant, independent predictors of future dementia were high age, low education, midlife hypertension, hypercholesterolemia, and obesity. The Dementia Risk Score predicted dementia well (AUC 0.77; 95% CI 0.71-0.83). The risk of dementia according to the categories of Dementia Risk Score was 1.0% for those with score 0-5; 1.9% for 6-7; 4.2% for 8-9; 7.3% for 10-11; and 16.4% for those scoring 12-15. When the cut-off >9 points was applied, the sensitivity was 0.77, the specificity was 0.63, and the negative predictive value was 0.98.

Interpretation: The Dementia Risk Score is a novel approach for developing a practical tool to predict dementia risk. We are currently further developing the risk score by adding new variables and testing its validity in the Kaiser Permanente population, US. The Dementia Risk Score highlights the role of vascular factors in the development of dementia, and may help to identify individuals who can benefit from intensive lifestyle consultations and pharmacological interventions.

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Prevalence and Natural Course of Aging-Associated Cognitive Decline in a Longitudinal Population-Based Study (ILSE): Preliminary Results of the Third Wave

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Objective: Little is known about prevalence and conversion from mild cognitive impairment (MCI) to dementia of subjects at risk in the general population.

Method: Within the population-based Interdisciplinary Longitudinal Study on Adult Development and Aging (ILSE), neuropsychological functioning was assessed in 500 community-dwelling subjects who were born during 1930–1932 in Heidelberg and Leipzig. The participants were carefully screened for physical and mental health in 1994 (t1), 1998 (t2) and are being re-examined since July 2005 (t3). MCI was diagnosed according to the "aging-associated cognitive decline" (AACD) criteria. All diagnoses including conversion to AD were result of a consensus conference under supervision of senior consultant in old age psychiatry.

Results: Until now, 249 patients (121 females, 128 males) of the cohort have been examined in T3. Mean age was 62.4±2.4 years at baseline, 66.7±1.1 years at t2 and 73.9±0.84 years at t3. At baseline, 13.4% of the subjects fulfilled the AACD criteria. In t2 AACD prevalence rates rose to 23.6% and in t3 to 26.1%. In t3 there where 11 (4,4%) patients who fulfilled the criteria for an Alzheimer's Disease (AD), and one (0,4%) for a Vascular Dementia (VaD).

Conclusions: As indicated by the longitudinal population-based study (ILSE), AACD is a frequent condition in the general population. While AACD prevalence increased in the longitudinal course, a subgroup of the AACD patients developed AD.

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Computer Based Cognitive Training With MindFit® Improved Cognitive Performances Above the Effect of Classic Computer Games; Prospective, Randomized, Double-Blind Intervention Study in the Elderly

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Background: Computer games training can improve cognitive performances (CP). Objective: To compare the effects of home-training with the recently developed MindFit® computer based package (CogniFit, LTD, Israel) to classic computer games on CP in elderly people.

Method: Self-referred volunteers, aged 50+, were randomly assigned to practice at home (3 months, 3 times a week for half an hour each session) with MindFit® package or with pre-chosen classic computer games. CP was assessed blindly at baseline and after 3 months by the computerized neuropsychological battery, NexSig®. Linear models were used to evaluate the differences in CP scores between and within groups.

Results: 121 people completed the protocol (34 dropped out during training): 66 in the Mindfit® group and 55 in the games group), which were similar in baseline characteristics. The MindFit® group improved significantly in total NexSig® score ($p<.0001$) and in all 8 sub-scores ($p<.0031$ in 7 sub-scores) while the games-group improved in total NexSig® score ($p=.0146$) and in 5 sub-scores ($p<.0472$). The MindFit® training group achieved higher post intervention total score, compared to the games group ($p=.0817$), especially in subjects with lower baseline CP (baseline scores modified the training effect).

MindFit® training with had a significant superiority in 3 cognitive domains: spatial short term memory ($p=.0001$), visuo-spatial learning ($p=.0012$) and focused attention ($p=.0019$).

Conclusions: Computerized cognitive training with MindFit® was better than classic computer games for improving cognitive abilities. These results demonstrate that home training with a specially designed computer-based program can improve several cognitive domains which have an important role in daily life such as driving. People with lower baseline CP gained more than those with normal cognition, thus demonstrating the potential therapeutic effect of home based computer games training in the elderly. Future studies should specifically assess the role of home based cognitive training on the rate of cognitive decline and the long term carry over effect.

This study was supported in part by CogniFit LTD, Israel

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Cell Models of Tauopathy Expressing the Tau Repeat Domain Show That Tau Aggregation is Toxic to Cells But is Reversible

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In order to study tau's mechanisms of neurodegeneration in Alzheimer's and other brain diseases we generated several cell models of tau pathology. Inducible N2a neuroblastoma cell lines were created that express different variants of tau when exposed to doxycyclin. Three variants of tauRD were chosen, based on the repeat domain of full-length htau40: the 4R wild-type repeat domain (K18), the 4R repeat domain with the deletion mutation delK280 known from frontotemporal dementia and highly prone to spontaneous aggregation (pro-aggregation mutant), and the 4R repeat domain with delK280 and two further proline point mutations that strongly inhibit aggregation (anti-aggregation mutant). The comparison of these wild-type, pro-aggregation and anti-aggregation tau constructs shows: (1) Tau aggregation into paired helical filaments is toxic to cells, (2) the degree of aggregation and toxicity strongly depends on tau's propensity for beta-structure, (3) soluble tau mutants that cannot aggregate are also not toxic; (4) tau phosphorylation in the repeat domain (at KXGS motifs) precedes aggregation but is not correlated with the degree of aggregation; (5) tau aggregates disappear when the tau expression is silenced, showing that aggregation is reversible; (6) tau aggregation can be prevented by adding certain low MW inhibitory compounds to the cells. Thus, the cell models open up new insights into the relationship between tau structure, expression, phosphorylation, aggregation, and toxicity that can be used in the screening drugs for AD and other tauopathies. - Supported by DFG.

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Nigral Degeneration and Caspase 3 Activation Resulting From Abarrant Splicing of Tau in a Mouse Model

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Tau protein regulates and stabilizes the assembly of microtubules by binding to them through 3 or 4 microtubule binding repeats (MTBR). Whether the tau protein has 3 or 4 MTBR is determined by the inclusion or exclusion of the second MTBR encoded by exon 10 of the TAU gene. TAU gene mutations that alter the splicing of exon 10 and hence the 4:3 MTBR ratio are causal in Frontotemporal Dementia and Parkinsonism linked to chromosome 17 (FTDP-17). The TAU N279K mutation has been identified in several families which present with parkinsonism, movement disorders, dementia,

neuronal and glial intracellular tau inclusions and neuronal cell loss.

We have engineered a human tau minigene construct that by design allows alternative splicing of tau exon 10. Using this construct we have created a transgenic mouse model that expresses human TAU protein with the N279K mutation under the regulation of the human TAU promoter. This first model of aberrant tau splicing recapitulates many of the disease symptoms that are seen in patients with the N279K mutation to include; motor and behavioral deficits, tau accumulation in neurons, neuronal processes and tufted astrocytes and neuronal cell loss. Furthermore, these mice present with degeneration of the nigrostriatal dopaminergic pathway suggesting a possible mechanism for parkinsonism. Additionally, activated caspase 3 immunoreactivity in both neurons and astrocytes implicates apoptosis as a pathway involved in neurodegeneration resulting from aberrant splicing of tau.

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The High-Affinity Hsp90-Chip Complex Recognizes and Selectively Degrades Phosphorylated Tau Client Proteins

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Background and aims: A primary pathological component of Alzheimer's disease (AD) is the formation of neurofibrillary tangles (NFT) composed of hyper-phosphorylated tau (p-tau). Expediting the removal of these p-tau species may be a highly relevant therapeutic stratagem.

Methods: To address our questions we utilized a variety of cell culture techniques, genetic knockdown (siRNA), biochemical approaches and in vivo models.

Results: Herein we report that Hsp90 inhibition degrades p-tau independent of heat shock factor 1 (HSF1) activation. CHIP, a tau ubiquitin ligase, is a critical mediator of this mechanism. Co-chaperones are also involved in Hsp90-mediated removal of p-tau; however those of the mature Hsp90 re-folding complex prevent p-tau degradation. This is the first demonstration that genetic blockade of the re-folding pathway promotes p-tau turnover through degradation. We also show that peripheral administration of a novel Hsp90 inhibitor promotes selective degradation of p-tau species in a mouse model of tau accumulation, further suggesting a central role for the Hsp90 complex in the pathogenesis of tauopathies.

Conclusions: When taken in the context of known high-affinity Hsp90 complexes in affected brain regions of AD patients, these data implicate a central role for Hsp90 in the development of AD and other tauopathies, and may provide a rationale for further therapeutic strategies.

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Distinct Phosphorylation Pattern of Peptidyl Prolyl Isomerase Pin1 During Neurofibrillary Degeneration in Alzheimer and Tau Transgenic Mouse

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The peptidyl-prolyl isomerase Pin1 isomerizes specific motifs of phoshorylated Serine or Threonine followed by Proline (pSer/Thr-Pro). Pin regulates the phosphorylation of Tau and APP. It has been recently reported that Pin1 is oxidized and down regulated in the hippocampus of Alzheimer's disease (AD). Therefore, Pin1 has been proposed to have a neuroprotective function against AD. Pin1 activity is regulated by phosphorylation at serine 16 (Ser16) by Protein Kinase A. Pin1 is also phosphorylated at Ser65 by Polo-like kinase 1 and is supposed to stabilize Pin1. We have also suggested that phosphorylation at Ser115 may be involved in the catalytic activity of Pin1. However Pin1 has numerous potential phosphorylation sites on serine, threonine or tyrosine which possibly regulate its function and/or metabolism. Moreover, the phosphorylation state of Pin1 has not been investigated in AD. In the present study, analysis of Pin1 phosphorylation was performed in Alzheimer's disease using 2D gel electrophoresis showing some changes. Moreover, this modified phosphorylation pattern was similarly observed in our Tau transgenic animal model in an age-dependent manner, consisting with the severity of tau pathology. To further investigate the relationships between tau levels and Pin1 phosphorylation, Pin1 isovariants were analyzed in SY5Y inducible Tau expression system. A similar change in Pin1 phosphorylation was observed after phospho-Tau accumulation. Together, our data suggest that altered phosphorylation of Tau, an early event in NFD, implicates Pin1 but also reversibly modified Pin1 phosphorylation state and most likely its function in AD and Tauopathies.

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Human Brain Tissue, Spinal Cord Tissue and CSF Available for Research Projects

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The aim of the PDS tissue Bank is to supply high quality samples of human brain and other tissues to scientists studying the causes and treatment of Parkinson's and related disorders in the UK and Worldwide. The Tissue Bank also aims to collect tissue in an ethical manner and in a way that it is most suitable for all research needs. The Tissue Bank currently has over 160 cases and samples are available from people who have had; Parkinson's disease; Parkinson's-like disorders (e.g. Multiple System Atrophy, MSA; Progressive supranuclear Palsy, PSP); and those who are healthy control donors. The tissue comes from registered prospective donors.

The majority of our tissue is collected within 24 and snap frozen and fixed tissue is available from each case. A detailed Neuropathological and clinical report is available on each case for the researcher.

We welcome applications for tissue and researchers are asked to complete and return a tissue request form, which outlines the project the tissue will be used for and details the tissue required. Each request is reviewed by an independent scientific panel, to verify the scientific merit and its possible

benefit to Parkinson's disease research. The research teams are also asked to provide a written report of the findings of research carried out on tissue supplied from the Tissue Bank.

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Amyloid Deposition and Glucose Metabolism in Parkinson's Disease Dementia (PDD): An [11C]PIB and [18 F]FDG PET Study

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Introduction: Neuropathological studies have reported varying amounts of amyloid pathology associated with PDD and dementia with Lewy bodies (DLB). 11C-PIB PET is a marker of brain amyloid deposition. Regional glucose uptake reflects synaptic activity and FDG PET studies have shown reduced glucose uptake in cortical association areas in Parkinson's disease with dementia. The relationship between amyloid load and brain metabolism in PDD is unclear.

Aim: To correlate amyloid deposition and glucose metabolism in PDD and to compare this with controls.

Methods: 8 control subjects and 6 PDD patients were recruited. Each subject had MRI and two PET scans (PIB and FDG) .Amyloid load was quantitated as 60-90' target:cerebellar uptake ratios (RATIO). Object maps were created using a standard probabilistic atlas. Cortical PIB and FDG uptake were assessed using region of interest analysis.

Results: None of the 6 PDD patients showed any increase in amyloid uptake but all of them had reduced glucose metabolism in frontal, temporal, parietal and occipital areas compared to controls (p value<0.03).

Conclusion: This pilot study confirms significant hypometabolism in the frontal, temporal, parietal and occipital cortex in PDD. In these subjects amyloid deposition did not contribute significantly to the pathogenesis of their dementia which is likely to arise from a combination of cortical Lewy body disease and neurotransmitter changes.

PDD	PIB	Frontal	Temporal	Parietal	Occipital
	Mean	1.05	1.08	1.05	1.08
	SD	0.09	0.09	0.07	0.07
Control	PIB				
	Mean	1.1	1.06	1.09	1.1
	SD	0.08	0.05	0.06	0.04
PDD	FDG				
	Mean	0.22	0.18	0.2	0.19
	SD	0.03	0.02	0.02	0.03
Control	FDG				
	Mean	0.28	0.26	0.28	0.3
	SD	0.02	0.02	0.02	0.02

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Matrix Metalloproteinase-3 (MMP-3) Cleavage of Parkinson's Disease-Related Gene Products: Its Implication on PD Pathogenesis

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Recently, several genes responsible for inherited form of PD have been mapped: two autosomal dominant disease genes, α-synuclein and LRRK2, and three genes for autosomal recessive PD, parkin, DJ-1 and PINK1. Previously we showed that matrix metalloproteinase-3 (MMP-3) plays an important role in dopaminergic neuronal degeneration in MPTP model. MMP-3 was localized in Lewy bodies (LBs) in the post mortem brains of PD patients. Since MMP-3 is a proteinase, we explored hypothesis that MMP-3 modifies or degrades PD-related gene products. We've found that MMP-3 can efficiently cleave α-synuclein, DJ-1, PINK1 and LRRK2. This modification was not mediated by other MMPs. Interestingly, MMP-3 results in different cleavage pattern of A53T, pathogenic α-synuclein mutant. Amino acid 1-91, 1-93 and 1-78 were significantly increased in A53Tsyn digested by MMP-3. Cell lines stably over-expressing each fragment revealed that each fragment differentially affects proteasomal and mitochondrial function. MMP-3 was induced in both primary mesencephalic culture or SN 4741 dopaminergic cells oxidative stress. After stress, SN4741 cells over-expressing α-synuclein showed increased fragmented peptides, which was reversed by pre-treatment with NNGH, a specific MMP-3 inhibitor. Under similar stress conditions, DJ-1 level was decreased by MMP-3-mediated degradation, which is reversed by NNGH pretreatment. Phosphorylation of Akt, down stream survival pathway of DJ-1, was decreased by oxidative stress and reversed by NNGH. Functional modification of PINK-1 and LRRK-1 by MMP-3 cleavage is now under investigation.

. The results strongly indicate that MMP-3 digestion of PD-related pathogenic gene products in vivo may play an important role in PD pathogenesis.

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Homocysteine and Cognitive Impairment in Elderly People

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Background and aims: Age and years of education are among the most relevant risk factors for dementia, but in recent years the role of homocysteine has also been investigated. There is evidence that increased serum homocysteine levels are associated with declining cognitive function and dementia. This report presents the relationship between homocysteine and cognitive function in healthy elderly people.

Methods: A sample population of 256 community-dwellers aged 65 years and over was recruited in three separate rural towns in Shimane prefecture, Japan, and 91 elderly were measured in two years. We divided the population as follows: 28 in an improved group (2-6 points increase, average age 72.9 years old), 45 in a no-change group (less than 1 point, 73.5 years old), and 18 in an aggravation group (2-6 points decrease, 73.9 years old) by the Revised version of Hasegawa Dementia Scale (HDSR)change of one year.

Results: Homocysteine was not correlated with age, Zung self-assessment depression scale or ADL scale. However, homocysteine was significantly related with HDSR($p<0.001$).

Homocysteine did not significantly change (10.9±4.7 to 11.7± 4.0) in the improved group, but did increase significantly ($p<0.001$) from 10.7±5.2 to 12.7±5.6 in the no-change group and 10.1±2.3 to 12.2±3.2 in the aggravation group. In a cohort study, homocysteine levels at baseline and increases in one year were found to be related to changes in cognitive function score.

Conclusions: These results suggested that plasma homocysteine has a close relationship with cognitive function.

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Mild Cognitive Impairment in Parkinson's Disease

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Introduction: Mild cognitive impairment (MCI) is presumed to be positioned between normal cognition and dementia. The prevalence and rate of conversion of MCI to dementia in PD remains unknown.

Objective: to study prevalence of MCI and establish the rate of progression to dementia in patients with PD.

Methods: 42 non-demented PD-patients (mean age 61, 36 + 8, 81, mean duration disease 4,38 +3,48) underwent neurological and clinical neuropsychological evaluations at baseline and 2-5 years later.

Results: At baseline assessment 9 PD-patients had MCI (21,4%). Remaining 33 (78,6%) patients did not meet Petersen's criteria for MCI but nevertheless showed some degree of cognitive deterioration in complaints and neuropsychological testing. We include them to the group of patients with subtle cognitive impairment (SCI). Of the MCI-patients 7 had multiple domain type (MCI-md), 2- amnesic type (MCI-a). At 2-5 years follow-up 14 (42,4%) patients with SCI converted to MCI (13 MCI-md, 1 MCI-a), 1 patient converted to dementia and 18 remained stable. 4 (57,1%) MCI-md patients became demented and 3 (42,1%) patients did not meet criteria to dementia. In patients with MCI-a 1 converted to dementia and 1 showed no progression. Patients with MCI-md demonstrated deficit of executive functions. MCI-a patients showed hippocampus dysfunction.

Conclusions: MCI syndrome in PD-patients is heterogeneous and is prone to convert to dementia within 2-5 years in the majority cases. Besides overt MCI we revealed more subtle cognitive impairment which sometimes is also characterized with progression.

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Atypical Familial Parkinson Syndrome in the Allgäu (Bavaria): Phenotype, Imaging and LRRK2-Screening

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Although pathogenetic mutations in six different genes have been discovered, most cases of sporadic as well as familial Parkinsonism are still unexplained.

We describe a large family originating from the eastern Allgäu in Bavaria, Germany. Examination of 26 adult family members of two generations included an interview with family history, physical examination, Mini Mental Status Test, Clock Drawing Test and Schellong Test.

Three of the family members showed clinical signs consistent with Parkinsonism and atypical pyramidal signs at the ages of 57, 66 and 71. Another branch of this family with at least one more patient with the diagnosis of Parkinson disease came to our attention recently. A variant phenotype was shown by a fifth patient (64 years of age) with psychosis and bilateral tremor since the age of 15. DAT-SPECT was denied by his legal representative.

Depression and dementia occurred among several relatives. Eight family members aged 35 to 66 exhibited soft signs of the familial syndrome whereas 14 members (age 22 to 71) were considered to be free of symptoms. Autonomic dysregulation was not a prominent feature. The inheritance pattern seemed to be autosomal dominant with variable penetrance.

DAT-SPECT in two patients revealed asymmetrically reduced uptake in the Caudate. As D2-receptor degeneration in the IBZM-SPECT was not significant, imaging was compatible with typical Parkinson Disease.

Results of mutation screening of LRRK2, tau and alpha-synuclein in this family are pending.

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Speech Characteristics of Idiopathic Parkinson's Disease

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Background: Change of the motor components in speech is one of the earliest and most frequent finding in Parkinson's disease (PD). Despite of its clinical importance, little is known about speech impairment in PD due to difficulty of evaluation. Speech can be influenced by various confounding factors affecting phonation, articulation, resonance, or respiration besides PD per se. We performed acoustic analysis of parkinsonian speech to define the characteristic speech pattern and to determine whether the acoustic parameters might be sensitive markers for clinical progression.

Methods: Twenty five patients with PD and their age- and gender-matched controls were recruited for the study. The acoustic analysis was done using Praat v. 4.4.26. We evaluated the maximum phonation time (MPT), jitter, shimmer, voice onset time (VOT), and fundamental frequency of speech. The Unified Parkinson's Disease Rating Scale and Hoehn and Yahr stage were used to evaluate the degree of motor disability in patients with PD.

Results: Significant decrease in the fundamental frequency and variability during free speech was observed in patients with PD compared with those of controls. Increase in VOT of stop consonants and decrease in MPT during sustained phonation of vowels were found in the patient group.

Conclusion : Characteristic speech patterns on the acoustic analysis might be helpful for clinical diagnosis of PD and can be sensitive markers for the disease progression.

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Clinical and Neuroimaging Study in An Overlap Case of PD and FTD

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Background: Cognitive and behavial impairment of Parkinson disease(PD) has been already discussed. It has been reported that SPECT and PET images are usuful for evaluation of patients with dementia and behavial disorder. To perform MRI is important to exclude atypical parkinsonisms, such as PSP, CBD, MSA, and FTD.

Objective:To investigate the neuroradiological characteristics and clinical course in an overlap case of PD and FTD.

Subjective:76 yaers old Japanese female who had been diagnosed as PD 5years ago and treated with levodopa/DCI ,cabergoline and selegiline,was presented with hallucination,delusion and behavial abnromality (a favorite change of a meal and clothes).

We adjusted her antiparkinsonian medication, after that her hallucination were reduced, in otherwise wearing-off phenomenon was frequently appeared and also behavial abnormality was continued. Neuroimaging studies such as MRI(1.5T), Tc99m-ECD-SPECT images and FDG-PET images.

Result: MRI showed left lateral temporal atrophy. Reduction of cerebral blood perfusion was recognized especially at bifrontal and left temporal lobes with Tc99m-ECD-SPECT images.Glucose metabolism was decreased in almost whole brain.

Two years later her parkinsonism was slightly worsened and preference of her meal were changed several times. Disinhibition ,frontal lobe signs and apathy were gradually worsening.

Conclusion: Though SPECT and PET studies are useful diagnostic tools in psychological disorders, repeated examination of MRI and reassessment of physical and psychological symptoms are important for diagnosis in this case.

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Assesment of the Olfactory Bulb Pathology in Parkinson's Disease

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A loss of the ability to smell is often reported as an early clinical symptom in Parkinson's disease (PD). Lewy bodies have also been reported to be present in the olfactory bulb and tract and there are significant increases in neuronal loss with longer disease durations (Pearce et al 1995). However, there is a lack of knowledge whether the neuronal loss correlates with alpha-synuclein deposition, or the presence of secondary pathology e.g. Alzheimer type, or the presence of activated microglia. This comparative study utilised olfactory bulbs from the UK Parkinson's Disease Society Tissue Bank at Imperial College, London from consented PD donors. Consecutive serial sections of fixed paraffin embedded olfactory bulb were stained with histological stains; haematoxylin and eosin, cresyl fast violet and immunohistochemical antibodies: alpha-synuclein, ubiquitin, tau, beta-amyloid and MHC class II (marker of activated micro-glia). Utilising stereological techniques the sections from each subject were scored for the numbers of neurons and for the presence Lewy bodies and Lewy neurites, neurfibrilary tangles and plaques and activated micro-glia. We found a positive correlation between the levels of alpha synuclein staining of Lewy bodies and neurites and the number of activated microglia within the bulb, however they were not consistently co-localised in the same areas of the bulb. The significance of these findings will be discussed in the light of the presence/absence of secondary pathology, age, disease duration and disease severity.

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Freezing of Gait and Executive Functions in Patients With Parkinson Disease

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Background: Freezing of gait (FOG) is a common and debilitating disturbance in patients with Parkinson's Disease (PD). FOG refers to paroxysmal events, usually lasting seconds, in which a subject is unable to initiate or continue locomotion occurring on a background of relatively good ability to move. To date, FOG pathophysiology has not yet fully explained.

A frontal lobe dysfunction or a disconnection between frontal lobe and basal ganglia, have been proposed to explain the occurrence of FOG. We explored frontal functions in PD patients by means of a battery of neuropsychological tests.

Method: 13 PD patients in early stage of disease (H&Y≤2.5) with freezing during on (FOG +) and 15 age-,H&Y score-, disease onset-, disease duration- and medication-matched PD patients without freezing (FOG-) were investigated; all patients were neither demented nor depressed. The assessment included UPDRS I-IV, FOG questionnaire, Stroop test, Frontal Assessment Battery (FAB), verbal fluency, Ten-point clock test (TPCT)

Results: There were no significant differences in UPDRS I-IV and MMSE scores between the two groups; FAB, verbal fluency and TPCT scores were significantly lower in FOG + patient compared with FOG - subjects (FAB: $P = 0.015$; verbal fluency: $P = 0.011$; TPCT: $P = 0.024$).

Conclusion: FOG seems to correlate with lower scores at frontal tests in patients with PD in early stage of disease

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Deficit of Automatic Auditory Change Detection in PDD: Mismatch Negativity Compared to Dementia With Lewy Bodies and Alzheimers Disease

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Objective: To investigate whether automatic auditory change detection, as

measured by the mismatch negativity (MMN) event-related potential waveform, differs in dementia associated with Parkinson's disease(PDD) and dementia with Lewy-bodies (DLB) as compared to Alzheimer's disease (AD), Parkinson's disease without dementia (PD) and healthy control subjects (HC).

Method: 17 DLB, 15 PDD, 16 PD, 16 AD patients and 18 HC subjects participated. The dementia patients were matched on overall MMSE score. A passive MMN paradigm with duration- deviant stimuli was presented. In addition, the subjects completed an oddball-distractor task in which they had to selectively respond to a frequency-deviant tone, while ignoring a frequent standard tone and a distractor stimulus consisting of white noise.

Results: The PDD patients had reduced MMN area and amplitude compared to the DLB group, as well as to the PD, AD and the HC groups. The MMN area correlated significantly with number of missed target stimuli in the oddball-distractor task, and the PDD group missed targets significantly more often than the DLB group.

Conclusion: The MMN and behavioral results taken together indicate that PDD patients may have a specific deficit of automatic auditory change detection that contribute to impairment in their ability to selectively attend and respond to deviant auditory stimuli. The results indicate that attentional functions may differ in the auditory modality for PDD vs. DLB.

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Clinical, Genetic and PET Longitudinal Study of a Multigenerational Family With Dopa-Responsive Parkinsonism

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Aim: To describe a large family with autosomal dominant (AD) parkinsonism

Background: Seven genes are implicated in autosomal inherited parkinsonism. However, there are families with no identifiable mutation, and their study is crucial to our understanding of Parkinson's disease.

Material and methods: Family members were evaluated clinically and by chart review. Subjects signed consent, and blood was drawn for DNA extraction. Genetic investigation included SCA 2,3,12, UCHL1 (Ile93Met), SNCA point mutations (A53T, A30P, E46K) and multiplications, and LRRK2 by gDNA sequencing. The proband underwent brain fluorodopa PET (FD-PET) scan.

Results: The 6-generation pedigree contains 108 subjects, 11 with a diagnosis of Parkinson's disease (9F), including 5 examined by us, with a 7-year follow-up. Mean age of onset was 52, with tremor-predominant asymmetric dopa-responsive parkinsonism. Evolution was slow, but severe motor fluctuations occurred. One patient required subthalamic nucleus deep-brain stimulation, with a good motor outcome. Two deceased subjects from another branch were reported to have postural tremor. One patient had mental retardation and schizophrenia and became demented, whereas two patients had mild learning difficulties. FD-PET showed severe asymmetric striatal tracer uptake deficiency, with antero-posterior gradient, consistent with Parkinson's disease. All genetic tests were negative.

Conclusion: Apart from a younger age of onset and a female predominance, the phenotype in most of our patients was indistinguishable from tremor-predominant idiopathic Parkinson's disease, as was the FD-PET scan. Essential tremor may have been associated. Known genetic causes of AD parkinsonism were excluded, thus a genome-wide linkage study is now in progress.

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Tau Gene Nucleotide Substitution Upstream of Exon3 in a Family With Dementia

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Background: Transcript of tau gene undergoes complex regulated splicing. The N-terminus is modulated by differential inclusion of exons 2 and 3. [Objective] To study possible association of tau gene nucleotide change prior to exon3 and dementia.

Methods: Three (I-2, II-2 and II-3) were affected. DNA was obtained from I-2, II-1 and II-3. All coding exons and surrounding intron sequences of the tau gene were examined.

Results: II-2 began to show disorientation, memory loss and behavioral abnormality at age 47. II-3 failed to distinguish his house from that of his mother-in-law at age 51. Since then disorientation and memory loss have gradually become prominent. Their mother, I-2 had been well until age 80 years, when she began to have memory loss, became unable to manage housekeepings. After having nursing care, she has been hospitalized for her violent conducts and excitement at night. MRI of II-3 showed bilateral temporo-fronto-parietal atrophy and ECD-SPECT showed decreased perfusion in medial and inferior frontal lobes. The tau gene of II-3 has no mutations except for heterozygous nucleotide substitution from a to g at -24th upstream of exon3. His affected mother (I-2) also had this substitution. 400 persons examined so far never had this change, nor did his healthy older brother (II-1). "-27th tct[a]ag -22nd" upstream of exon 3 is conserved among species of chimpanzee, mouse, bull, and chicken.

Conclusions: An new nucleotide substitution of at -24th upstream of tau gene exon3 was detected in a family, requiring further study on the pathogenicity.

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Cholesteryl Ester Transfer Protein (CETP) Gene and Cholesterol Metabolism in Dementia

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Dementia is becoming a major concern, especially in western countries, and Alzheimer's disease (AD) is the most common form of dementia. High plasma cholesterol levels (T-CHOL) are believed to be a risk factor of dementia, and comparisons of plasma T-CHOL levels between healthy control subjects and AD patients have shown conflicting results. Cholesteryl ester transfer protein (CETP) is primarily involved in transferring cholesteryl esters from HDL cholesterol (HDL-C) to triglyceride(TG)-rich lipoproteins. Genotypic variation in the CETP gene has been associated with CETP mass and activity, and higher plasma HDL-C and apolipoprotein A1 levels. We have investigated the genotype and allele distribution of the CETP Taq1B polymorphism in Control and AD patients, as well as its influence on the levels of blood lipid parameters. A total of 347 subjects were included in the study, 174 controls and 173 patients with AD. Our results showed no significant differences in allele and genotype frequencies between control and patients with AD. We found genotype-related variations in T-CHOL and LDL-C levels in controls and in AD patients, being the B2B2 genotype the lowest lipid levels. There is a clear interaction between CETP genotype and the diagnosis that affects T-CHOL and LDL-C levels. Thus, AD patients with B1B1 genotype present higher levels of T-CHOL and LDL-C than controls with the same genotype. The data presented show a genotype- (CETP Taq1B polymorphism) phenotype (plasma lipid parameters) relation in control subjects vs. AD patients.

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Validity and Reliability of the "Resource Use in Dementia" (RUD) Instrument

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Background: Studies of cost-effectiveness consists of two basic components: costs and consequences. Both these components need to be valid and reliable in order to obtain trustworthy results.

This presentation focus on the development of the Resource Utilization in Dementia instrument (RUD).

Methods:

Validation approaches

1. Language
2. Care concepts
3. Caregiver time assessments
4. Reliability
5. Utilization of formal care resources

Results:

1. Language: RUD is validated in Swedish, Danish, Norwegian, Finnish, German, Dutch, France-French, Canadian French, Belgian French, Italian, UK English, US English, Canadian English, South African English, Australian English, Spain-Spanish, US Spanish, Greek.

2. Validation of care concepts in and between countries in terms of face validity has been done in Sweden, Denmark, Finland, Germany, The Netherlands, UK, France, Italy, Spain, USA, Canada, South Africa, Australia.

3. Validity of caregiver time in terms of Personal ADL, Instrumental ADL and supervision has been studied both in institutions and homes (golden standard: time measurement with a clock vs time estimates and diaries. The Crohnbach´s alpha in institutions for PADL-time was 0.89, for IADL 0.45, and for supervision 0.67. The correlation between dementia severity and time use is high. Figures from home settings showed similar values (paper submitted)

4. The intra-rater reliability is high for almost all items (Cronbach's alpha and intraclass correlation coefficient >0.9).

5. There is a high correlation between interview-based data and register data regarding hospital and district nurse visits (0,86-0,96), but somewhat lower for GP visits.

Conclusion: RUD is a valid and reliable instrument for the assessment of resource use of demented persons.

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Long-Term Prevalence of Dementia in Parkinson's Disease. A 12-Year Prospective Study

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Background and aims: A wide range of prevalence of dementia in Parkinson's disease (PDD) has been reported and few longitudinal studies of PDD exist. The aim of this study was to examine the 12-year cumulative prevalence of dementia in patients with Parkinson's disease (PD).

Methods: Patients were recruited from an epidemiological study of PD in the county of Rogaland, Norway. A semi-structured caregiver-based interview, cognitive rating scales and neuropsychological tests were used to diagnose dementia according to DSM-IIIR criteria at baseline and at follow-up evaluations 4 and 8 years later, and then annually up to 12 years. Subjects with cognitive impairment at disease onset were excluded.

Results: 233 PD patients were included. A total of 139 (60%) patients had developed dementia at the end of the study period. Twenty percent died with unknown dementia status, 10% had died without dementia, and 10% were alive and without dementia at study end.

Conclusions: 60% of this representative PD cohort had developed dementia after the 12-year study period.

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Searching for the Person Legally Authorized to Give Substitute Consent for Research Purposes: Preliminary Work for the Score Study

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Background: Knowledge on advanced Alzheimer's disease (AD) cannot progress without involving affected individuals in research. In several jurisdictions, national regulations/guidelines allow the enrolment of decisionally incapacitated adults in research with third-party consent, deferring to states/provinces for defining who may act as substitute decision-makers (SDMs).

Aims: To select provinces and design case vignettes for a cross-Canada investigation of various groups' knowledge regarding substitute consent for AD research.

Methods: We reviewed relevant provincial statutes and held a workshop with key informants to clarify who, in selected provinces, is legally authorized to consent in hypothetical research situations.

Results: We found significant differences in legislation across provinces. One expressly excludes research from its health care consent legislation. Three others have no legislation explicitly addressing the issue, rendering unclear whether the authority of SDMs for health care extends to the research context. Substitute decision-making for research is possible in other provinces through three legal mechanisms. From these findings, four provinces were chosen for the cross-Canada study. Vignettes were then drafted to capture important differences on whom legal authority to make substitute decisions for research participation is conferred in these provinces. Vignettes involve a decisionally incapable AD patient who is solicited for research. They also feature a family member whose legal status varies across vignettes. The key informants were instrumental in better determining who, if anyone, is authorized to consent in each situation.

Conclusion: Designed to capture various legal regimes, the proposed vignettes could be useful to other researchers investigating substitute consent for research purposes.

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Knowledge and Practices Regarding Substitute Consent for Research in Advanced Alzheimer Disease: A Comparison Between Quebec and France

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Background: Alzheimer's disease (AD) gradually deprives patients of their ability to give valid consent for research participation. Many jurisdictions allow the enrolment of decisionally incompetent research subjects with the consent of a legally authorized substitute decision-maker, provided certain conditions are met.

Aims: Using a common postal questionnaire, we surveyed all researchers in aging in Quebec and France to 1) assess their knowledge concerning the rules enacted in their jurisdictions to protect incapacitated adults who may be approached to participate in research on AD, and 2) investigate how researchers secure consent for subjects lacking decisional capacity.

Methods: Knowledge was assessed by asking respondents to identify the person who is legally authorized to consent in various hypothetical situations.

Results: Response rates were 68.1% for Quebec and 45.4% for France ($p<0.001$). Knowledge about the legislation governing substitute consent was poor in both samples, especially in situations involving an incompetent person who did not have a legal representative. Knowledge was worse among French researchers ($p<0.001$). In both Quebec and France, most adults who lack decisional capacity have not had a legal representative appointed. In such cases, nearly two-thirds of the researchers approach the family for consent, a practice authorized in France since August 2004 but prohibited in Quebec.

Conclusions: These findings underscore the need to better educate researchers in aging about existing measures to protect adults who lack decisional capacity. Such efforts could improve both knowledge of and compliance with legal provisions that limit the participation of AD patients in clinical research.

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Evaluation of the Caregivers Functioning of Alzheimer`S Disease Patients Treated With Donepezil and Valproate

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Background: Alzheimer`s (AD) disease is a common neurodegeneratieve disorder of the central nervous system, characterized clinically by loss of cognitive function. Behevioral symptoms (Behevioral and Psychological Symptoms of Dementia – BPSD) in AD patients occur frequently over the course of the disease. Efficient treatment ob BPSD symptoms is essential for caregivers of AD patients.

Objective: The aim of the study was to assess the influence of valproate in AD patients treated with donepesil on BPSD symptoms and the influence of the reduction of this symptoms on cargivers functioning

Methods: The study included 45 patients, 29 woman and 16 men, aged 64 to 79. BPSD symptoms progression was measured according to Neuropsychiatric Inventory (NPI). All patients were treated with donepesil 10 mg a day and valproate 1000mg a day. In the caregivers group 70% were spouses, 20% children and 10% non-relatives. Cargivers condition was assessed on the strength of Assessment of Caregivers Quolity of Life scale (ACQLI). Observation period spanned 3 months.

Results: 40 patients manifested significant reduction in agitation, 34 disinhibition, 30 anxiety, 28 irratibility and aberant motor behevior. No significant differences were demonstrated in relation to the hallucinations and delusions.

Assessment caregivers condition in ACQLI scale demonstrate improvement in 40 caregivers.

Conclusiones: Patients treated with valproate on the strenght of NPI scale manifested BPSD symptoms reduction. This beneficial effect improved also caregivers functioning, decresed their emotional tension and discouragement as well as permit to reduce tranquillizers and sedative drugs.

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Association Between Green Tea Consumption and Cognitive Function in Elderly People: From Shimane Epidemiological Study

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Green tea is rich in polyphenols and acts as an antioxidant in the biological system. Experimental and animal evidence shows that green tea exerts potent activities of neuroprotection and amyloid precursor protein processing, but few human data are available. We examined the association between green tea consumption and cognitive function in elderly people recruited in Shimane prefecture. Shimane is the best senior citizen prefecture in Japan.

Design: A total of 234 community-dwellers aged 65 years and over were recruited. The subjects completed a self-administered questionnaire that included questions about the frequency of green tea consumption. We evaluated cognitive function by using the Mini-Mental State Examination (MMSE) and measured biochemical factors in blood of subjects.

Results: Subjects were divided into three groups by tea consumption per day: 2 cups and over/d group (104 peoples), less than 2 cups/d group (24 peoples), and no drink/d group (106 peoples). The MMSE score was 27.1±2.5 for 2 cups and over/d group, 25.8±2.4 for less than 2 cups/d group, 26.2±2.6 for no drink /d group, respectively. Both the score and plasma vitamin C level were significantly higher in 2 cups and over/d group than in no drink/d group.

Conclusion: A high consumption of green tea and the plasma vitamin C level were associated with a lower prevalence of cognitive function in elderly people. Green tea and vitamin C may affect the cognitive function in elderly people synergistically.

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Fish Oil Consumption and Cognitive Function in Elderly People: From Shimane Cohort Study in Japan

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A Japanese traditional feeding pattern is Japanese food mainly on fish meat, which is rich in n-3 polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA). DHA, one of the predominant n-3 PUFAs in the brain, is essential for normal prenatal CNS development. Although epidemiological studies in European and USA suggest that dementia is less frequent in populations that consume fish oil, no data in Japan were reported. We examined the association between fish oil consumption and cognitive function in healthy elderly people.

Design: A total of 256 community-dwellers aged 65 years and over were recruited in Shimane prefecture and 96 elderly people were measure in two years. The subjects completed a self-administered questionnaire that included questions about the frequency of fish meal consumption. We evaluated cognitive function by using the Hasegawa dementia rating scale (HDRS) and measured biochemical factors in blood of subjects.

Results: Subjects were divided into three groups according to the changing score of HDRS for one year: 29 in an improved group (2-6 points increase), 48 in a no-change group (less than 1 point), and 19 in an aggravation group (2-6 points decrease). Consumption of n-3 PUFAs was significantly higher in an improved group than in an aggravation group. Regression analysis revealed a positive correlation between n-3 PUFA contents in red blood cells and the HDRS score.

Conclusion: Consumption of n-3 PUFAs was associated with the improvement of cognitive function in elderly people, suggesting that consumption of fish may protect the declining cognitive function and dementia.

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Serum Antioxidants and Fish Fatty Acids Are Not Related to Risk of Alzheimer's Disease. A 35-Year Follow Up

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Background and Aims: It has been suggested that antioxidants and polyunsaturated fatty acids are important in maintaing brain function intact in high age. There is some evidence from epidemiological studies of that a high intake of fish fatty acids may lower the risk of Alzheimer's disease (AD) and dementia in general, but the results are far from clear-cut. We investigated the relations between serum concentrations of antioxidants and fish fatty acids measured in mid-life and the risk of AD up to 35 years later.

Methods: The Uppsala Longitudinal Study on Adult Men (ULSAM) started in 1970-4. At baseline (age 50 years), proportions of fatty acids in serum cholesterol esters, serum antioxidants and traditional cardiovascular risk factors were analysed in 2009 men. Thirty-five years later, the cohort has been re-examined four times and since the age of 70 years, with regard also to cognitive function.

Results and Conclusion: In December 2005, 99 men had been diagnosed as AD according to the NINCDS-ADRDA and DSM-IV criteria. Another 89 men had other dementia disorders. Baseline serum levels of beta carotene, alpha-tocopherol, selenium, retinol and proportions of fish fatty

acids (EPA, 20:5 n-3 and DHA, 22:6 n-3) did not differ between those who had developed AD, or dementia, and the rest of the cohort. Serum beta carotene, selenium and DHA levels were higher in men with university education. Our results do not support the hypothesis that a "healthy diet" in midlife protects from late-life dementia.

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International Differences in Resource Use and Costs of Care in Alzheimer's Disease: Baseline Data From the Ictus Study

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Background and aims : This study aimed to estimate the costs of formal and informal care of patients with Alzheimer's disease, to compare care costs across European countries and identify potential differences in cost patterns between countries and regions.

Methods: 1385 patients diagnosed with Alzheimer's disease were enrolled from memory clinics in 12 European countries in a prospective observational study. Resource utilization data was captured with the RUD Lite (Resource Use in Dementia) instrument. Subjects were also assessed with a battery of instruments capturing e.g. cognitive function, activities of daily living (ADL), neuropsychiatric disturbances and caregiver burden.

Results: At baseline the mean annual cost of care per participant was estimated to €9228 (95% conf.interval: 8438€ – 10020€). 49% were costs of informal care, 28% direct medical costs and 24% community care costs. There were substantial differences in total resource utilization and in the balance between formal and informal care between Northern, Western and Southern Europe. ADL scores were strongly associated with formal care costs while instrumental ADL scores correlated strongly with informal care costs.

Conclusions: Costs of dementia is high across European countries, and ADL function is a strong determinant of care costs. Formal care service use is lower and informal care higher in Southern Europe compared to Western and Northern Europe. Differences in resource utilization patterns are important to consider in international studies of dementia care costs as well as in economic evaluations of new treatments for Alzheimer's disease.

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Functioning Evaluation of Caregivers of Patients With Alzheimer`S Disease in Private and Professional Life in the Years 2001-2005

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Introduction and aim of the study: Alzheimer's disease is a chronic, neurodegenerative disease of the nervous system which is characterized by gradual loss of cognitive abilities, deterioration of functioning in everyday life as well as the presence of neuropsychiatric symptoms. Alzheimer's disease affects not only patients but also their families. Patients' caregiver is most frequently his spouse, usually an elderly person and quite frequently suffering from various complaints herself.

Material and methods: The studies included caregivers of 45 patients with Alzheimer's disease. There were 23 women and 2 men among caregivers . 45% caregivers were their spouses, 45% their children and 10% strangers. ACQLI (Assessment of Caregivers Quality of Life) survey included questions with the answer: true or false referring to various spheres of caregiver's life and the ADL-10 scale (Activities of Daily Living) included questions concerning everyday life activities.

Results: The performed studies revealed a correlation between the applied treatment and patients' psycho-physical well-being as well as caregiver's well-being. Caregivers' psycho-physical tensions decreased with the change of index in ADL scale, they had less neurotic, tension and depressive symptoms. More frequent alcohol abuse was stated in male caregivers and drug abuse in female caregiver.

Conclusions: Caregivers of patients with Alzheimer's disease demand medical care, neuropsychological assistance and frequently pharmacotherapy in relation to the symptoms

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Apolipoprotein E and Lifestyle Determinants of Dementia: A Population Based Study

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Background: The risk of dementia and Alzheimer's disease (AD) is suggested to result from an interaction between genetic and environmental factors. The aim of this study was to investigate the putative effects and interactions between the apoE ε4 allele and lifestyle related risk factors for dementia and AD.

Methods: Participants of the Cardiovascular Risk Factors, Aging, and Dementia (CAIDE) study were derived from random, population-based samples previously studied in 1972, 1977, 1982, or 1987. After an average follow-up of 21 years, 1449 individuals (73%) aged 65 to 79 years were re-examined in 1998.

Results: The apoE ε4 allele was an independent risk factor for dementia even after adjustments for lifestyle and vascular factors (OR 2.83, 95% CI 1.61-4.97). Physical inactivity, alcohol drinking, and smoking at midlife increased the risk of dementia and AD particularly among the apoE ε4 carriers. Further, among the apoE ε4 carriers low to moderate dietary intake of polyunsaturated fats at midlife, as well as

moderate to high intake of saturated fats were associated with increased risk of dementia and AD. Also the summarised effect of the lifestyle related factors was more pronounced among the apoE ε4 carriers.

Conclusions: Physical inactivity, dietary fat intake, alcohol drinking, and smoking at midlife are associated with the risk of dementia and AD, especially among the apoE ε4 carriers. The apoE ε4 carriers may be more vulnerable for various environmental and lifestyle related factors. Thus, lifestyle interventions may significantly modify the risk of dementia, particularly among the genetically susceptible individuals.

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Serum Cholesterol, Lipid-Lowering Drugs and Cognition: 21-Year Follow-Up Study (CAIDE)

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Background: Previous results from the Cardiovascular Risk Factors, Aging and the Incidence of Dementia (CAIDE) study indicate that high serum total cholesterol (TC) at midlife is a risk factor for mild cognitive impairment (MCI) and dementia/AD, and that TC decreases after midlife especially in subjects who develop MCI/dementia.

Objectives: To analyse the relationship between midlife TC, late-life TC, TC changes after midlife, lipid-lowering drugs, and various cognitive domains in non-demented persons.

Methods: Participants of the Cardiovascular Risk Factors, Aging and Dementia (CAIDE) study were derived from random, population-based samples previously studied in a survey in 1972, 1977, 1982 or 1987. After an average follow-up of 21 years, 1449 individuals (72 %) aged 65 to 79 participated in a re-examination in 1998. 61 subjects were diagnosed with dementia and were excluded from the present study.

Results: After controlling for socio-demographic and vascular factors, a U-shaped pattern of association between midlife TC and poorer memory performance was observed. Late-life TC was not significantly associated with any cognitive domain. Subjects with a more pronounced TC decrease after midlife had significantly poorer memory performance compared to those with little or no TC change. Lipid-lowering treatment was associated with better memory and psychomotor speed, even after adjustment for baseline cholesterol level, cholesterol changes over time and other confounding factors.

Conclusions: Serum TC seems to be associated not only with dementia but also with memory performance in non-demented persons, while lipid-lowering treatment seems to have a positive effect on cognitive functioning.

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A Data Mining Approach for the Diagnosis and Differentiation of Dementia Types

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Diagnosis and differentiation between dementias has been a clinical exercise since Alzheimer described his two initial cases, with the ultimate diagnostic criterion being post-mortem verification of the clinical diagnosis. There is a need to improve diagnostic accuracy. Techniques, which might be used to support diagnoses, should also be practical in terms of cost, time and ease of use.

This study used data mining algorithms to automatically classify dementia types. Cognitive Drug Research collected computerised cognitive test data on 1842 patients diagnosed with dementia, included in clinical trials, and diagnosed with Alzheimer's disease (AD), dementia with Lewy bodies (DLB), Parkinson's disease dementi (PDD), Vascular dementia (VAD) and Parkinson's disease (PD). The data of the PD patients was used as a control group.

Three different classification tasks were designed: (a) discrimination between three macro-classes (PDD-DLB, AD-VAD, PD); (b) specific discrimination between PDD and DLB; and (c) specific discrimination between AD and VAD. Seven classification algorithms were tested, assessing their performance via cross-validation.

Depending on the algorithm and on the task, the classification accuracy ranged between 70% and 80%. The data of the first visit led generally to better accuracy for classification tasks (a) and (b).

The use of computerised cognitive testing provides a quick, reliable, inexpensive and non-specialist method to support clinical diagnoses of dementia. The technique can also monitor both the response of a patient to treatment, and progression of their illness over time. Further refinement of the algorithms used and inclusion of more cognitive parameters is expected to improve accuracy.

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Factors Associated With Increased Alzheimer's Disease Severity at Diagnosis; Data From the ICTUS Study

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Background And Aim: Earlier diagnosis of Alzheimer's disease (AD) has been shown to have a positive influence on quality of life despite current limited treatment options. Research aiming at a positive diagnosis of AD in the preclinical stages of the disease is gaining pace. In the real world however patients are still presenting at varying stages of the disease spectrum. The aim is to determine factors

associated with disease severity (determined by MMSE score) at time of diagnosis in cross sectional data from a prospectively recruited longitudinal cohort of patients with mild to moderate AD.

Methods: A total of 1,380 patients with probable AD were recruited between 2003 and 2005 in 29 European AD centres and comprise the ICTUS cohort. All patients had MMSE scores between 10 and 26 at baseline. Baseline data was used to identify characteristics (demographics, test findings, and co morbid conditions) influencing risk of increased disease severity at diagnosis.

Results: New patients with a date of diagnosis being equal (-0 to 3 months) to that of study inclusion were included in the analysis (n=473). Factors associated with increased risk of more severe AD (MMSE 10-20) at diagnosis were older age (p=0.038), female sex (p, 0.0001), low educational level (p=0.002), low income (p=0.0098), and living separately from caregiver (p=0.011).

Conclusion: In this cohort increased age, female sex, low educational level, low income, and living independently from caregiver are all factors associated with increased risk of presenting with more severe AD. Results of the multivariate analysis are awaited.

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Factors Associated With Increased Time to Diagnosis of Alzheimer's Disease; Data From the ICTUS Study

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Background & Aim: Earlier diagnosis of Alzheimer's disease (AD) has been shown to have a positive influence on quality of life despite current limited treatment options. In the real world patients often present years after the onset of their AD symptoms. The aim is to determine factors associated with increased time to diagnosis of AD in cross sectional data from a prospectively recruited longitudinal cohort of patients with mild to moderate AD.

Methods: A total of 1,380 patients with probable AD were recruited in 29 AD centres in Europe. Time to diagnosis was defined as the time between date of symptom onset and date of diagnosis in years. Baseline data was used to identify characteristics associated with increased time to diagnosis.

Results: New patients with a date of diagnosis being equal (-0 to 3 months) to that of study inclusion were included in the analysis (n=473). The median time to diagnosis was 2 years. Factors associated with longer time to diagnosis (>2years) were younger age at symptom onset (p=0.0003), income ≥750 euros/month (p=<0.0001), higher NPI score at diagnosis (p=<0.0001) and lack of diagnosis of depression prior to diagnosis (p=0.0344). Time to diagnosis was not associated with MMSE, CDR, or ADL/IADL scores at diagnosis.

Conclusion: In this cohort younger age at symptom onset, higher income, higher NPI score at diagnosis and no prior history of depression are all factors associated with a longer interval between symptom onset and diagnosis. Results of the multivariate analysis are awaited.

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Quality of Life Among Dementia Patients in Special Care Units and in Traditional Integrative Nursing Homes

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Background and aims: Two-thirds of all residents in nursing homes in Germany suffer from some type of dementia. Cognitive impairment is frequently accompanied by behavior problems that can have a considerable negative impact upon the quality of life of the affected individual. New concepts of special care have been developed for dementia patients in the city of Hamburg. In an evaluation study we investigated whether or not dementia patients residing in the model institutions in Hamburg exhibited a better quality of life than their counterparts residing in traditional nursing homes in the city of Mannheim.

Methods: In a prospective longitudinal study, over 80% of all residents (n = 744) of the model program in Hamburg (admission criterion: mobile dementia patients with behavior problems) were examined and compared to a group of nursing home residents with dementia who were receiving traditional integrative care.

Results: Controlling for confounding variables, for dementia patients in special care units as compared to a reference group in traditional integrative care, the level of volunteer caregiver involvement was higher and there was more social contact to staff, fewer physical restraints, more involvement in home activities, and more frequent use of psychiatrists.

Conclusions: Significant differences for a number of indicators of the quality of life point in favor of the model program in Hamburg. Future evaluation studies ought to examine not only the general efficacy of types of care designed especially for dementia patients but also the efficacy of the respective individual components (i.e. caregiver ratio).

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Prevalence of Cognitive Dysfunction in Old Patients Admitted in Emergency Room

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Objective:Presence of cognitive dysfunction in elderly patients with physical problem imposes major diagnostic problem on part of the physician. Symptoms of cognitive dysfunction is mostly silent and are very often overlooked which in turn make the patient's condition more critical. This study highlights the major symptoms of cognitive dysfunction among elderly patients from the emergency department of internal medicine.

Method: The sample study comprised 100 patients who were examined at out patient basis, using Mini Mental State Examination(MMSE). One fourth of the subjects were identified with clear symptoms of cognitive dysfunction.

Results: There was a positive correlation between age and degree of cognitive dysfunction. However, with education, this

trend declined. Female patients were more affected as compared to their male counterparts. In order of importance symptoms of cognitive dysfunction were observed more frequently in patients with neurological disorder(50%), followed by those with renal disease(41.6%), hepatic and GI disease(40%), and those with pulmonary disease(26%). Those with cardiovascular diseases were least affected (13%).

Conclusion: Findings of this study have implications for early diagnosis, easier management and better prognosis of physical illness.

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Prevalence of Affective Disorders in a Population-Based Sample of German Adults: Preliminary Results of the Third Wave

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Objetive: Affective disorders are among the most frequent conditions in the elderly, but only a few epidemiological studies are available for the German population. We therefore established the prevalence and clinical course of affective disorders within the Interdisciplinary Longitudinal Study on Adult Development and Aging(ILSE).

Methods: Prevalence of affective disorders were investigated in 500 community-dwelling subjects from the birth cohort 1930/32 of two German urban regions. Participants were carefully screened for physical and mental health. In all subjects, the structured clinical interview according to DSM-III-R (SCID) was applied. The first examination wave (t1) was performed in 1994, t2 in 1998. Currently we are completing the t3 which was initiated in July 2005.

Results: Until now, 249 patients (121 females, 128 males) of the cohort have been examined in T3.

Mean age was 62.4±2.4 years at baseline (t1), 66.7±1.1 years at t2 and 73.9±0.84 years at t3.

Lifetime prevalence rates of MDD rose from 8.1% at t1 to 11.2% at t2. Preliminary analyses of the current examination wave led to lifetime prevalence rates of 15.3%. The point prevalence of MDD was 0.7%, 0.5% and 4.4% for t1, t2 and t3 respectively.

Conclusions: These preliminary results show an important increase in the point prevalence rate of MDD in the third wave and emphasize the high prevalence of affective disorders in elderly people. Since this condition also affects general health and may contribute to an increased risk of developing dementia, these results emphasize the clinical importance of affective disorders in the elderly.

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I.PR.E.A. - Italian Project on Epidemiology of Alzheimer's Disease: Preliminary Results of the Cross Sectional Phase

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Dementia and cognitive decline are extremely common among elderly subjects. Recently, much attention has focused on early diagnosis, in order to help choosing appropriate strategies for delaying/preventing further cognitive decline and to allow earlier rehabilitation and maintain functional autonomy as long as possible.

I.PR.E.A. is a multicenter community-based prospective study. It was designed with the main objective of improving knowledge on AD and dementias in Italy, where few national-level data are available on preclinical AD. It was aimed at estimating the prevalence and incidence of Alzheimer's disease (AD) in the preclinical phase, the natural history of cognitive decline without dementia in the Italian population, and identifying risk factors/health determinants related or associated with various health outcomes.

It consisted of a cross-sectional and a longitudinal phases. This is the first prospective study on the preclinical phase of AD in Italy.

The cross-sectional phase was performed in a random sample of 4785 subjects aged 65-84 years selected from the registries of 12 Italian rural and urban municipalities. The study population was examined through: personal and informant interviews, physical and neurological examination, laboratory tests and an extensive neuropsychological battery.

The participation rate was 64.2%, mean age ±SD 73.8±5.5 (versus 75±5.7 among non participants); mean education ±SD was 6± 4.05 yrs (versus 5.4±3.8 for non-participants). The response rate was lightly higher in men (52.4%). Institutionalized subjects were 0.5% of the total examined population.

Methodology, study design and preliminary data of the cross-sectional phase will be discussed.

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Direct Costs of Alzheimer's Disease in Korea : A Survey of National Health Care System

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Background and aims: Alzheimer's disease has a lifetime prevalence of 7.1% age over 65 years in Korea. However, the economic costs of Alzheimer's disease have rarely been assessed. The aims of this study were to examine the health care utilization and the current trend of the direct costs of Alzheimer's disease in Korea.

Methods: National Health Insurance System (NHIS) covered up to 96.6% in the year 2004. We reviewed the Health Insurance Review and Evaluation Statistics from 2000 to

2004, a large database of inpatient and outpatient treatments of health insurance and medical aids.

Results: Treated prevalence of Alzheimer's disease from 2000 to 2004 has been rapidly increased by 30% every year. Annual incremental rate of direct costs for Alzheimer's disease was 18% from 2000 to 2004. Inpatient care was about 3-fold higher than outpatient care .The annual NHIS cost of managing Alzheimer's disease in the year 2004 was estimated to be about 76,982,000 (U.S. dollars), including nursing costs. Pure medical costs of Alzheimer's disease account for 0.16% of total medical expenses and 4.7% of all mental health costs.

Conclusion: Our survey demonstrates the current trend of the medical costs of Alzheimer's disease in Korea. Nowadays, Korea has rapidly progressed into an aging society. Policy pressures increasingly demand the improvements of cost-effectiveness of Alzheimer's disease. Thus, more attention should be directed at the accumulation of reliable evidences of the economic burden of Alzheimer's disease.

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The Analysis of Factors Influencing the Intensity of Grief of Family Caregivers of Alzheimer's Patients

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Presently, most AD patients in Poland are cared for by family caregivers. Caregivers often experience grief. The analysis of determinants of grief is of special interest in Poland because this country is undergoing social changes and structural economic reforms.

The goal of this paper was to determine the relation between the intensity of caregiver grief and the care recipient's level of cognitive and functional impairment. The study involved 48 caregivers from Poznan. Along with questions concerning demographic characteristics, the respondents were administered the Polish version of Clinical Dementia Rating® and MM – CGI – 50. CDR® is administered as a structured interview with the caregiver concerning the care recipient's impairment level in memory, orientation, judgment & problem solving, community affairs and personal care. MM – CGI – 50, which is a grief inventory for caregivers of demented patients, includes three subscales: Personal Sacrifice Burden, Heartfelt Sadness and Longing, and Worry and Felt Isolation. Both questionnaires came from Thomas Meuser of Washington University at St. Louis. The statistical analysis showed that the age of subjects ranged from 24 to 84 years and most of them provided care for their mothers and husbands. Also, the intensity of grief correlated with cognitive impairment. It was also related to the type of family relation between the subject and the care recipient. Respondents caring for their spouses have high scores on the subscale of Heartfelt Sadness and Longing while adult children of care recipients score high on Personal Sacrifice

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Development of a Dementia Risk Score in the Kaiser Permanente Population

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Background & Aims: Recent work from the CAIDE study has illustrated that a summary score of age, education and vascular risk factors in midlife predicts 20 year dementia risk. Development of a risk score may help target individuals susceptible to dementia, thus it is important to test it in larger diverse populations. The goal of this study was to both validate and further develop the CAIDE risk score in a large (N=10,350) diverse population of members of an integrated health care delivery system.

Methods: Kaiser Permanente (KP) multiphasic health study was in 1964-68 and included a clinical exam. Participants were ages 40-45 at time of exam, and remained members of KP. Dementia diagnoses were from inpatient & outpatient records (January 1, 1994-January 16, 2006). Age, education, midlife obesity, hyperlipidemia,& hypertension were used to create the risk score. Beta-coefficients from each variable were summed to create a score (range 0-15).

Results:1644 participants were diagnosed with dementia. The probability of dementia according to the risk score was 15% for those scoring 0-5; 17% for 6-7; 20% for 8-9; 20.2% for 10-11; and 23.5% for 12-15.Those with scores of > 12 had a 2.32 fold increased risk of dementia versus those with scores of 0-5 (HR=2.32, 95% CI 1.7-3.2). Central obesity and diabetes increased the predictability of the risk score.

Conclusions:A risk score may provide a clinical tool for predicting dementia and highlights the role of modifiable risk factors.

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Assessment of Severity of Parkinson Disease in Clinical Practice. Is It Always in Accordance With the Hoehn and Yahr Classification?

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Background: The progression of Parkinson's disease (PD) is usually assessed with the Hoehn and Yahr (H&Y) scale. Nevertheless, it is not always used in clinical practice as the physicians, after their initial evaluation, frequently base their assessment on patient symptoms and classify the disease as mild, moderate or severe.

Aim: The aim of this study was to correlate the physician's classification of disease severity in PD patients with that based on the H&Y scale.

Methods: The demographic characteristics and extended medical history of PD patients were recorded and the stage and severity of the disease in each patient was evaluated by using the physician's criteria, the H&Y scale and the Schwab & England scale. The severity of symptoms, as defined by the physicians and the patients, were correlated.

Results: 500 patients were evaluated (50.9% male), their mean age (years + SD) was 69.7+ 8.3. and 74.3% of them had been diagnosed with PD <1 year before. The physician's

evaluation was in accordance with the H&Y scale in only 72.2% of the cases. 24.6% of the patients were classified as being at a lower stage of the disease and only 3.2 % at a higher stage using the H&Y scale compared to the physicians evaluation.

There is a 24,6% tendency to overestimate the severity of PD, when the H&Y scale is not used.

Conclusion: Physicians may overestimate the severity of PD if appropriate diagnostic tools are not used in clinical practice. This could possibly lead to less than optimal disease management.

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What Determines Health Related Quality of Life (Hrqol) in Patients With Dementia and Their Caregivers?

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Background and Purpose: The optimal instrument for evaluation of the effectiveness of pharmacological and non-pharmacological therapies for dementia is a matter of debate. There is widespread agreement that maintenance and improvement of HRQOL constitutes an essential intervention target. However, due to the inherent cognitive impairment in dementia, assessment of HRQOL remains difficult.

Methods: We developed and validated two questionnaires based on DEMQOL and other instruments: One self rating questionnaire for the patient, and one for the caregiver to rate his HRQOL and the assumed HRQOL of the patient. The questionnaires were distributed by the German Alzheimer Society to 301 caregivers and 179 patients with dementia.

Results: Most patients (61%) were female, mean age was 79 years (caregivers: 64 years). At the time of the investigation the mean duration of illness was 45 month. Fifty seven month had past since onset of first symptoms. Fifty percent of the patients reported their HRQOL as good, one percent as very good, thirty eight percent as moderate and eleven percent as bad. HRQOL of the patients was not associated with age or duration of illness. However, HRQOL was correlated with worries about cognitive impairment (r=.346**; p<0,01), memory difficulties (r=.356**; p<0,01), and depression (r=.263**; p<0,01). Fast progression of dementia was associated with a decreased HRQOL in the caregiver (r=-.268**; p<0,01).

Conclusions: These results indicate that any treatment that slows down the progression of the illness could increase the HRQOL of the patients and their caregivers.

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Patients With Dementia With Lewy Bodies Have More Impaired Quality of Life Than Patients With Alzheimer's Disease

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The primary aim of this study was to compare Quality Of Life (QoL) in patients with Dementia with Lewy Bodies (DLB) and patients with Alzheimer's Disease (AD). The secondary aim of this study was to investigate determinants of QoL in DLB.

34 patients with DLB at the Neuropsychiatry clinic, University Hospital MAS, Malmö, Sweden, were included in a cross sectional study. These patients were matched to 34 patients with AD. Two QoL instruments, the EQ-5D instrument and the Quality of Life-Alzheimer's disease (QoL-AD) instrument, were applied in this study. Both instruments were administered to both patients and caregivers.

The DLB patients in this study have significantly lower QoL than the AD patients regardless of instrument or whether patient or caregiver-reported QoL was used. Furthermore this study shows that important determinants of quality of life in DLB include NPI score, independency in I-ADL, whether the patient is living with the caregiver and the presence of apathy and delusions.

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Patients With Dementia With Lewy Bodies Utilise More Resources Than Patients With Alzheimer's Disease

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The annual resource utilisation in a patient with Alzheimer's disease has been estimated to be on average 172000 SEK (18300 €), ranging from 60700 SEK (6460 €) in mild dementia to 375000 SEK (39900 €) in severe dementia.

The purpose of this study was to compare resource utilisation and costs in patients with dementia with Lewy bodies (DLB) and Alzheimer's disease (AD), and to assess determinants of costs of care in DLB.

34 patients with DLB were included. The patients were matched with respect to age, gender and Mini Mental State Examination (MMSE) score to 34 patients with AD. Both groups were examined using Resource Utilisation in Dementia (RUD Lite), MMSE and the Neuropsychiatric inventory (NPI). The DLB patients were additionally examined using the Disability Assessment for Dementia Scale (DAD).

Costs of care in patients suffering from DLB was on average 348000 SEK (37500 €) per year compared to 169000 SEK (18200€) in the AD group (p<0.001)

Within the DLB group, care costs correlated significantly (rc=2.77, p<0.001) with dependency in instrumental activities of daily living measured with DAD, whereas MMSE and NPI were not significantly correlated to resource use in the DLB group.

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How Frequent Are Cognitive Impairments Among PD Patients in Germany? - Results From the GEPAD Study

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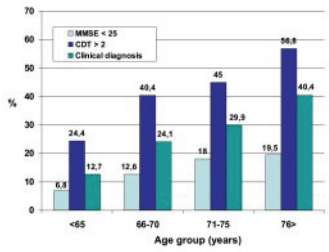
Background: Non-motor complications, such as dementia, frequently occur during the course of Parkinson's disease (PD) and increase the personal and socioeconomic burden. Estimates of the frequency of these complications have been varying considerably, especially PD dementia (PDD), for which prevalence data between 20 and 80% have been reported. For Germany, there are no estimates available so far at all.

Methods: GEPAD is a large, nationwide, cross-sectional study of dementia in an unselected sample of 1449 outpatients with parkinsonian syndromes who visited neurologist offices in Germany on a specified study day. All patients were assessed with standardized diagnostic scales for PD and cognitive impairment. Aside the clinician's appraisal of dementia, cognitive impairment was additionally assessed with the Mini-Mental State Exam (MMSE), and Clock Drawing Test (CDT). The present analysis is based on a subsample of 873 patients who met the UK Brain Bank criteria for PD.

Results: Frequencies of cognitive impairments ranged from 14% with MMSE, to 27% of all patients diagnosed with dementia by the physicians. With the CDT, the estimated overall frequency was at 41.8%. All estimates increased with age (figure 1) and PD severity.

Conclusion: Cognitive impairment is frequent in PD patients, related to PD severity, age, and psychopathological conditions. There is evidence that the MMSE overlooks a substantial proportion of demented patients if a suggested cut-off (≤24) is chosen.



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Reproductive Period and Cognitive Functioning in Postmenopausal Women

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Background and aims: Estrogen-replacement therapy has been suggested to exert protective effects against Alzheimer's disease, but the effects of natural exposure to estrogen on cognitive disorders have rarely been studied. We hypothesized that women with a prolonged reproductive period have a higher exposure to natural estrogen, and that these women have a better cognitive function, which this study was designed to test.

Methods: The study cohort comprised 1,552 women aged 60 to 84 years who were registered in the Ansan Geriatric Study in South Korea. We conducted a cross-sectional analysis using their data on the age at menarche, age at menopause, number of children, age at first delivery, and age at last delivery. All subjects were clinically assessed for dementia and cognitive impairment.

Results: The risk of cognitive impairment was associated with a longer reproductive period and being younger at menarche. After adjusting for sociodemographic factors, the risk of cognitive impairment in menopausal women was negatively correlated with the length of the reproductive period (adjusted OR, 0.98), and the risk of cognitive impairment was positively correlated with the age at menarche (adjusted OR, 1.06–1.07).

Conclusions: Overall, our findings support the hypothesis that a longer reproductive period and being younger at menarche are associated with a lower risk of cognitive impairment.

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A Multi-Domain Micro-Simulation Economic Modeling Framework in Alzheimer´s Disease

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Background: Progressive decline across multiple domains (e.g. cognition and physical functions) in Alzheimer's disease constitutes a challenge for economic modelling. Existing models stratify patients into artificial cohorts using single domains (typically either cognition or care setting) thereby neglecting important explanatory variables and limiting the extent to which individual variability can be modeled. There is a need for a more dynamic model that captures individual variability and small changes across multiple domains relevant for Alzheimer's disease progression.

Objective: To develop a stochastic multi-domain micro-simulation model for evaluation of cost-effectiveness and long-term outcome in Alzheimer's disease.

Methods: Key disease indicators (e.g. cognitive function, functional abilities - ADLs and care setting) are simulated over time for individual patients using regression functions derived from longitudinal observational data. Micro-simulation of each individual patient enables incorporating individual variability over time into the model, i.e. the disease progression can depend on individual characteristics and previous progression rates. The disease indicators together with patient characteristics are used to predict the incremental benefit of competing treatment options.

Conclusions: The proposed model provides a dynamic simulation framework completely based on regression functions. This enables inclusion of all relevant disease indicators and incorporation of individual variability into disease progression functions.

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The Clinical Chracteristics of Cognitive Impairment in Dementia Prevention Center of Kang-Dong Sacred Heart Hospital in 2005-2006

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Objectives: Early detection of Alzheimer's Disease (AD) or Mild Cognitive Impairment (MCI) is important to prevent from disease progression and socioeconomic burden.

Methods: Our team consisted of 2 psychiatrists, 1 neurologist, 1 rehabilitation doctor, 1 psychologist, 1 nurse, 1 social worker, and 1 nutritionist. We have diagnosed cognitive impairment according to NINDS-ADRDA for AD, NINDS-AIREN for vascular dementia (VaD), modified Neary criteria for Fronto-Temporal Lobar Degeneration (FTLD), and ICD-10 criteria by Seoul Neuropsychological screening Battery (SNSB) comprised of tests for 5 domains(Attention, language, memory, visuospatial function, and frontal lobe function).

Results: From October 2005 to November 2006, Total 128 patients have visited our Center and have been assessed for cognitive function by Mean age was 74.3±9.4 years-old and 34 persons were uneducated. Mean periods of education was 5.5±5.3 years (men:9.7±5.2, women:3.5±4.1). Mean Korean Mini-Mental Status Examination(K-MMSE) score was 19.8±5.8 points. The distribution of cognitive impairments showed that 16(12.5%) in MCI, 42(32.8%) in AD, 17(13.3%) in Vascular dementia (VaD), 17(13.3%) in mixed type with AD and VaD, 5(3.9%) in FTLD, 2(1.6%) alcohol related Dementia, 7(5.5%) in depression, and 22(17.2%) in other causes of cognitive impairment.

Conclusions: Because we did our best to find out MCI and early AD, The proportion of MCI in our center was higher than previous studies. Many patients and caregivers were gratified with Consult by social worker and nutritionist. In the future, we would cooperate with public health system for early detection of MCI and AD to the population of the district.

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Cholinesterase Inhibitors and All-Cause Mortality in a Population-Based Cohort of Patients With Alzheimer's Disease

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Background & Aims: To examine the effect of time-varying use of Cholinesterase Inhibitors (ChEIs) on all-cause mortality among patients with Alzheimer's disease (AD).

Methods: A retrospective cohort study of AD patients was conducted using an administrative database from Saskatchewan, Canada. The cohort was defined as all patients receiving an index ChEI prescription during the first year of the Special Access Program, from Dec 2000 to Dec 2001, and followed for 4 years. Kaplan-Meier analysis was used to estimate the risk of all-cause death. Cox regression was used to assess potential confounding factors.

Results: A total of 1080 patients had an index prescription during the inception year. The mean (sd) age was 79.8 (7.1) years, 63% were women, and baseline mean (sd) MMSE and FAQ scores were 20.8 (4.4) and 17.5 (7.7), respectively. Over the follow-up period, there were 4545 time intervals of patients taking ChEIs and 4333 time intervals of patients not taking ChEIs. The one-year risk of death for patients taking ChEIs was 4.5% compared to 10.3% for patients not currently taking ChEIs; absolute risk reduction of 5.8% (95% CI: 3.1% to 8.5%, p-value<0.001) and relative risk reduction of 56%. Cox regression did not identify any confounding or effect modification.

Conclusions: Using a population-based cohort of patients with AD, mortality was significantly lower during the time intervals that patients were taking ChEIs. If this observation can be replicated in other databases, such a finding could have important pharmacoeconomic and health policy implications.

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Mortality Rates and Risk Factors in Community Based Dementia Patients

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Objectives: The aim of this study was to investigate mortality rates and risk factors in dementia by longitudinal study in a rural cohort.

Methods: A total of 114 subjects with clinically diagnosed dementia were followed up for eight years from 1997 to 2005. Their mortality was compared with sociodemographic and clinical variables using the Cox proportional hazards models after adjusting age, sex, and education.

Results: During follow-up, the mortality rate of subjects was 80.2% and the mean(SD) duration of survival from at

diagnosis until death was 4 years. Mortality in subjects with dementia depended on old age (relative risk[RR]:1.05; 95% confidence interval[CI]:1.01-1.08), male (RR:1.61; CI:1.00-2.59), low Clinical Dementia Rating scale (RR:1.54; CI:1.14-2.10), low Activities of Daily Living (RR:0.72; CI:0.59-0.89), low Instrumental Activities of Daily Living (RR:0.83; CI:0.75-0.92), no physical activity (RR:0.44; CI:0.28-0.70), smoking (RR:1.74; CI:1.05-2.89)

Conclusion: Mortality in dementia depended on age, sex, CDR, ADL, IADL, physical activity, smoking. These findings have important implications that contribute to make the disease management of dementia patients.

Key words: dementia, mortality, risk factors

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The AIBL Flagship Study of Ageing: The Australian Biomarkers Lifestyle and Imaging (AIBL) Flagship Study of Ageing

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Keywords: Alzheimer's disease, biomarkers, neuroimaging

Background: AIBL is a 3-year longitudinal cohort study which aims to improve understanding of the pathogenesis and diagnosis of Alzheimer's disease (AD) using neuropsychological, neuroimaging and biomarker techniques, and to examine lifestyle and dietary factors associated with AD and healthy ageing.

Methods: 1000 volunteers (minimum age 60 years) will be recruited, comprising 200 participants from the following groups: 1) AD, 2) mild cognitive impairments, 3) healthy volunteers (ApoE4+), 4) healthy volunteers (ApoE4-), 5) "memory complainers" (i.e. healthy volunteers reporting subjective memory complaints). At baseline and 18-months, all participants will receive a clinical/neuropsychological assessment and blood biomarker analysis, with a sub-group also receiving [C-11]PIB-PET and MRI scans. Participants will also complete questionnaires assessing diet and exercise patterns, with a sub-group receiving actigraph accelerometer measurement of activity levels, and Dual Energy X-Ray Absorptiometry measures of body composition.

Results: Recruitment commenced in October 2006. Patterns of change in individual measures (neuropsychology, neuroimaging and biomarkers) will be examined within each population group. Changes in neurpsychological measures will be correlated with neuroimaging and biomarker measures to establish convergent validity.

Conclusions: This is the largest study of its kind ever undertaken in Australia, will identify neuroimaging, biomarker and neuropsychological measurements of longitudinal changes in a large cohort, and will enhance knowledge of lifestyle and dietary factors associated with AD and healthy ageing.

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CSF Neurofilament Proteins in the Differential Diagnosis of Dementia

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Background: Neurofilament (NF) proteins are major cytoskeletal constituents of neurons. Increased CSF NF levels may reflect neuronal degeneration.

Objective: To investigate the diagnostic value of CSF NF analysis to discriminate in relatively young dementia patients between frontotemporal lobe degeneration (FTLD) and early-onset Alzheimer disease (EAD; onset ≤ 65 years of age), and in elderly dementia patients between dementia with Lewy bodies (DLB) and late-onset AD (LAD; onset > 65 years of age).

Methods: In CSF of 28 FTLD (mean age 63.2 ± 9.1 years), 37 EAD (61.2 ± 4.5 years), 18 DLB (72.3 ± 8.5 years), and 33 LAD patients (75.6 ± 4.2 years), and 26 control subjects (60.9 ± 7.2 years), we analysed NF light chain (NFL), phosphorylated NF heavy chain (pNFH), amyloid β42 protein (Aß42), total tau (t-tau), and tau phosphorylated at threonine 181 (p-tau181).

Results: CSF NFL levels were significantly higher in FTLD patients (20.6 ± 18.0 pg/ml) compared to EAD patients (8.5 ± 8.2 pg/ml), and diagnostic accuracy improved in combination with CSF levels of p-tau181 and Aβ42 (sensitivity 86%, specificity 100%). CSF pNFH levels were significantly elevated in DLB (182 ± 151 pg/ml), LAD (157 ± 102 pg/ml), and FTLD (142 ± 90 pg/ml) compared to controls (79 ± 20 pg/ml), however, no significant differences were found between the dementia groups.

Conclusions: In the diagnostic workup of relatively young dementia patients, CSF NFL levels may play a role in de discrimination between FTLD and EAD, especially in combination with Aß42 and p-tau181 analysis.

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An Increased Risk of Conversion From Mild Cognitive Impairment (MCI) to Dementia Among Individuals With Cognitive Multidomain Impairment

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Background: MCI individuals can develop demonstrable cognitive impairment, without crossing the threshold for dementia. They have an increased risk of developing dementia, however not all convert to dementia, and some can revert to normal.

Objective: To determine whether MCI patients with impairment in memory and other domains are more likely than those with memory impairment alone to develop dementia.

Methods: The longitudinal study consisted 105 individuals with the diagnosis of MCI according to Peterson's et al., criteria. All subjects received annual clinical and psychometric examinations for up to mean 3 years. Diagnosis were made for dementia according to DSM III-R.

Results: The mean age of the group was 69.3 years with SD of 7.2 in 2001-2002. Of the 105 individuals, 42 (40%) had amnestic MCI, and 63 (60%) had multidomain MCI. 23 incidence cases of dementia developed during three years follow-up. Mean time to conversion was slightly shorter in multidomain group compared with amnestic group - 29.3 months (SD 9.4) vs 30 months (SD 14.6). The subjects with multidomain MCI were more likely to had dementia than those with amnestic MCI: (n)=18, 28.6% vs (n)=5, 11.9%; Chi-square=9.2; d.f.=2, p=0.0097.

Conclusions: Over the three years follow-up, the presence of memory impairment and impairment in another cognitive domain was significantly associated with an increased risk of conversion from MCI to dementia. We conclude that currently used diagnosed criteria for MCI characterized heterogeneous population of patients with an increased risk of developing dementia.

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One Carbon Metabolism and Incidence of Dementia

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Background and Aims: This study investigated prospective associations with incident dementia and cognitive decline, both for baseline levels of folate, vitamin B12 and homocysteine and for changes in these levels over the study period.

Methods: Of 625 elders without dementia at baseline, 518 (83%) were followed over a 2.4 year period and were clinically assessed for incident dementia and Alzheimer's disease (AD). Serum levels of folate, vitamin B12 and homocysteine were assayed and MMSE was measured both baseline and follow-up assessments.

Results: Only baseline folate levels predicted incident dementia and AD. The onset of dementia was, however, associated with a decline in folate and vitamin B12 levels and an increase in homocysteine levels over the follow-up period. The association between increasing homocysteine level and incident dementia was partly accounted for by folate and vitamin B12 changes and all associations were partly accounted for by weight change.

Conclusions: Incident dementia is more strongly associated with changes in folate, B12 and homocysteine than with previous levels. These changes may be secondary to other somatic manifestations of dementia, such as weight loss.

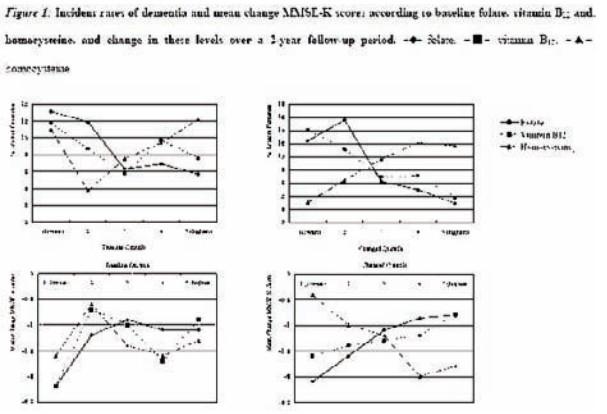

Figure 1: Incident rates of dementia and mean change MMSE-K scores according to baseline folate, vitamin B_{12} and homocysteine, and change in these levels over a 2-year follow-up period. -◆- folate, -■- vitamin B_{12}, -▲- homocysteine

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Generation of Tissue Micro Arrays (TMA) Specific for Alzheimer's Disease and Related Neurodegenerative Disorders

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The generation and validation of tissue microarrays (TMA) allows for the rapid, and simultaneous screening of target expression in tissues from different disease states, using in situ hybridization and immunohistochemical techniques. We generated a TMA from 10 different anatomical cerebral regions derived from brains of individuals with Alzheimer's disease or related neurodegenerative disorders (14 disease and 7 age matched control tissue autopsies).

Three TMAs were constructed using 0.8 mm sections from neuropathological and anatomically well defined areas of interest each from formalin-fixed paraffin embedded brain blocks. The areas was selected based on functional and morphological importance in AD.

All 3 TMA blocks were analyzed with immunohistochemistry using antibodies against common AD biomarkers such as Abeta, AT-8, Vimentin and GFAP, a potential biomarker namely myeloperoxidase (MPO), and histological methods such as Congo Red and Bielschowsky.

The results obtained demonstrated a high reproducibility that can be used in the routine diagnostic and research procedure, thus less time consuming and less amount of conventional slides that need to be analysed (1 TMA slide = 200 normal slides).

We conclude that the derived TMA can serve as a valuable tool for validation of tissue for diagnostic and research purposes by using different histological techniques such as immunohistochemistry and In situ hybridisation.

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Metabonomics and Proteomics Biomarkers Investigations in Neurodegenerative Disorders

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Our Proteomics Group is mainly focused in the investigation of differential protein expression profiles and metabolites pattern in neurodegenerative diseases. We have actively established a set of novel mass spectrometry investigations in order develop potential assays for molecular biomarkers identification and characterisation . 2D Electrophoresis approach has been complemented by MALDI-TOF mass spectrometry protein profiles either in the screening of complete protein extracts and in direct analysis of tissue samples. Novel bioinformatics tools have been developed to specifically analysed MALDI-TOF-MS molecular profiles and possibly outline spectra differences.

We have investigated the cerebral spinal fluid (CSF) of Alzheimer Disease affected patients versus subjects without cognitive impairment (Biroccio et al., Proteomics, 6(7), 2006). Our results indicated a differential distribution of TTR post translational modifications which might be implicated in amyloid peptides catabolism.

Our attention have been also focused in trying to assess analytical standards to implement clinical proteomics studies for neurodegenerative diseases (Del Boccio et al., Ann Neurol. 2006 Sep 27 on-line e-pub) especially in the employment of linear MALDI-TOF mass spectrometry.

Moreover, given the possible involvement of additional non protein biomarkers in neurodegenerative disorders we are pursuing metabonomics investigations. Characteristic CSF and serum metabolite profiling of different define clinical syndromes are collected by a high resolution LC-Q-TOF-MS method followed by multivariate statistical analysis. Such an experimental strategy outlines the possibility to discriminate different clinical groups based on principal component analysis (PCA) of 2,251 different metabolites.

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CSF Biomarkers Do Not Differentiate Between the Cerebellar and Parkinsonian Phenotypes of Multiple System Atrophy

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Background: Multiple system atrophy (MSA) can clinically be divided into the cerebellar (MSA-C) and the parkinsonian (MSA-P) variants, depending on the initial predominant signs and symptoms. However, neuropathological examination does not reveal major differences between the two clinical subtypes. It is unknown whether its variation in clinical expression is also reflected by a different underlying neurochemical profile.

Methods: We analyzed brain specific proteins and neurotransmitter metabolites in cerebrospinal fluid (CSF) of 26 patients with MSA-C and 19 with MSA-P, matched for age and disease duration, as well as in an age-matched control group.

Results: No differences were found in age and disease duration between both MSA-phenotypes. No differences were found in the levels of the various axonal or brain specific proteins and neurotransmitter metabolites between MSA-P and MSA-C.

Discussion: our results suggest that the clinical distinction between the two clinical MSA phenotypes is not reflected by the neurochemical composition of CSF, which, in turn, is in line with neuropathological observations. Possibly, our findings suggest a comparable global course and rate of disease progression, as well as neuropathological deterioration, in MSA-C and MSA-P.

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Cerebrospinal Fluid Neurofilament Concentrations in Dementia Syndromes

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Background: Cerebrospinal fluid (CSF) levels of amyloid β42 and tau proteins have been extensively studied as potential biomarkers for Alzheimer's disease (AD). However, to distinguish (late-onset) AD from dementia with Lewy bodies (DLB) and (early-onset) AD from frontotemporal dementia (FTD) still remains a challenge.

Objective: We investigated the potential values of CSF neurofilament protein analysis to distinguish AD from FTD and DLB.

Methods: 70 patients with AD, subdivided in 37 patients with early-onset AD (EAD) and 33 patients with late-onset AD (LAD), 18 patients with DLB, 28 patients with FTD and 26 control patients were included. Determination of NF light chain (NFL) and phosphorylated heavy chain (pNFH) levels in CSF was performed using home-made sandwich ELISAs.

Results: CSF levels of NFL were higher in FTD patients (mean 20.6 pg/ml) compared to control subjects (6.6 pg/ml; $p<0.001$) and AD patients (12.3 pg/ml; $p<0.05$), especially compared to EAD patients (8.5 pg/ml; $p<0.01$). However, sensitivity (82.1%) and specificity (70.3%) were moderate for the discrimination between FTD and EAD. CSF levels of NFL in DLB patients and LAD patients were comparable. CSF levels of pNFH were elevated in LAD (157 pg/ml; $p<0.05$), FTD (142 pg/ml; $p<0.05$), and DLB (182 pg/ml; $p<0.001$) compared to controls (79 pg/ml). However, no differences in CSF pNFH were found between DLB and LAD patients, or between FTD and EAD patients.

Conclusions: In conclusion, CSF NFL levels are elevated in FTD compared to EAD, but its application as a potential biomarker awaits confirmation in a larger patient population.

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Sequential Imaging Analysis Using MIBG Scintigraphy Revealed Progressive Degeneration of Cardiac Sympathetic Nerve in Parkinson's Disease

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Background and Aims: Functional imaging using [123I]metaiodobenzylguanidine([123I]MIBG) cardiac scintigraphy enables the visualization and quantification of a myocardial postganglionic sympathetic nerve terminal. As in Parkinson's disease, MIBG uptake is reduced at an early stage of the disease in almost all patients with a clinical severity of Hoehn and Yahr II or higher, it is recognized as a valuable tool to identify patients with Parkinson's disease early in the course of the disease.To clarify the utility of the imaging technique as a biomarker of sympathetic neurodegeneration in patients with Parkinson's disease, we studied sequential imaging analysis.

Methods:From the outpatients visiting our service, 43 patients met the UK Parkinson's Disease Society Brain Bank criteria were enrolled in our present study. MIBG scintigraphy was performed after informed consent from each patient. None of the patients had ingested any medications that was reported to influence MIBG metabolism. Planar images were taken 15 min (early images) and 3 h (late images) after intravenous injection of 123I MIBG. We analyzed these images and created region of interest. M refers to average count of the mediastinum and H refers to that of heart. We measured the ratio of H/M on both early and late images.

Results: Sequential imaging of the entire cohort showed a mean (SD) decline from baseline in late image of MIBG H/M ratio of 3.0% (0.06) at the second imaging. Mean interval between two imaging was 270 days.

Conclusion: We demonstrated the progressive decline of cardiac MIBG uptake using sequential imaging analysis in patients with PD.

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Transcriptional Profiling of Inflammatory and Apoptotic Genes in Peripheral Blood Mononuclear Cells From Patients With Alzheimer Disease

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Previous studies in our lab have shown that caspase 8, 9 and 3 were more activated in peripheral blood mononuclear cells (PBMCs) from Alzheimer's disease (AD) patients than in those from aged-matched controls. In order to investigate mechanisms underlying this phenomenon we performed the gene expression profiling of a specific panel of genes associated to apoptosis and inflammation in PBMCs from AD patients. Methods. PBMCs from 12 AD patients and 12 aged-matched controls were stimulated with IL-2 20 U/ml and PHA 2 µg/ml. After 24 h in culture, RNA was extracted and then analyzed for expression of 300 genes belonging to the apoptotic and inflammmatory pathways by means of a commercial gene-array (SuperArray, Bioscience). Results. A number of the assayed genes were found to be modulated. Particularly, TNF receptors and ligands showed a one/two-fold increase in AD patients in respect to age-matched controls. Moreover, also antiapoptotic genes of the Bcl-2 superfamily were found to be upregulated in PBMCs of AD patients in respect to aged-matched controls. In addition, cyclin dependent kinase 2 (CDK2) and the cytochrome P450 isoform, CYP1A1, were upregulated more than two-/three-folds, respectively in AD patients in comparison with controls. Difference in the expression of some genes was confirmed by real time PCR. Conclusions. Our findings suggest that important changes in the expression of genes associated to apoptosis, cell cycle and inflammatory response could be detected in AD patients at peripheral level, confirming our previous observations and supporting the hypothesis for an ongoing systemic dysregulation in AD.

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Amyloidogenic Peptides in Alzheimer Disease

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In the predominant "sporadic" late-onset forms of Alzheimer Disease (AD), the role of specific mutations appear of minor importance. ALZAS (Alzheimer Associated Protein), a short peptide with a sequence of 79 amino acids, partially homologous to the Aβ42, expressed from a nested gene located within the APP (Amyloid Precursor Protein) region on chromosome 21, is suggested to be involved in the APP dysfunction and pathogenesis of AD. ALZAS gene was identified using a procedure called "disease gene discovery by positional searching". It is expected to be expressed and activated in humans by several factors before the onset of clinical AD and may be a missing link in the pathogenesis of AD. ALZAS is expressed in human brain tissue and lymphocytes. ALZAS transcriptional unit is derived from exon 16 and 17 sequences comprising the codons translating to the Aβ peptide and the entire APP transmembrane signal sequence, and from APP intron 17 sequences that are not transcribed in the APP gene. The independent expression of ALZAS has been confirmed. In post-mortem AD brain: it is located intracellularly in neurons and in the inner membranes of cerebral vessels and correlated significantly with Braak and NIA-R classification scores of AD. In ELISA-pilot studies, ALZAS peptide and a specific ALZAS-autoantibody (IgG) could be detected in serum samples of patients diagnosed for AD or mild cognitive impairment. Whether ALZAS represents an indicator in a dynamic process of equilibrium between both

peripheral and brain degenerative changes and for induction of amyloidosis awaits further elucidation.

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Accurate Pre-Symptomatic Diagnosis in Blood, Effective, Side Effect-Free Prevention, and a New Pathology Model of Alzheimers Disease

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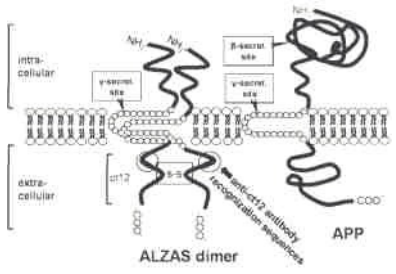
We have partially characterized two mechanistic intimately related biomarkers for Alzheimer's disease. These molecules are an AD-specific beta amyloid precursor protein (ALZAS) and an auto-antibody directed against ALZAS. ALZAS is translated from APP exon 16 and 17 plus it contains 12 amino acids encoded by sequences in APP intron 17. These 12 amino acids (ct12) make ALZAS unique. The auto-antibody is directed against ct12 and is found in subjects who are pre-symptomatic for AD. Such subjects may present another neurological disease e.g., schizophrenia, MCI, some forms of depression, non AD dementia. To distinguish AD from non-AD subjects we detect above molecules in specific ELISAs. The level of the auto-antibody at different stages of the disease led us to propose that the presence of the antibody prevents onset and modulates development of clinical AD whereas absence of the antibody allows AD to progress. We further propose that this activity can be mimicked by a vaccine when this becomes necessary as the natural defense declines with advancing age. Finally, we can present a model of the disease pathology on the basis of ALZAS which is strikingly consistent with the evidence-based hypothesis by Marchesi (2005), see figure.



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Identifying Relevant Clinical and Pathological Measures to Better Target Microarray Analysis of Alzheimer's Disease

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Background and aims: Basic researchers must often decide which clinical measures (metrics) are the most relevant to their study, and these decisions can have profound influences on downstream analysis. This is particularly true when those metrics are used to define microarray-based transcriptional profiles. In a prior microarray study of Alzheimer's disease (AD), we correlated gene expression levels with pre mortem Mini-Mental Status Exam (MMSE) scores and post mortem neurofibrillary tangle (NFT) counts (Blalock et al., 2005). However, several other metrics were also available (e.g., height, weight, age, gender, amyloid- both diffuse and plaque, Braak staging).

Methods: In the present work, I use a bioinformatic approach to test for the most suitable metrics by building a list of genes and proteins previously reported in the literature to have been altered in the AD brain ('AD list'). I hypothesized that the best biomarkers for detection of AD-related changes in the brain would find the greatest proportion of genes within the 'AD list'. Each metric was assigned a statistical probability based on its ability to detect 'AD list' genes using Pearson's correlation.

Results: MMSE and NFT counts were highly significant detectors of genes within the 'AD list', while other measures, such as body weight, performed no better than chance. Interestingly, measures of amyloid beta content were also poor detectors of genes within the 'AD list'.

Conclusions: This approach may aid investigators interested in less biased selection of useful metrics for microarray analyses.

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Elisa Test for Alzheimer's Disease (AD).Theoretical Premises and Practical Achievements

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Background:The AD diagnose has not so far support in physical and laboratory findings. At this Conference is presented the evidence that the condition may pe reproduced experimentally. s

Objectives:To use the antibodies of experimental induction of AD for developing a immunological diagnostic test according to ELISA model and enabling thus a laboratory positive diagnostic.

Methods: The raising of AD-like condition is possible as shown in any vertebrate, creating thus a multitude of AD-like sera each specific to species but with common functional properties.The situation in which the antigen to be looked for is not yet identified suggests as technical solution Sandwich type of Elisa. Taking advantage of the cross-immunity of the Abs anti480 Abs an optimal combination of two species was chosen for coating and for detection. A linear calibration curve was obtained in sequential dilutions of antigen 480.

Results:The results show constant and constant and significant differences between normal serum and 'AD human serum expressed in optical density values, aproximately three times higher

Conclusion:A laboratory solution for evidence of AD condition was found by ELISA test

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Decreased Alpha-Synuclein in Cerebrospinal Fluid as a Potential Diagnostic Marker for Parkinson Disease

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Background and Aims: There is ample biochemical, pathological and genetic evidence that the metabolism of α-synuclein (α-syn) protein plays a crucial role in the pathogenesis of Parkinson disease (PD). We examined whether quantification of α-syn in cerebrospinal fluid (CSF) is potentially informative in the diagnosis of PD.

Methods: We developed a specific ELISA system and measured the concentration of α-syn in CSF from 33 patients with PD (diagnosed according to UK PD Society Brain Bank criteria) and 38 control subjects including 9 neurologically healthy individuals.

Results: We found that PD patients had significantly lower α-syn levels in their CSF than the control groups ($p<0.0001$) even after adjusting for gender and age. Age was independently associated with lower α-syn levels. Logistic regression analysis showed that reduction in CSF α-syn served as a significant predictor of PD beyond age and gender alone (area under ROC curve, $c=0.882$). Furthermore, we observed a close inverse correlation between α-syn levels in CSF and assigned Hoehn and Yahr score in this cohort of 71 living subjects ($p<0.0001$), even after adjusting for age.

Interpretation: These findings identify in the quantification of α-syn from CSF a potential laboratory marker to aid the clinical diagnosis of PD.

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Biomarkers for Clinical Development in Alzheimer's Disease

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In the recent years, transcriptomics and neuroproteomics analyses studies have revealed that AD pathophysiology resides mostly in both neurodegeneration and inflammation. However, as the basic molecular events in the initiation and progression of AD are still poorly understood, the gap between disease onset and clinical diagnosis remains.

Analysis of clinical success rate from first-in-man clinical trials to registration showed that CNS compounds, including those targeting AD, have one of the lowest success rates compared to other therapeutic areas mostly due to lack of efficacy. Clinical biomarkers have emerged as a critical tool to help define disease and to determine pharmacological response with a strong focus both on diagnostic biomarkers to track disease at its initiation or when only mild symptoms occur and on prognostic biomarkers to evaluate disease severity and progression and also patients at risk who would most benefit from novel treatments.

The optimal biomarker features include identification of a universal feature of AD pathophysiology, independence of the clinical symptoms, high specificity and sensitivity, non invasive, allowing repeated measurements, low cost and easy to test. Biomarkers combinations also help to achieve all/most of these components for their use as symptomatic or presymptomatic diagnostic tools. In drug development, safety and efficacy biomarkers contribute to the understanding of drug response variability enabling go/no go decisions.

In our presentation, the AD molecular and neuroimaging biomarker landscape will be reviewed focusing on the inflammatory and neurodegenerative features and applications of biomarkers in clinical development decision making.

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CSF Tau Proteins in Evaluation of Patients With Suspected Dementia

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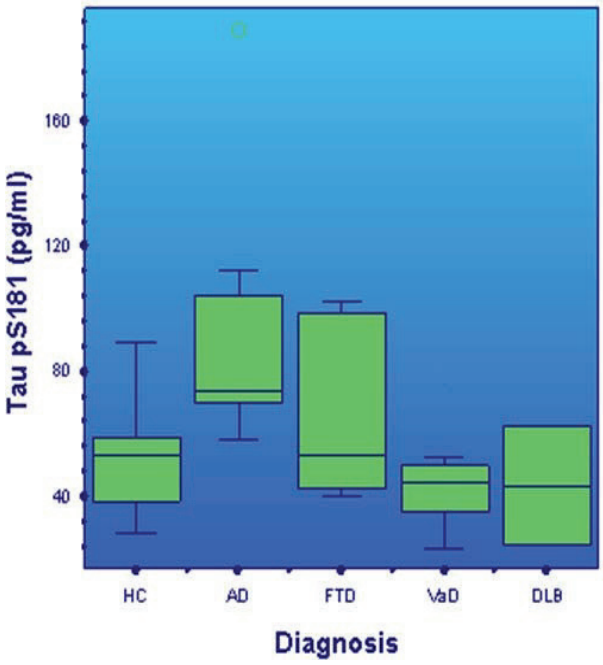
We tried to evaluate the diagnostic value of CSF total tau protein (t-tau), tau protein phosphorylated at threonine 181 and serine 199 (p-tau181 and p-tau199) in early-onset Alzheimer's disease (AD) versus healthy controls (HC) and other primary causes of dementia, such as frontotemporal dementia (FTD), vascular dementia (VaD), diffuse Lewy-body disease (DLBD) and Creutzfeld-Jacob disease (CJD). CSF levels were measured using commercially available ELISA kits (t-tau and p-tau199 - BioSource, Camarillo, CA, USA and p-tau181 - Innogenetics, Ghent, Belgium). Mean CSF t-tau and CSF p-tau181 levels were significantly elevated in AD patients compared to FTD, VaD and HC. P-tau181 showed best sensitivity and specificity (AD vs DLB: sens. 91%, spec. 94%; AD vs VaD: sens. 91%, spec. 95%), while p-tau199 showed low specificity (25-30% when sensitivity was set at 85% or greater). We concluded that total tau and p-tau181 represent useful biological markers for distinguishing AD from other primary causes of dementia, suggesting that CSF examination of tau proteins should become a part of routine diagnostic procedure for patients with suspected dementia.



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Detection and Validation of Biomarkers for Alzheimer's Disease in Cerebrospinal Fluid Using High-Resolution Proteome Analysis

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Background and aims: Dementias are diagnosed late in the course of the disease with poor sensitivity and specificity regarding differentiation between various forms. Future treatments will have to start earlier in the disease process to avoid disability. Hence an early identification of patients at risk to develop dementia is needed. Established biomarkers for Alzheimer disease based on disease models like tau, phosphotau and abeta peptides are currently tested on their ability to detect Alzheimer pathology in possible early disease stages like Mild cognitive impairment, but new diagnostic tools may enable us to detect dementias even earlier and guide our understanding of the diseases toward new treatment options.

Methods: Sophisticated high-resolution proteome analysis of cerebrospinal fluid was performed by capillary-electrophoresis coupled mass-spectrometry.

Results: The investigation of CSF from patients with various neurodegenerative diseases like AD, FtD, vascular dementia etc. resulted in the definition of disease specific polypeptide patterns allowing the differentiation of dementias from healthy volunteers with high sensitivity and specificity (90%).

Blinded assessment of the disease specific biomarker patterns onto more than 100 patient samples indicated that dementias such as AD and FtD can be detected with high sensitivity and specificity. Additionally this approach allowed the identification of synaptic biomarkers, previously not linked with Alzheimer disease, like Testican-1 and ProSAAS fragments pointing toward new battlefields beside plaques and tangles.

Conclusion: The data presented regarding the prospectively investigated MCI cases indicate that CEMS can be used to identify incipient AD cases in MCI cases with high confidence.

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Abnormal APP Processing in Platelets of Patients With Alzheimer's Disease: Correlations With Membrane Fluidity and Cognitive Decline

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Background and Aims: Previous studies have implicated platelet APP as a candidate biomarker for Alzheimer's disease (AD). Platelets contain more than 95% of the circulating APP, and enclose the enzymatic machinery for the APP metabolism yielding both soluble APP and amyloid-β peptides. The objective of this study is to compare the ratio of 130kDa to 110kDa fragments of APP in platelets from patients with AD, mild cognitive impairment (MCI) and elderly controls.

Methods: Western-blot analysis (22C11 monoclonal antibody) and DPH anisotropy of platelet membranes of AD (n=23), MCI (n=25) and control subjects (n=30).

Results: APP ratio in AD: 1.01±0.21, Controls: 1.24±0.21, and MCI: 1.18±0.21 (AD vs. Controls: p=0.001; AD vs. MCI: p=0.027). No significant differences were found between MCI and controls (p=0.904). Correlations between the APP ratio and DPH anisotropy: r=0.3 (p=0.01), and with cognitive scores: total MMSE score: r=0.33, p=0.003; total CAMCOG score: r=0.37, p=0.001; memory subscore of the CAMCOG: r=0.38, p=0.001.

Conclusions: The APP ratio was significantly reduced in AD as compared to controls and MCI patients. In addition, we found positive correlations between the APP ratio and DPH anisotropy (as a measurement of membrane fluidity), and with certain parameters of global cognitive and memory performance. Our results are in agreement with the limited available literature on this subject.

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Nitric Oxide and Aminopeptidase a Activity Are Inversely Correlated in Plasma of Experimental Hemiparkinsonism

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Central and peripheral Ang II, its functioning being regulated by Aminopeptidase A (AP A), interacts with nitric oxide (NO) and dopamine (DA). APs and DA exhibit important asymmetries in brain involving the nigrostriatal system. In addition, there also are asymmetries in the peripheral response to experimental hemi-parkinsonism. This

work analyzes in normotensive (WKY) and hypertensive (SHR) rats, the effect of injections of 6-hydroxydopamine or saline into the left or right striatum, on plasma AP A activity and NO concentration. Results demonstrate that there exist an inverse correlation between plasma AP A activity and NO, particularly in right-DA-depleted WKY and left-DA-depleted SHR rats. NO is released in part through the autonomic innervation of the vessels, so reflecting autonomic activity. Consequently, the observed changes after left or right experimental hemiparkinsonism may be due to asymmetries in the autonomic innervation of vessels. Since there is a marked inverse correlation between NO and AP A, changes in AP A may be also due to asymmetries in the autonomic innervation of vessels.

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Diagnostic Accuracy of the Clapping Test and Primitive Reflexes in Parkinsonian Disorders

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Background and objective: To determine the diagnostic value of the clapping test, which has been proposed as a reliable measure to differentiate between progressive supranuclear palsy (where performance is impaired) and Parkinson's disease (PD, where performance is said to be consistently normal) and its association with primitive reflexes.

Methods: We evaluated the clapping test in 44 patients with PD, 48 patients with atypical parkinsonism (AP) and 149 control subjects. Clapping performance was scored as follows: score = 0 (normal performance): Subject claps exactly three times following the verbal instruction and after having observed the examiner clapping three times. Score = 1: Following the verbal instruction, the subject claps more than three times. Score = 2: Even before receiving the instruction, the subject already spontaneously imitates the examiner. Various primitive reflexes were also tested.

Results: All control subjects had a completely normal clapping test compared to 71% of the PD patients, and 37% of the patients with AP. 18% of PD patients had a score 1 and 11% a score 2. Of the AP patients 42% had a score 1 and 21% a score 2. Clapping test was only correlated with snout reflex in the patients with PD.

Conclusion: There was a large overlap in the percentage of patients with an abnormal test performance in the patients with PD and AP, which limits the use of this test in the differential diagnosis of parkinsonian disorders. Possibly a disinhibited mirror neuron circuitry (imitation circuitry) may be part of the underlying pathophysiological mechanism

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Leukocyte Mapk Activity Associated With the LRRK2 G2019S Mutation and Parkinson's Disease

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Background: The 6055G>A mutation in the leucine-rich repeat kinase 2 gene (LRRK2) produces a G2019S substitution, located in the mixed-lineage kinase domain of Lrrk2, and leads to autosomal dominant Parkinson's disease (PD). We hypothesized that this mutation would probably alter mitogen-activated protein kinase (MAPK) signal transduction cascades. Such changes would probably be detectable in a variety of tissues.

Methods: We compared the activation and total amounts of the signal transduction proteins Src, HSP27, p38 MAPK, JNK and ERK, in leukocyte extracts from patients with G2019S-associated PD, healthy G2019S-carriers, patients with idiopathic PD, and healthy individuals.

Results: Phosphorylation of Src, HSP27 and JNK was significantly reduced in leukocyte extracts from patients with G2019S-associated PD compared to healthy controls. Similarly, phosphorylation of Src and HSP27 was reduced in healthy G2019S carriers and patients with idiopathic PD. Significant reductions in total Src were also found in these three groups compared to the controls.

Conclusions: Leukocyte extracts from patients with PD were found to have significant changes in key signal transduction proteins, though the effect seemed to be most pronounced in patients with the G2019S mutation. Changes in MAPK-signalling may therefore be common to PD pathophysiology, regardless of aetiology. Such changes may also be measurable in the blood of asymptomatic G2019S-carriers. This could be important for neuroprotective strategies to delay onset or slow the progression of PD, and may be potential biomarkers.

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Beta-Amyloid Containing Complexes in Human Cerebrospinal Fluid

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According to the beta-amyloid (Abeta) cascade hypothesis it is an imbalance in the Abeta metabolism that eventually leads to plaque formation in the brain and development of Alzheimer's disease (AD). The concentration of free Abeta is determined not only by the production and clearance of the peptide but also by its potential complex formation capacity with other proteins and their

concentrations. Data will be presented showing that a fraction of Abeta in cerebrospinal fluid (CSF) is bound in complex. First, when CSF is diluted in a buffer containing detergent prior to quantification not only the calculated concentration of Abeta but also the raw signal is increased. This suggests a dissociation of a complex, which exposes epitopes on Abeta that would otherwise be invisible in an immunoassay. Second, when CSF is subjected to size exclusion chromatography followed by Abeta1-40 and Abeta1-42 quantification on the fractions there is a signal in two peaks for both peptides, one at >100kD (the exclusion limit for the column) and the other at a fraction below the lowest molecular weight standard (6.5 kD). The former most likely represents complex bound Abeta while the latter corresponds to free peptide. Identification of the interaction partners would allow for specific assays towards these to be developed and tested for their usefulness as biomarkers for AD.

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Similar Pharmacogenetic Patterns of CYP2C19 in Spanish and Colombian Populations

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Background: The CYP2C19 isoenzyme is responsible for the formation of active or inactive metabolites of important drugs. The CYP2C19 gene is polymorphic, with a series of alleles that codify for enzymes with null or minimum catalytic activity. Moreover genotypic or phenotypic studies of this gene do not exist in the South American Mestizo and Spanish Caucasian populations.

Aims: The objective of this investigation was: a) to determine the prevalence of alleles CYP2C19.1, CYP2C19.2, CYP2C19.3, CYP2C19.4, CYP2C19.5, CYP2C19.6 and CYP2C19.8 in DNA samples of Spanish and Colombian populations; b) to compare the genotypes and allelic frequencies found in these ethnic groups.

Methods: Genotyping was performed using minisequentiation and real-time PCRs in 189 and 183 DNA samples obtained from Spanish Caucasian as well as Colombian Mestizo individuals.

Results: Within the Colombian subjects we found 83.6% of carriers of the two wild-type alleles (extensive metabolizer, EM), 15.3% of heterozygous of one nonfunctional allele (intermediate metabolizer, IM) and 1.1% of carriers of two nonfunctional alleles (poor metabolizer, PM). Within the Spanish subjects we found 77% of carriers of both native alleles (EM), 21.8% were heterozygous of one nonfunctional allele (IM) and 1.1% were homozygous 2/2 (PM).

The Hardy-Weinberg equilibrium was confirmed for the genotype frequencies of both populations.

Conclusion: Both allelic and genotypic prevalence of the CYP2C19 gene are statistically similar among the two ethnic groups. This data suggests that there are no differences in pharmacogenetic responses among Colombian Mestizos and Spanish Caucasians, in relation to drugs metabolized by the CYP2C19 enzyme.

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CYP2D6 in the Spanish Population With Dementia

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Background: The CYP2D6 gene codes for an enzyme involved in the biotransformation of commonly prescribed drugs. The CYP2D6 gene is highly polymorphic, with a series of alleles that codify for enzymes with null or minimum catalytic activity. The frequently used drugs for dementia treatment are substrates of the CYP2D6 isoenzyme. In addition, allelic variation of the CYP2D6 gene has been suggested to be associated with CNS disorders.

Aims: a) To determine the prevalence of CYP2D6.3, 4, 5, 6, 7, 8 and 10 alleles and the multiplication of the CYP2D6 gene in healthy subjects and in patients with dementia in the Spanish population. b) to assess a potential relationship between CYP2D6 polymorphisms and dementia.

Materials and Methods: Genotyping was performed by long-PCRs, minisequentiation and real-time PCRs in 110 and 88 DNA samples obtained from patients with dementia and healthy subjects, respectively, in the Spanish population.

Results: Results show that between 5,6 to 9% of total subjects were poor metabolizers and approximately 6% were ultrarapid metabolizers.

The Hardy-Weinberg equilibrium was confirmed for genotype frequencies of both groups. The distributions of alleles, genotypes and phenotypes of the CYP2D6 among dementia patients and controls were not significantly different.

Conclusions: The data suggests that the treatment of patients with dementia can be improved via the phamacogenetic assessment of drug metabolic pathways.

The similar genotype frequencies found in patients with dementia and control subjects indicate that the CYP2D6 is unlikely to represent a risk factor for dementia susceptibility in the Spanish population.

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Validation of Housekeeping Genes for the Normalization of Qpcr Gene Expression Studies in Human Brain Tissue

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Quantitative real-time reverse transcription PCR (qPCR) is increasingly used to analyse gene expression in neurodegenerative diseases. However, identification of valid reference genes requires careful analysis to ensure that their expression is not altered by the disease process.

We examined the expression stability of 9 potential reference genes in post-mortem brain tissue from subjects with one of three neurodegenerative diseases and from matched

controls to validate their use for normalization of data. Gene expression stability was analysed using the MS Excel applet 'geNorm', developed by Vandesompele et al (2002) relying on the principle that the expression ratio of two theoretically perfect reference genes would be equal in all samples, regardless of disease state.Total RNA was extracted from the snap frozen post-mortem brain tissue obtained from the rapid autopsy program at The Netherlands Brain Bank, and reverse transcribed for subsequent qPCR studies using an ABI 7500 Real-Time PCR System with relevant TaqMan® Gene Expression assays. Gene expression was analysed in cerebellum and medial temporal cortex from 23 individuals. Mean cycle threshold (Ct) values for each sample and gene of interest were transformed to raw, non-normalized quantities prior to analysis in geNorm, where reference gene stability was defined as the average pairwise variation of a particular gene with all other candidate genes.

This study identified sets of valid reference genes for Alzheimer's Disease, Parkinson's Disease, and Dementia with Lewy Bodies which will be invaluable for normalization of gene expression data in future studies.

Vandesompele, J et al (2002) Genome Biology 3, 0034.1-0034.11

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Development of Cost-Effective, Homogenous 384-Well High Throughput Screening Assays for A-Beta(1-42) & A-Beta(1-40) Using Alphascreen Technology

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Beta-Amyloid peptides (A-beta) are the major constituent of amyloid plaques, one of the hallmark pathologies of Alzheimer's Disease (AD). Therefore accurate and precise quantitation of these peptides in biological fluids is a critical component of AD research. The current gold standard assay for analysis of A-beta peptides is ELISA which, although highly sensitive and of proven utility, is a multi-step, labour-intensive assay that is difficult to automate. To overcome these limitations, we have developed and optimised simple, sensitive, homogenous 384-well assays for A-beta(1-42) and A-beta(1-40) using Alphascreen technology. The assays are capable of detecting A-beta peptides at concentrations <10pg/ml and, using a final assay volume of 20ul, routinely generate z' values >0.85. The Alphascreen format has the key advantages that it requires substantially less "hands on" time, has higher precision, is easier to automate, has higher throughput and is cheaper to run than the gold standard ELISA assays. We will present data detailing the optimization of these assays and show their utility for measuring A-beta peptides in cell culture medium or from guanidine extracted brain samples.

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Inducible Nos (iNOS) and Peroxynitrite Determination Reveal Continous Inflammation in Parkinson's Disease

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Background and Aims: Abnormalities in immune functions in PD patients have been reported. Inducible (i)NOS is expressed in brain glial cells and invading monocytes/macrophages in response to a variety of injuries. To determine the possible involvement of iNOS and peroxynitrite, markers of oxidative-nitrative stress and inflammation, in the pathogenesis of PD, we were examined the expression of inducible NO synthase (iNOS), and the production of peroxynitrite in PD patients and in normal controls.

Methods: PBMC was isolated from 15 patients with sporadic PD and 15 controls. The mean age at examination of the patients and controls was 61.5 years and 59 years. A diagnosis of PD was verified according to PD Society Brain Bank criteria. Determination of iNOS and 3-nitrotyrosine proteins was performed in protein extracts by Western blotting. The densitometric analysis of Western blots was performed using a computerized densitometric system.

Results: In unstimulated and LPS-stimulated PBMC from PD patients levels of iNOS and 3-nitrotyrosine were higher than in unstimulated and LPS stimulated PBMC from HC ($p< 0.001$).

Conclusion: Our results support the involvement of peripheral mononuclear cells in PD pathogenesis highlighting by iNOS and 3-nitrotyrosine expression. These results suggest that increased peroxynitrite production, a strong oxidant resulting by rapid interaction between superoxide anion and increased iNOS expression and NO formation, may have an important involvement in mechanisms of activation and regulation of inflammatory processes in Parkinson's disease and iNOS is a target for the design of effective therapeutic strategies.

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Utility of Pronucleon Peptides in Rx-Dx Screening for Alzheimer's Disease

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Background & Aims: We have developed novel peptides that mimic conformational regions on target amyloid proteins associated with neurodegenerative diseases such as Alzheimer's Disease. The peptide ligands, called PronucleonTM peptides, are matched to particular conformational regions of the target amyloid proteins and upon binding undergo a conformational change from alpha helical to β-sheet.

Methods: We are able to monitor these conformational changes through engineered fluorophores conjugated to the N- and C-terminus of the peptide that form excimers when induced to β-sheet by the presence of the target protein. Assay reactions are conducted in a 96-well plate format using a TECAN scanning spectrofluorometer.

Results: We have shown the utility of this assay in measuring β-sheet rich Aβ proteins in biological fluids from AD disease states. Post-mortem collected cerebrospinal fluid clinical samples from confirmed AD cases were distinguishable from age matched cognitive normal adults. The PronucleonTM peptides have been shown to be directly recruited into growing Aβ fibrils and are currently being evaluated in a defined AD drug screening effort. Size exclusion chromatography of Aβ40 and Aβ42 aggregates are being evaluated to ascertain the sensitivity of the AD PronucleonTM peptides for a given aggregate state of Aβ.

Conclusions: Because the PronucleonTM peptides form a direct interaction with the target substrate, they have dual utility as both a diagnostic screen for Alzheimer's Disease as well as for use in a drug screening assay.

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Discovery of Alzheimer's Disease Biomarkers From Whole Blood Splice Variant Expression Analysis. A New Resolution in Transcriptome Investigation

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Alzheimer's Disease (AD) and related cognitive disorders would highly benefit from the development of specific biomarkers for an early detection of the pathology. Progress in "omics" technologies is giving us the ability to expand from hypotheses driven towards exhaustive genome investigation.

Gene expression profiling from whole blood represents an attractive new avenue for biomarkers discovery. There are several lines of evidence why the expression of specific genes could be altered in the blood of AD patients. First, alterations in platelets and lymphocytes physiology have been reported in AD. Second, changes in the central clock of AD patients could be communicated to the peripheral clocks. Third, several polymorphisms in AD predisposition genes will impact on gene expression which will provide a read-out in blood.

Alternative RNA Splicing is mainly responsible for generating transcripts and proteins diversity. 80% of human genes undergo alternative splicing regulation with more than six splice events per gene on average. Thus, gene expression profiling via current commercial microarrays only provides a limited and incomplete picture of transcriptomes. We have developed novel technological tools to move from a gene-centric to an exon-centric expression analysis and take into account splice variants participation. This enhanced view of transcriptomes led to initial successes in correctly classifying early breast cancer patients (AACR, 2006) and has now been applied to the discovery of AD related biomarkers from whole blood. Progress in the analysis of 50 AD patients, 50 matched controls and 20 non AD related CNS disorders will be presented.

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Discovery and Early Validation of Peripheral Biomarkers of Alzheimer's Disease

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Peripheral biomarkers of Alzheimer's Disease (AD) could have important clinical utility, providing increased certainty in the positive diagnosis of AD, particularly in the early phase of disease progression where a patient with mild cognitive impairment may have either incipient AD, or a benign form of mild cognitive impairment. Biomarkers may also identify disease mechanisms so highlighting potential disease-modifying therapies capable of slowing down or arresting the degenerative process and providing a means of monitoring pharmacodynamic action. In order to identify such biomarkers an extensive and well characterised cohort of AD patients and matched controls was assembled, facilitating genetic and genomic analysis. Previous studies have reported CSF biomarkers with good sensitivity and specificity, however lumbar puncture is an invasive procedure, requiring aseptic techniques and performance by qualified and skilled medical staff. Peripheral samples such as plasma are a much preferred sample source. In this study a number of plasma samples from Alzheimer patients were extensively matched with control samples and subjected to analysis by both gel based and non-gel based proteomics. This lead to the identification of a number of proteins, which showed a significant statistical association with the disease. Using a range of different assays, a number of these proteins have undergone initial validation in the original cohort and secondary validation in an independent cohort. This has resulted in a number of encouraging potential peripheral biomarkers of AD. These data will be presented and discussed in the context of the disease.

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CSF Biomarkers and Apoe in MCI, AD and Related Disorders: Usefulness for Diagnosis and Correlation With Clinical Phenotypes

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The use of CSF biomarkers has improved accuracy of diagnosis of AD, while is uncertain their usefulness in differentiating among different clinical phenoypes of AD and related disorders. Moreover, neuropathological series had shown AD pathology in 30% of normal pressure hydrocephalus (NPH) patients.

Aβ1-42, t-tau and p-tau181 CSF levels were measured in 10 MCI, 50 AD, 23 FTLD, 7 NPH and 14 age-matched controls. Correlations with the presence of ApoE e4 allele and with different clinical phenotypes were evaluated.

Aβ1-42 was decreased in AD versus controls and FTLD (P<0.001), in MCI versus controls (P=0.04) and in NPH versus controls, FTLD (P<0.001) and AD (P<0.02). t-tau was higher in MCI, AD and FTLD than in controls and NPH (P<0.01). In AD p-tau181 was higher (P<0.01) and Aβ1-42/ p-tau181 ratio lower than in other groups (P<0.01). Controls with ε4 had higher levels of t-tau, and early onset AD (EOAD) with ε4 allele had higher p-tau181 (P=0.018). Positive correlation between t-tau and age was found in controls and FTD, while inverse correlation was present in AD patients (P=0.01). No correlation between biomarkers and clinical, neuropsychological or neuroradiological variables was found.

This study confirms the usefulness of CSF biomarkers in AD and MCI diagnosis but not in phenotypical differentiation. An interaction between ε4 allele and p-tau181 has been found in EOAD. ε4 correlate with higher levels of t-tau in controls, suggesting that this allele could have a role in neurodegeneration in healthy elderly. CSF of NPH shows low Aβ1-42 but normal t-tau and p-tau181.

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Olfactory Function in the Korean Elderly Population: Changes With CDR

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Background and Objective: The aim of the study is to investigate the changes of the olfactory function in the Korean elderly population related to cognitive decline by using CDR (Clinical Dementia Rating scale) and KVSS Test (Korean Version of Sniffin' Sticks Test).

Methods: 41 subjects between the ages of 65-85 years, were included in this study. All subjects were screened to exclude conditions affecting olfactory function. These subjects were divided into three groups according to CDR. 23 subjects (CDR=0) belonged to Group 1, 9 (CDR=0.5) to Group 2 and 9 (CDR=1) to Group 3. KVSS Test was composed of olfactory threshold test and olfactory identification test. A one-way analysis of variance (ANOVA) was used to compare smell characteristics among the groups.

Results: We have found significant differences between Group 1 and Group 3 and between Group 2 and Group 3 (p <0.001) in olfactory identification test. Score in olfactory identification test was lower in Group 3 than in Group 1 and Group 2. There were no significant differences between scores of subjects in Group 1 and those in Group 2 on olfactory identification test. Scores in olfactory threshold test were not different in Group 1, Group 2 and Group 3 (p >0.05).

Conclusion: These results suggest that the decrease of the scores in olfactory identification test is more associated with the cognitive deficit than that in olfactory threshold test is. They also indicated that in Group 3 (CDR=1), there is a drop of the scores in olfactory identification test.

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Lymphocyte Activation Induces An Increase in BACE1 Expression

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BACE1 plays a pivotal role in the generation of amyloid ß-peptide (Aß) through the proteolytic processing of the amyloid precursor protein (APP). BACE1 protein expression is mainly restricted to brain and pancreas although little constitutive BACE1 mRNA levels have been reported in many tissues.

We have demonstrated the presence of BACE1 expression in peripheral blood lymphocytes (PBLs) from human blood samples. BACE1 protein was analyzed by immunofluorescence and western blotting in PBLs purified from a series of AD patients and non-AD individuals. Whereas BACE1 protein expression in PBLs from non-AD individuals was found to be rather low, PBLs from some sporadic AD patients showed higher amounts of BACE1. BACE1 expression was not increased in PBLs purified from familiar AD-patients. We also analyzed the study of BACE1 expression in platelets, since its presence in platelets has been previously reported.

Then, we addressed the role of the lymphocyte activation in BACE1 expression. We treated PBLs isolated from healthy individuals with interleukin-2 (IL-2), concanavalin A (ConA) or bacterial lipopolyshaccaride (LPS). We found a significant increased in the BACE1 expression when PBLs were treated with ConA and IL-2. These data suggest that BACE1 expression in PBLs from AD patients can be the result of pro-inflammatory signals arriving to the blood from the brain.

Supported by the Spanish Ministerio de Sanidad (FIS-PI041242; Red-HERACLES) and Generalitat de Catalunya (GSR2005-266).

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The Göteborg MCI Study: Intra-Individual Cerebrospinal Fluid Biomarker Levels Are Highly Correlated in Repeated Measurements Two Years Apart

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The Göteborg MCI study is a longitudinal study of patients with mild cognitive impairment (MCI) that involves repeated analyses of biological markers for neurodegeneration at baseline and every second year upon enrolment. Here, we report the first cerebrospinal fluid (CSF) biomarker results of the 2-year follow-up. CSF total tau (T-tau), phospho-tau181 (P-tau) and beta-amyloid1-42 (Abeta42) levels were determined using the Luminex xMAP technology in 78 MCI patients and 15 cognitively stable control individuals at

baseline and after 2 years. During follow-up 10 patients progressed to pure Alzheimer's disease (AD) and 8 progressed to other or mixed dementias, while 60 remained stable. Baseline T-tau and P-tau levels were significantly elevated in the MCI-AD group as compared to the stable MCI patients and the control group ($p<0.05$), while baseline Abeta42 was significantly lower ($p<0.001$). Stable MCI patients were biochemically indistinguishable from controls. Likewise, biomarker levels in the MCI-AD group did not differ from those in patients who progressed to other or mixed dementias. The biomarker levels at baseline and after the 2-year follow-up were highly correlated in the whole material (Pearson R values between 0.74 and 0.94, $p<0.001$). These correlations resisted subgroup analyses in a robust manner. We conclude that intra-individual biomarker levels are remarkably stable over 2 years and that biomarker changes occur at least 2 years prior to conversion to AD in MCI.

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A Novel Candidate Biomarker in AD; Fibrinogen Gamma-A Precursor

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The prospect of early detection and treatment, to slow progression, holds hope for Alzheimer's disease with increasing average lifespan. The aim of the present study was to investigate candidate CSF biological markers in patients with mild cognitive impairment(MCI) and AD and compare them with age-matched normal control subjects. We classified patients by three groups: normal controls without cognitive dysfunction, MCI and AD. The AD group was subdivided into three groups by clinical severity according to a well known clinical scale for dementia, clinical dementia rating. We demonstrated a gradual increase in CSF fibrinogen gamma-A chain precursor protein in patients with mild cognitive impairment and AD compared to the age-matched control subjects. The CSF-fibrinogen gamma-A chain precursor protein was elevated in the MCI group; its expression was more prominent in AD group, and correlated with disease severity and progression. The expression level of fibrinogen gamma-A chain precursor protein was observed to be very weak in age-matched control subjects. These findings suggest that the CSF level of fibrinogen gamma-A chain precursor may be a promising biomarker for the progression of MCI to AD.

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Assessment of a 72-Marker Panel in the CSF of Pathologically Confirmed Alzheimer's Patients and Controls Within the Optima Cohort

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A key goal within the Alzheimer's disease (AD) research community is the early clinical detection and diagnosis of AD in patients who are at risk, or present with symptoms, of the disease. Much effort in the field is aimed at identifying biochemical biomarkers. In a collaborative effort between the Oxford Project to Investigate Memory and Ageing (OPTIMA) and Merck Research Laboratories, we have combined a panel of 9 CSF biochemical assays for APP catabolites (Aβ40, Aβ42, Aβx-40, Aβx-42, sAPPα, sAPPβ), tau (tTau, pTau-181), and CSF BACE activity, along with a panel of 63 measurable markers from the RulesBasedMedicine human Multi-Analyte Profile (xMAP v1.6). These biochemical markers were measured in the CSF of pathologically confirmed AD (N=27) and clinically evaluated control cases (N=29). In total, 72 markers were analyzed by both univariate and multi-variate methods. Univariate results will be presented which extend the literature findings beyond common markers like Aβ42, tTau and pTau-181. Optimal multi-analyte panels of sizes ranging from 2 to 12 analytes were determined for classifying AD and healthy control subjects using the Simulated Annealing Algorithm and Genetic Algorithm, within the framework of Linear Discriminant Analysis. The relationship between some of these markers and memory impairment using the Random Forests Analysis will also be presented. Taken together, the findings obtained from this post-mortem confirmed case analysis help extend the breadth of marker knowledge beyond the amyloid cascade and suggest the use of non-amyloid centric CSF markers in support of Alzheimer's disease biomarker research.

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in Vitro Characterisation of PIB Binding to White Matter

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Background and aims: 11C-PIB –PET has been demonstrated to be a suitable biomarker for assessing cortical Abeta burden in vivo. Notably, there is marked PIB retention in frontal and temporal cortex, posterior cingulated/precuneus and the caudate nuclei of Alzheimer's disease (AD) patients and to a lesser extent, within the white matter of all subjects. Whilst in vitro binding studies have previously characterised PIB binding to Abetaplaques within the grey matter of brains of AD subjects, PIB binding to white matter in AD or age-matched control subjects (AC) subjects has not been addressed. We therefore characterised the in vitro binding of 3H-PIB to white matter from AD and AC.

Methods: In vitro saturation studies were carried out utilising 5-500 nM 3H-PIB and 40ug of grey or white matter brain homogenates from the frontal cortex and centrum semiovale of AD and NC subjects, respectively. Non-specific

binding was established by incubation with 1 µM of non-radioactive PIB.

Results: Despite no significant binding to AC grey matter, 3H-PIB bound to AD grey matter in a saturable manner exhibiting nanomolar affinity (KD =2nM). 3H-PIB failed to show specific binding to white matter in both AD and AC subjects.

Conclusions: In vitro binding studies suggest that 3H-PIB does not specifically bind to white matter. Hence, 11C-PIB white matter retention in PET studies probably reflects slower brain kinetics rather than specific PIB binding to white matter.

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Pyrrolidone Carboxyl Peptidase Activity as a Serum Marker of Alzheimer's Disease

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Alzheimer type dementia (ATD) is a neurodegenerative disorder characterised by the progressive deposition of senile plaques in brain, related to an augmentation of beta-amyloid deposits. Some shorter derivatives of A-beta start with a glutamyl residue which under certain conditions are cyclized forming pyroglutaminated, more neurotoxic forms of A-beta. These derivatives are potential substrates of pyrrolidone carboxyl peptidase (Pcp). This enzyme may be measured in serum and may be considered as a biochemical marker for diagnosis of ATD. Pcp activity has been analysed in serum of ATD patients and elderly healthy volunteers. ATD diagnostic has been made using NINCDS-ADRDA criterion, together with a neurological exploration and complementary assays. Cognitive scores (Minimental, Blessed and FAST) allowed the clasification of patients by their evolutive situation. Serum Pcp activity was increased in patients with ATD. Furthermore, there was a correlation between serum Pcp activity, the time of evolution of the illness and the values obtained with the cognitive stage FAST. Although the origin of serum Pcp activity is unknown, it may be derived form the excision of the membrane-bound form. The increase observed may indicate a lost of Pcp activity in cellular membranes of brain cells and suggests a decreased capacity to degrade the pyroglutamyl-ended forms of A-beta, contributing to the fibrillogenesis and the formation of amyloid plaques. Furthermore, we propose Pcp as a key enzyme in preventing the formation of senile plaques, in the biochemical diagnosis of ATD and also may be considered as a specific pharmacological target in the treatment of ATD.

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Proteasome and Caspase Activity in Lymphocytes of Patients With Parkinson's or Alzheimer's Disease: Disease- or Drug-Related Changes?

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Impaired activity of the ubiquitin-proteasome (UP) system, a multicatalytic complex degrading poly-ubiquitinated proteins, has been recently suggested to intervene in the pathogenesis of neurodegenerative disorders characterized by intracellular formation of protein aggregates - such as Parkinson disease (PD) or Alzheimer disease (AD) – by inducing pro-apoptotic conditions. To test this hypothesis, while verifying, at the same time, whether such changes are detectable peripherally, we measured the activity of proteolitic UP core, proteasome 20S, and of pro-apoptotic caspases 3 and 9 in peripheral blood lymphocytes (PBLs) of PD and AD patients. We found that proteasome 20S activity is reduced in PBLs of treated PD patients vs. healthy controls, while marked increases in caspase-3 and caspase-9 activity were found. Increased caspase-9 activity was also detected in PBLs of untreated PD patients. PD duration and severity (UPDRS score) were inversely correlated with proteasome 20S activity and directly correlated with caspase-3 activity. An inverse correlation was also observed, in PD patients, between caspase-3 and proteasome 20S activities. No significant changes in proteasome 20S or caspase activities, nor correlations between biochemical and clinical variables, were found in AD patients. In conclusion, decreased proteasome activity, possibly related to caspase activation, is present in PBLs of PD (particularly those treated with dopaminergic agents), but not AD patients, suggesting that these variables may be considered for the development of peripheral biomarkers of PD. The exact influence of dopaminergic stimulation on these variables remains to be clarified.

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Vascular Risk Factors and Endothelial NOS3 Polymorphism Variants in Alzheimer's Disease

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Oxidative and vascular factors are implicated in Alzheimer´s pathology. In the vascular system, nitric oxide is generated by endothelial nitric oxide synthase 3 (eNOS3) and has an important role in vascular tone. Recently, a significant association between G894T and e4a/b polymorphisms in eNOS3 gene and Alzheimer disease (AD) was found. The presence of the T allele and the 4a variant are associated with vascular risk and the GG genotype increases the risk of developing late-onset AD. In order to know the influence of eNOS3 polymorphisms on AD-associated vascular parameters, we analysed Total-cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol, triglycerides, apolipoprotein E levels, nitric oxide (NO) and 1-42 beta-amyloid levels according to eNOS3 polymorphisms in the serum of 39 patients with AD and 28 control subjects. We have not found any association between the genetic variants of eNOS3 gene and serum nitric oxide levels. However, we found a strong positive correlation between HDL-cholesterol

and NO in AD patients with the T allele of the G894T polymorphism. The same HDL-cholesterol/NO correlation is observed in the absence of the 4a allele in both AD and healthy subjects. HDL-cholesterol and NO are suggested to have atheroprotective effects. It´s likely that eNOS3-related variants contribute to deteriorate brain function in AD due to cerebrovascular alterations.

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A Gene Expression Signature in Blood to Accurately Detect Alzheimer's Disease

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Alzheimer's disease (AD) is today the leading cause of dementia. An accurate, convenient and objective test to detect AD is much desired but currently, no definitive diagnostic test for AD exists. An accurate test based on peripheral blood as the clinical sample would be in line with the desired criteria. We have previously presented results from a pilot study suggesting that a blood-based gene expression based test potentially can be developed for AD [Abstract number: 162960, 2005 12th IPA, Stockholm, Sweden]. We have more recently also shown results from a large-scale study using ABI Human Whole Genome microarrays that confirm our initial findings and identifies a novel blood-based gene expression signature that identified patients with AD at an early stage with high specificity (91%), sensitivity (84%) and accuracy (87%) [Abstract number: O3-01-01, 2006, 10th ICAD Conference, Madrid, Spain]. The signature could also distinguish AD from Parkinson disease. Results from an initial validation on the RT-PCR based TaqMan Low Density Arrays® (TLDA®) (ABI) with a subset of the gene expression signature was also presented. This initial non-optimised test still retained high accuracy (85%) showing that TLDA® potentially can be used as a diagnostic platform.

A more extensive validation of the gene expression signature from optimised assays on TLDA® has now been performed on an independent cohort of 100 patients. Results from this validation will be presented. In addition we will also present results from a similar validation with a selected assay using the customised microarray system Codelink® (GE Healthcare).

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Translational Biomarker Discovery in Rhesus Monkey CSF and Plasma

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The conscious cisterna-magna ported (CMP) rhesus monkey model was established to enable simultaneous, repeat sampling of CSF and plasma to investigate APP metabolites as surrogate markers to predict human exposure required for amyloid lowering efficacy. The CMP rhesus monkey model has shown similar baseline Aβ40 / Aβ42 values to human in both CSF and plasma. Responsiveness to γ-secretase inhibition, γ-secretase modulation, and other potential AD therapeutics has also been demonstrated. This unique primate model has become extremely valuable to expand the traditional panel of analytes and for identification of novel biomarkers. A multianalyte CSF panel has been established using ELISA assays to measure Aβ40, Aβ42, sAPPβ, sAPPalpha, and tau analytes, while a panel of Aβ fragments can be measured using mass-spectrometric analysis. CSF proteomic analysis was used to evaluate drug responsive biomarkers. A robust readout was observed with 3 diverse Aβ lowering agents across 6 monkeys which correlated in time to maximum Aβ42 lowering. The relative signal among animals was consistent in a full time-course study up to 24 hrs after compound administration. In addition, a panel of 90 analytes measured in rhesus CSF demonstrated cross-reactivity in a human multi-analyte panel (MAP)in an external laboratory. Univariate analysis of these data yielded significant differences for three proteins beyond the known CSF Aβ40 and Aβ42. In addition to AD markers, this model has been useful in biomarker identification with central vs. peripheral CNS compounds for other disease targets.

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Plasma A-Beta Peptides and Apoe in Sporadic Alzheimer's Disease and Mild Cognitive Impairment

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Background: Plasma Ab levels have been examined in sporadic Alzheimer's disease (AD) patients yielding conflicting results. The results of several recent studies suggest that elevated plasma Ab1-42 levels may be detected several years before the onset of symptoms, though the value of that effect in predicting progression to dementia in mildly cognitively impaired (MCI) subjects is not known.

Subjects & Methods: Levels of Ab1-40 and Ab1-42 were measured in plasma from 54 patients with AD, 39 subjects with MCI and 35 controls using a commercially available ELISA.

Results: Mean plasma Ab1-42 levels were significantly higher in MCI as compared to both AD ($p<0.001$) and control subjects ($p<0.001$) while levels of Ab1-40 did not differ between the groups. In contrast to some earlier reports no correlations were observed between Ab species levels and age or MMSE scores. However, Ab1-42 were significantly lower in subjects carrying at least one apoliprotein e4 allele. Employing ROC curve analysis we found that the maximum accuracy in discriminating MCI versus both controls and AD subjects has been achieved using a cut-off value of 3,8.

Conclusions: Mean plasma levels of Aβ peptides differ between AD, MCI and control subjects though their usefulness in the differential diagnosis of AD is doubtful. Further studies

are needed to establish the value of Ab peptides levels in identifying patients with MCI and (possibly) in prediction of their progression to clinically overt AD.

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Plasma A-Beta Levels as Predictors of Clinical Response to Rivastigmine Treatment in Alzheimer's Disease

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Background: Clinical response to cholinesterase inhibitors treatment of patients with Alzheimer's disease is highly unpredictable and predictors of good response poorly studied and understood.

Subjects & Method: Fifty four highly selected AD subjects were included and treated with rivastigmine for 6 months. Clinical evaluations included MMSE and Clock Drawing Test for screening, CDR for the estimation of severity and ADAS-cog has been chosen to assess treatment impact on cognitive deficit. Plasma A-beta levels have been measured using a commercially available sandwich ELISA colorimetric assay. All subjects have been treated with rivastigmine with initial dose of 3mg/day, dose escalations possible every 4 weeks with maximum dose allowed of 12 mg/day.

Results: Mean ADAS-cog after 6 months of rivastigmine treatment has not changed, however, response was highly variable as about 25% of subjects improved 3 or more points, 50% showed no change and 25% worsened 3 or more points. Improvement in ADAS-cog score after 6 months correlated negatively with initial ADAS-cog score and progression rate before treatment. A-beta1-42 (but not 1-40) levels were elevated significantly in responders group defined as improved 3 or more points on ADAS-cog. Multiple linear regression showed that initial ADAS-cog score and change in plasma A-beta 1-42 levels after initial treatment but not apoliprotein genotype best predicted response to rivastigmine within 6 months of treatment.

Conclusion: Measuring A-beta 1-42 level change in plasma after initial (up to 4 weeks) treatment with cholinesterase inhibitor rivastigmine treatment might be useful in predicting clinical response after 6 months.

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Identifying the Potential Cis-Acting SNPs of the AD Candidate Genes

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AlzGene (http://www.alzforum.org) provides collection of genetic association studies performed on AD phenotypes. There are total 355 genes and 1036 SNPs studied. These candidate genes are strong candidate genes for Alzheimer's disease (AD) because of their known biological functions. However, although these genes were not new candidate genes for AD, almost of SNPs of the genes studied were not functional SNPs and there are only about 2.9 SNPs per gene were studied. Therefore, there is need to systemically characterize these genes. In our study, we will only focus on studying allelic expression SNPs selected from these genes. Briefly, we systematically determine the characteristic motifs common in promoters of AD candidate genes and their co-expressing genes, and then identify those "cis-acting" SNPs (the allelic expression SNPs) which co-localize on the AD candidate gene promoters with any of these characteristic DNA motifs. The fact that Co-expressed genes usually share some cis-regulatory elements has been strongly supported by previous classic works by clustering the expressions. Similarly, AD candidate genes and their co-expressed genes are supposed to share and regulated by some common cis-regulatory elements. Here at first, we strikingly found that there is very significant co-expression among the 355 Alzgene members. Furthermore, the common sequence features of these significant gene groups have been determined. Finally, comparing with the significant motifs, we could identify the significantly overlapped SNPs on promoters of the 355 Alzgene members.

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Amnestic Mild Cognitive Impairment and Alzheimer Disease is Impaired in Navigation in Two Dissociated Spatial Reference Frames

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Background: Many patients with Alzheimer€™s disease (AD) experience spatial disorientation, explained by both parietal and temporal lobe damage. Deficits in spatial navigation appear also in MCI. We developed human real space analogue of the Active Allothetic Place Avoidance (AAPA) task for rats, where the rats were shown to be impaired by unilateral hippocampal inactivation.

Methods: Comparison was made among subtypes of MCI and AD. The MCI subtypes were amnestic vs. non-amnestic, single- vs. multi-domain and hippocampal vs. non-hippocampal. Test was performed on slowly rotating circular arena 3 m in diameter. Subjects were instructed to avoid one or two independent sectors of the arena, indicated by loud warning sound. The sectors were defined by two cues on the arena wall and/or on the rotating arena floor. The test consisted of five parts. In the first two parts the subject got no information concerning the two frames of reference and should therefore infer him/herself the position of the sectors by the warning sound. In the second half of the test, the subject was first trained to recognize both frames and then use this information to avoid them on rotating and stable arena.

Results: The MCI were impaired after the training on a rotating arena but not on stable arena, suggesting a specific effect of dissociation of the reference frames.

Conclusions: Our results suggest impairment in orientation in two dissociated reference frames from the early signs of AD. Supported by GACR grants 309/05/0693 and 309/06/1231, MSMT ŒR 1M0002375201 and research project AV0Z50110509.

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Imperacer - New Prospects in CSF Biomarker Detection

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Background: Imperacer™ is a proprietary biomarker detection and monitoring technology focused on the analysis of low abundant and hardly detectable biomarkers. The Imperacer™ technology combines the advantages of immunoassays with the exponential signal amplification of PCR resulting in a typically 100 – 10,000-fold increase in sensitivity compared to standard immunoassays. Additional advantages of Imperacer™ include minimized sample volume requirements, a very broad linear dynamic range and high tolerance against drug and biological sample effects.

Results: We show the high sensitive detection of Alzheimer's Disease (AD) related biomarkers in femtogram/ml concentrations. AD is characterized as a protein misfolding disease due to the accumulation of abnormally folded amyloid beta protein and also considered a tauopathy. However, several proteins are involved in the pathogenesis and diagnosis of neurodegenerative disorders like AD. Some of these biomarkers are only available in lowest concentrations in CSF during the early-stage of the disorders, other could only be detected in biopsies samples. Imperacer™ address these challenges by improving the sensitivity in any biological sample type and also by allowing to switch from one biological sample to another easier accessible one. Furthermore the sample size for testing is a critical issue, here Imperacer™ could decrease the sample volume to less than 1µl still showing an improved sensitivity compared to standard immunoassays. Moreover Imperacer™ enables the detection and establishment of new biomarkers.

Conclusion: These results show a potential new approach in early-stage diagnosis of Alzheimer's Disease and prognosis of related dementias, including parkinsonian movement disorders, and ways for future developments.

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Differentially Expressed CSF Amyloid-Beta Peptides in Alzheimer's Disease, Dementia With Lewy Bodies and Parkinson's Disease

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Objectives: Diagnosis of dementias based on established clinical criteria is often difficult, biomarkers for applicable diagnostic testing are most warranted. A recently established quantitative urea-based Aβ-sodium-dodecylsulphate-polyacrylamide-gel-electrophoresis with western immunoblot (Aβ-SDS-PAGE/immunoblot) revealed a highly conserved amyloid-beta (Aβ) peptide pattern (Aβ1-37, 1-38, 1-39, 1-40, 1-42) in cerebrospinal fluid (CSF).

Methods: We used the Aβ-SDS-PAGE/immunoblot to investigate CSF of 23 patients with Alzheimer's disease (AD), 21 with dementia with Lewy bodies (DLB), 21 with Parkinson's disease dementia (PDD) and 23 non-demented disease controls (NDC) for disease-specific Aβ peptide patterns.

Results: An oxidized and probably alpha-helical form of Aβ1-40(Aβ1-40ox) aside the aforementioned Aβ peptide pattern was constantly observed in all CSF samples.

AD and DLB shared a significant decrease of CSF Aβ1-42 and an elevation Aβ1-40*. AD presented with pronouncedly decreased CSF Aβ1-42, whilst carboxterminally shorter Aβ peptides tended to be increased as compared to DLB and PDD. The ratio of Aβ1-42 to Aβ1-37 and Aβ1-38, respectively, discriminated all diagnostic groups from each other, except DLB from PDD. The percentage increase of Aβ1-40* relative to the sum of all investigated Aβ peptides was most prominent in DLB. The sensitivity for detection of DLB was 81% with a specificity of 71% and 70% among PDD and all investigated patients, respectively.

Conclusions: CSF Aβ peptide patterns reflect disease-specific interactions of each ongoing neurodegenerative dementia process with APP metabolism as distinct neurochemical phenotypes in AD, DLB and PDD and come closest to the requirements for a biomarker candidate.

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Analysis of Low-Abundant Proteins in Human Cerebrospinal Fluid

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Alzheimer`s disease (AD) is the most common form of dementia affecting the elderly. AD brains are characterized by the presence of senile plaques and neurofibrillary tangles (NFT). NFT are neuronal inclusions that consist of paired helical filaments (PHF), which contain as the main protein component a modified form of the microtubule-associated tau protein. In recent years, combined clinical examinations and measurements of the biochemical markers tau and Aβ in cerebrospinal fluid (CSF) have become a valuable diagnostic tool for AD with specificity above 80%.

Our work aims to identify novel biomarkers in CSF. Difficulties arise from dominant proteins present in this body fluid, which mostly prevent detection of low-abundant proteins. The concentration range of proteins in biological fluids spans nine to twelve orders of magnitude, with 85-90% of the total protein content being mass represented by only six proteins that is serum albumin (HSA), IgG, IgA, haptoglobin, antitrypsin and transferrin. Thus, we have tested several chromatographic techniques to deplete abundant proteins in CSF samples. The multiple affinity removal system (MARS, Agilent Technologies GmbH, Germany) quantitatively removed the six most abundant proteins, mentioned above. The IgY-12 Proteome Partitioning Spin Column (Beckmann-Coulter GmbH, Germany) removed additionally IgM, fibrinogen, apolipoprotein (A-I and A-II), α-1 acid glycoprotein and α-2 macroglobulin. The depleted samples were analyzed by two-dimensional gel electrophoresis and western blotting using monoclonal antibodies (mAbs). To

improve the sensitivity membranes, blotting techniques and staining procedures were optimized.

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Immunodetection of Doubly Phosphorylated Tau-Epitopes in Relation to Alzheimer´S Disease

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The microtubule-associated protein tau is the most abundant MAP in the brain. Its function is the stabilisation of the microtubules, the cell skeleton. The protein is regulated by reversible phosphorylation by MAP-kinase and phosphatases. In Alzheimer€™s disease (AD) the tau protein becomes abnormally hyperphosphorylated subsequently forming insoluble intracellular aggregates known as neurofibrillary tangles (NFTs). NFTs are composed of paired helical filaments (PHF) and are considered to be one of the major pathological hallmarks of the disease.

Phosphorylated proteins like the tau protein are typically characterized with phosphorylation-dependent monoclonal antibodies (mAbs). Two promising disease specific doubly phosphorylated epitopes on PHF-tau have been selected to produce mAbs using short phosphopeptides corresponding to relevant tau sequences. The generated monoclonal antibodies recognized the neighboring phosphorylation sites Thr212/Ser214 and Thr231/Ser235. The exact binding motifs of the obtained six mAbs were mapped with non-, mono-, and double-phosphorylated peptides as well as terminally shortened sequences. Thus, two mAbs for each epitope were obtained recognizing only the double-phosphorylated sequence of the peptides and PHF-tau without significant cross-reactivity towards normal human tau and bovine tau in ELISA or Western-blot analyses. This proved that double phosphorylation is sufficient to distinguish PHF-tau from all other tau versions. In addition, two further generated antibodies recognized specifically phospho-Thr231 and phospho-Ser235 independent of the phosphorylation status of the nearby phosphorylation site. Finally, all mAbs tested were useful for the immunohistochemical labeling of abnormally phosphorylated tau in the neocortices from autoptic brains of AD cases.

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Quantitative Measurement of Alzheimer's Disease Related Biomarkers Using Multiplexed Immunoassays

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Alzheimer's disease (AD) is a leading cause of memory impairment and dementia. Amyloid plaques consisting mainly of ABeta peptide, generated by proteolytic cleavage of the amyloid precursor protein (APP), are the hallmark pathology of AD. Since the CSF is in direct contact with the extracellular space of the brain, biochemical changes in the brain may be reflected in the CSF and thus the APP processing cascade may prove a rich source for potential biomarkers of AD. The full-length APP is cleaved by either alpha-secretase or beta-secretase, releasing sAPPąor sAPPb. Cleavage by Beta-secretase followed by gamma-secretase produces ABeta peptides. Using a novel set of immunoassays, the three ABeta peptides, ABeta38, ABeta40, and ABeta42 can be quantified in a manner that achieves maximum information from minimum sample. Using 4G8 as a detection antibody and peptide-specific capture antibodies, a triplex peptide assay has been developed that can be used to simultaneously quantify ABeta38, ABeta40, and ABeta42 in a variety of sample types, including human CSF and human or rodent brain cell lysates. Singleplex assays, utilizing 6E10 as capture are extremely sensitive and measure individual peptide levels in human CSF and plasma. Variability in the quantification of absolute levels of ABeta peptide has been limited in these assays through the use of peptide-specific monoclonal antibodies, critical sample handling and collection guidelines, and assay protocols optimized for distinct biological matrices. Accurate quantification of these AB peptides, along with other key biomarkers for AD, may allow for earlier and more precise diagnosis of AD.

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Comparative Platelet Proteome Profiling From Dementia and Parkinson's Disease Patients

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Biomarkers are needed to assist in the diagnosis and medical management of neurodegenerative disorders, including Alzheimer's disease (AD) and Parkinson's disease (PD). Peripheral blood platelets mirror alterations in the central nervous system of AD and PD patients, like changed processing of amyloid precursor protein, increased membrane fluidity mitochondrial dysfunction and increased oxidative stress. We used a multiplex quantitative proteomics method, DIGE (2D differential in gel electrophoresis) to identify alterations in the platelet proteome which would be suited for a routine diagnosis from blood of AD and PD by a protein chip.

From 25 dementia patients, 20 idiopathic PD patients and 50 age- and sex-matched individuals alterations of the platelet proteome as well as the level of platelet oxidative stress measured by thiobarbituric acid assay was investigated.

Accordingly to literature we could measure significantly alterations of amyloid precursor protein processing in peripheral blood platelets of dementia patients as well as increased platelet lipid peroxide levels of the dementia and PD patients compared to control individuals.

Mass spectrometric analysis of the changes observed in two-dimensional electrophoresis identified a number of proteins previously implicated in the pathology of these neurodegenerative disorders, including Apolipoprotein E, glutathione S-transferase omega, monoamine oxidase B as

well as unknown like tropomyosin and phospho glycerol dehydrogenase.

These findings suggest that blood platelets are a rich source for biomarkers of Alzheimer's and Parkinson's disease and this roster of proteins may enable to develop a diagnostic protein chip that could eventually assist in clinical diagnosis and therapy of AD and PD.

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Effect of Cerebral White Matter Changes on Clinical Response to Cholinesterase Inhibitors in Dementia

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Background: Cerebral white matter changes (WMC) represent cerebrovascular disease (CVD) and are common in dementia. Cholinesterase inhibitors (ChEIs) are effective in Alzheimer's Disease (AD) with or without CVD, and in Dementia with Lewy Bodies/Parkinson's Disease Dementia (DLB/PDD). Predictors of treatment response are controversial.

Objective: To investigate the effect of WMC severity on response to ChEIs in dementia.

Method: CT or MRI brain scans were rated for WMC severity in 243 patients taking ChEIs for dementia. Raters were blind to patients' clinical risk factors, dementia subtype and course of illness. Effects of WMC severity on rates of decline in cognition, function and behaviour were analysed for 140 patients treated for nine months or longer. Analysis was performed for this group as a whole and within diagnostic subgroups AD and DLB/PDD. The main outcome measure was rate of change in Mini Mental State Examination (MMSE) score. Secondary measures were rates of change in scores on the Cambridge Cognitive Examination (CAMCOG), Instrumental Activities of Daily Living (IADL) and Clifton Assessment Procedures for the Elderly – Behaviour Rating Scale (CAPE-BRS).

Results: There was no significant correlation between severity of WMC and any specified outcome variable for the cohort as a whole or for patients with AD. In patients with DLB/PDD, higher WMC scores were associated with more rapid cognitive decline.

Conclusions: Increased WMC severity does not predict response to ChEIs in AD, but may weaken response to ChEIs in patients with DLB/PDD.

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Brain Spect in Mild Cognitive Impairment in Comparison to Subjective Memory Complaint. Findings From the Descripa Multicenter Study

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Eighty patients undergoing brain SPECT (Tc-99m-ECD) were grouped according to neuropsychology: i) subjective memory complaints-SUBJ:19 patients, 8 females, mean age:68.9±5.8; mean MMSE:29.0±1.14); ii) objective memory deficit (amnestic MCI-aMCI:41 patients, 19 females, mean age:70.8±7.7; mean MMSE:27.7±1.51); iii) objective deficit other than memory (non amnestic MCI-naMCI:20 patients, 16 females, mean age:68.5±7.1; mean MMSE: 28.5±1.32). After normalization in the MNI space, six Volumetric Regions of Interest (VROIs) were identified in each hemisphere (Pick Atlas, SPM2) and were normalized on whole cerebellum (CRB). CRB was normalized on whole brain for reference. ANOVA for repeated measures was performed on 13 VROIs (2*6+CRB) among the 3 groups. A significant effect of group (p=0.006) and VROI (p=0.0001) was found, with a nearly significant interaction between group and VROI (p=0.059). aMCI group showed lower values than SUBJ and naMCI group at right Hippocampus (p=0.0005), both posterior Parietal cortices (p=0.004), right Temporal cortex (p=0.01), both posterior Cingulates (p=0.02). naMCI group did not significantly differ from either aMCI or SUBJ groups. Factorial analysis on neuropsychology identified Factor 1 (word list learning and delayed recall) and Factor 2 (TMT-A and B). Factor 1 significantly correlated with several VROIs, mostly left Parietal cortex (r=0.41;p=0.001) and right hippocampus (r=0.38;p=0.001). aMCI group showed low brain perfusion in critical cortical areas, whereas naMCI cannot be distinguished by SUBJ, thus underscoring its heterogeneity. Using SPECT in aMCI group versus SUBJ and naMCI subjects taken together, 77.5% of aMCI patients were correctly identified. Brain SPECT is useful in aMCI, while naMCI needs to be further characterized.

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Study on the Bioaccumulation by Aluminum Administration With or Without Amino Acids

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Research on both animals and humans has linked aluminum with neuro-cognitive dysfunction such as Alzheimer's disease. Aluminum ion binds strongly to physiologically important compounds such as the phosphate group of adenosine triphosphate and to blood serum proteins. Aluminum ion may also bind with some amino acids and pass through the blood-brain barrier more easily than aluminum ion alone.

We reported that oral administration of water containing aluminum ion and specific amino acids glycine or glutamic acid to mice over a long period promoted aluminum ingestion, but the presence of tryptophane did not give any appreciable influence on aluminum incorporation1,2). The authors considered that the material in some drinking water and food additives combine to the aluminum and may promote the absorption of aluminum.

The present report is concerned with aluminum absorption and excretion to urine (and excrement) of mice. Our results indicate that the amounts of aluminum decreased in the urine or excrement of the mice to which proline, serine or leucine including aluminum ion had been administered. These results suggest that aluminum may form chelate compounds with the above amino acids and accumulate in the body more easily than aluminum only.

1) H. Aikoh and T. Shibahara, Abstract of 7th International Conference AD/PD 2005, p.179.

2) H. Aikoh and T. Shibahara, Physiol. Chem. Phys. & NMR(2005) 37:65-69.

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EGCG Reduces Neurotoxicity by Beta-Amyloid Through Down-Regulation of C-Abl Kinase Activity

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Alzheimer's disease (AD) is the most common form of neurodegenerative disease nowadays. The neuropathological hallmarks of AD are the accumulation of protein deposits in the brain as amyloid beta (Aβ) plaques. The mechanisms involved in the Aβ-mediated neurotoxicity are not known thoroughly, but lots of evidences suggested that oxidative stress may play a key role. Aβ neurotoxicity was also found to be attenuated by numerous antioxidants. Increasing data point to a group of antioxidants, such as polyphenol compounds, can scavenge ROS and protect cells from oxidative damage.

Using the model of endogenous Aβ-induced neurotoxicity, we evaluate the neuroprotective effects of tea polyphenols. In our study, we demonstrate (-)-epigallocatechin-3-gallate (EGCG) treatment of MC65 cells, a human SK-N-MC cells transfected with β-amyloid precursor protein, leads to a significant reduction in apoptosis. We find that non-receptor tyrosine kinase c-abl activation is involved in cell signals that regulate neuronal death, and EGCG may display neuroprotection effects through down-regulation of c-abl kinase activity and nuclear subcellular localization. Furthermore, in vivo study also revealed decreased production of Aβ and β-amyloid plaques in the brain of APP transgenic mice (Tg2576), which is corrected with the reduction of c-abl kinase activity. In conclusion, these results indicate that EGCG not only prevents Aβ production, but also plays a role in blocks amyloid-induced neurotoxicity by inhibition of c-abl activity. These results raise the possibility that EGCG supplementation may provide a therapeutic strategy for the treatment of AD.

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The Case for Cdk5 as a Protective Agent in the Development of AD

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Cdk5 is an atypical cyclin dependent kinase. It is structurally related to other Cdk enzymes yet it plays no role in a normal cell cycle. Rather, the targets of Cdk5 are usually identified as cytoskeletal proteins such as neurofilament and tau. Work from our lab has shown that Cdk5 does regulate the cell cycle, albeit in a negative way. Mature neurons enter a non-mitotic state after emigration from the ventricular zone; any influence that forces the cell to re-initiate a cell cycle leads to death rather than division. In Cdk5-deficient neurons, the cell cycle is not efficiently suppressed, resulting in ectopic re-expression of cell cycle proteins and unscheduled DNA synthesis. This aberrant mitotic activity can be blocked by transfection of wild-type Cdk5. Subcellular localization of the kinase also plays a role in this regulation as cell cycle suppression is associated with a nuclear location for Cdk5 while DNA synthesis is associated with a cytoplasmic location. Cultured Cdk5 deficient neurons have low constitutive levels of tau phosphorylation, suggesting a major role for Cdk5 in the maintenance of normal levels of phosphotau. When stimulated by Aβ1−42, however, neurons lacking Cdk5 develop high levels of AT8 and PHF1 immunoreactivity, suggesting a separate kinase is responsible for the pathological alterations of tau that are associated with AD. The appearance of immunoreactivity can be blocked by lithium and other GSK3β inhibitors suggesting that this latter kinase is more directly involved in tau-related disease pathology.

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Evidence for Pathogenic Involvement of LRP / RAGE in Alzheimer Brains

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There is significant evidence to suggest that a damaged or dysfunctional blood-brain barrier [BBB] may contribute to the pathogenesis of some AD lesions. An indication of altered BBB integrity / regulatory capacity could be an altered expression of capillary endothelial LRP and / or RAGE. Cortical samples were taken from the superior temporal and calcarine cortices of ten confirmed AD brains. Coronal sections were cut, with multiple contiguous sections for each

case, and stained using immunohistochemistry techniques for tau protein, beta- amyloid n-terminus [8- 17, 40 & 42] , LRP and RAGE. Segments of cortex were randomly selected in each case and section areas of ten field- diameters, contiguous, full and of comparable cortical widths were observed at 250x magnification. The densities of neurofibrillary tangles (NFTs), senile plaques (SPs) and beta-amyloid, LRP and RAGE positive capillaries were recorded. Independent T-test analysis revealed that there was no significant difference between the two cortices for either LRP or RAGE activity in AD brains. There were significant negative correlations between the densities of some SP isoforms and both LRP and RAGE-positive capillaries in both cortices [p>.001, Spearman's]. In both cortices there were significant positive correlations between LRP-positive capillaries and amyloid- positive capillaries and larger vessels [p>.001, Spearman's]. These results suggest that an altered LRP, especially, and / or RAGE BBB capillary expression in lesion prone regions of AD brains may contribute to the pathogenesis, severity and / or recovery from SP lesion development.

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Dual Role of Kinins in Prostaglandin Sysnthesis Regulation in Primary Rat Astrocyte Cultures

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Kinins are important mediators of peripheral and probably brain inflammation. They act via B1 and B2 receptors. Kinins have been shown to induce synthesis of prostaglandins (PGs), major mediators of inflammation. Release of pro-inflammatory molecules by astrocytes has been implicated in a number of neurodegenerative diseases including Alzheimer's disease (AD). Furthermore, functional differences in cell signaling induced by kinin receptors have been linked to AD. Apart from the well recognized immediate effects, recent studies indicate that kinins may be involved in induction of protein synthesis, pointing to long-term effect of kinins. We examined the role of bradykinin (BK, a B2 agonist) and a B1 agonist in long-term regulation of basal and lipopolysaccharide (LPS)-induced synthesis of PGs in rat astrocytes. Exposure to B1 agonist (0.01-100 nM, 15h) or BK (1-100 nM, 15 h) enhanced basal PGE2 synthesis by about 2 fold, as measured by radioimmunoassay. Also, BK (100 nM) increased LPS-induced PGE2 production by 1.5 fold. However, the B1 agonist (100 nM) reduced LPS-induced PGE2 production by 35%. Both BK and B1 agonist also augmented COX-2 levels about 2 fold. On the other hand, while BK elevated LPS- induced COX-2 levels by 2 fold, the B1 agonist decreased LPS-induced COX-2 to control levels. The rise in PG and COX-2 was abrogated by cycloheximide, a protein synthesis inhibitor, suggesting de novo synthesis. Our results imply, for the first time, a dual role of kinins in regulation of inflammatory mediators in astrocytes.

"This research was supported by THE ISRAEL SCIENCE FOUNDATION (grant 500/04)".

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Up-Regulation of Hsp27 Affects Cell Cycle Progression and Tau and Neurofilament Phosphorylation

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Several heat-shock proteins are up-regulated in various neurodegenerative disorders, like Alzheimer's disease (AD), and one of these proteins is Hsp27. Hsp27 belongs to a family of small heat-shock proteins that are activated by, for example, oxidative and heat stressors. One hallmark of AD is neurofibrillary tangles that are mainly composed of abnormally hyperphosphorylated tau. This hyperphosphorylation is attributed to changes in kinase and phosphatase activities. But tau is not the only protein that is hyperphosphorylated and aggregated in AD, the phosphorylation state of neurofilament proteins is also deregulated. Many of the kinases that are known to phosphorylate tau also phosphorylate neurofilament proteins to varying degrees.

Here we investigate the overexpression of Hsp27 in AD brains and the consequences of Hsp27 overexpression in N2a mouse neuroblastoma cells using FACS analysis, Western and dot blots and immunohistochemistry. The affects on other proteins implicated in AD, like the kinases glycogen synthase kinase-3beta and p70 S6 kinase, are also investigated with focus on the regulation of tau and neurofilament protein phosphorylation and cell cycle progression.

Hsp27 overexpression affects specific phosphorylation sites in tau; decreased phosphorylation is seen for the tau site Ser202, while phosphorylation is increased at Thr212 and Ser214. But no significant effect on neurofilament protein phosphorylation was found. We also observed a shift in cell cycle phases, from S-phase to the earlier G1-phase, in the cells transfected with Hsp27.

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Interaction Sites Between Alpha B-Crystalline and Beta-Amyloid Mapped by Solution-State Nmr Spectroscopy

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Amyloid β-peptide (Aβ), a of 39 to 42 residue peptide, is a major fibrillar component of neuritic plaques in Alzheimer's disease (AD) brains and is related to the pathogenesis of the disease. In the brain of AD patients, Aβ co-precipitates with the small heat shock proteins (sHSPs) αB-crystalline and Hsp27. Recently, it was reported that αB-crystalline prevents amyloid fibril formation in vitro, but at the same time enhances the neurotoxic effect on cultured neurons. We report here the identification of the chemical groups of Aβ which are involved in interactions between Aβ and αB-crystalline by means of solution state NMR spectroscopy. We find that

especially the hydrophobic core region of Aβ is involved in intermolecular interactions with αB-crystalline. In the presence of αB-crystallin, Met35 in Aβ becomes efficiently oxidized. In order to quantify the redox properties of the different complexes consisting of Aβ / αB-crystallin / copper, we suggest an NMR assay which allow to estimate the electrochemical properties indirectly by monitoring the rate of glutathion (GSH) auto-oxidation. The oxidation of the side chain Met35 in Aβ might account for the increased neurotoxicity and the inability of Aβ to form fibrillar structures, which has been observed previously in the presence of αB-crystallin.

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Role of M1 Acetylcholine Receptors (AChRs) in Amphetamine-Induced Neurotoxicity in Striatum (ST) and Prefrontal Cortex (PFC)

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Oxidative stress has often been implicated in dysfunctional states and neurodegeneration in ST and PFC. In the rat model of acute AMPH neurotoxicity (5mg/kg ip; 3x in time intervals of 2h) an increase of striatal ACh release is associated with elevated nitric oxide (NO) synthesis and raised generation of lipid peroxidation (LPO) in PFC and ST. The interrelationship of these processes is not well understood and the role of ACh and AChRs has hardly been investigated yet.

In our study, the rise in NO (determined by electron paramagnetic resonance) and LPO production by AMPH was prevented in PFC and ST by M1/M4 AChR blockade with pirenzepine (30µg icv) and/or pre-treatment of the ST with microinjected MT7 (2µg), a selective and irreversible M1 antagonist. Activation of M1/M4 AChRs with McNA-343 (200µg icv) enhanced generation of NO by about 70% and LPO about twofold in the striatal and cortical tissue. The increases in these parameters of oxidative stress were prevented by pirenzepine (30µg), L-nitroarginine (100mg/kg ip) and quinacrine (40mg/kg ip). These results indicate that striatal M1/M4 AChRs stimulate NO synthase and phospholipase A2 (PLA2), inducing oxidative stress leading to phospholipide breakdown. M1 AChRs via NO synthesis and PLA2 activation play an important role in the neurotoxic effect of AMPH in ST and PFC. Probably the hypercholinergic state induced by AMPH leads to the M1 AChR activation. The described neuronal damage-promoting pathway may be of importance in neurological disorders such as dementias, Parkinson's disease and addiction.

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Role of the C-AbL/p73 Pathway in Niemann Pick C Neurodegeneration

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Niemann Pick type C (NPC) disease is a fatal autosomic recessive disorder, caused by mutations in the npc1 or npc2 genes, and characterized by the accumulation of free cholesterol in endosomes and lysosomes. NPC patients present a markedly progressive neuronal loss, mainly of cerebellar Purkinje neurons. Several evidences suggest that apoptosis is involved in Purkinje cell death; however it is not known how the apoptotic program is activated in NPC. We propose that the pro-apoptotic p73 transcription factor and the c-Abl kinase, that stabilizes p73, are involved in NPC neuronal death. The c-Abl/p73 system has been implicated in neuronal death induced by amyloid, redox and endoplasmic reticulum stress.

We analyzed p73 and c-Abl expression in postnatal wild-type and NPC (NPC1 -/-) mice cerebellar sections. In wild-type mice p73 and c-Abl signals were associated to Purkinje neurons. At 4 weeks of age, NPC mice showed p73 and c-Abl staining in Purkinje neurons and as the disease progresses both signals decreased due to the dramatically loss of these cells. To further analyze the participation of the c-Abl/p73 system in NPC neuron death we modulated this pathway in vivo in the NPC mice. Our results show that regulation of the c-Abl/p73 pathway reduces weight loss, neurological symptoms and cerebellar apoptosis and increases Purkinje neuronal survival of NPC mice. Conclusions: the c-Abl/p73 pathway is involved in NPC neurodegeneration.

Supported by: Ara Parseghian Medical Research Foundation, Fondecyt Grants # 1040782, 1040799, 1030415.

Alvarez and Zanlungo contributed equaly to this work.

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Tauopathy-Related PS1 G183V Causes Aberrant Splicing and Decreases Secretase Activity Without Affecting - Amyloid Secretion

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Background and Aims: In a family with Pick type tauopathy but no A?-plaques, we earlier identified a PS1-G183V mutation at the 3' splice signal of intron-6. Recently,

we identified this mutation in trans with a PS1-P49L mutation in a familial patient. We showed that both G183V families are descendants of a common founder and that mutations in PGRN were absent. Here we aimed at understanding how G183V altered transcript splicing and whether spliced-variants or full-length mutant protein (PS1G183V-FL) contribute to loss of PS1 function.

Methods and Results: Cloning/sequencing of brain PS1 mRNA showed that 20-25% transcripts were alternatively spliced producing shorter transcripts with predicted proteins having only first 2 transmembrane domains (PS12TM). Direct brain lysate immunoblotting and pulse-chase/immunoprecipitation on lymphoblasts did not identify any predicted truncated-protein, and consistently, PS1 and its endoproteolytically-cleaved fragments were reduced by about ?20% in brain. A serial tau-solubilization assay showed that pathological tau (Tau-64/60) was recovered in the more soluble fractions and normal tau was reduced, however, we did not identified any obvious alteration in the Akt/PI3K pathway. Further biochemical analysis showed that PS12TM failed to complement the γ -secretase defect in murine PS-/- fibroblasts. In contrast, PS1G183V-FL integrated into a γ-secretase complex but had reduced activity towards N-cadherin and Notch processing. Consistent with absent Aβ pathology, PS1G183V-FL had no detectable effects on γ-secretase-dependent generation of Aβ in 2 different cellular-systems.

Conclusions: Although the precise mechanism by which G183V causes Pick's disease remains elusive, our data suggest that G183V allele has APP independent reduced -secretase activity.

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Overexpressing Tau Mutants in Neuroblastoma Cells Leads to Tau Conformational Changes Associated With the Early Steps of Tau Nucleation

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Alzheimer's disease is mainly characterized by degenerating neurons displaying aggregates of the microtubule associated protein Tau. Even if aggregated Tau is always hyperphosphorylated and abnormally phosphorylated, the question of whether the phosphorylation alone or in combination with additional cofactors trigger Tau aggregation is not well elucidated yet. The discovery of Tau mutations in FTDP-17 dementia has allowed for generating animal models, which recapitulate the main features of Tau pathology. However, cellular models overexpressing Tau mutations provided some controversial results. Regarding that, we undertook analysis of 4RTau bearing FTDP-17 mutations in a neuronal cell context by overexpressing, in a human neuroblastoma cell line, Wild-type Tau or two mutated Tau (P301S or S305N) which we previously described as displaying opposite effects regarding Tau binding to microtubule. First, analysis of cell structure show that Tau overexpression leads to cytoskeleton remodelling independently of mutations. Secondly, Western blot analysis did not reveal any hyperphosphorylation linked to Tau mutation, at the studied epitopes (AD2, AT270, 12E8). However, 2D experiment approach showed a difference in migration pattern between wild-type and mutated Tau, suggesting some conformational modifications. Finally, even if no filaments were observed, these modifications appear to be concomitant with the beginning of nucleation with mutated Tau as displayed by electronic microscopy assays. These results suggest that beside Tau mutations, that can mimic some conformational changes, additional cofactors are required to lead to Tau aggregation into PHFs, and place these models at a crucial point to study the very early events of Tau nucleation.

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Skin Test for Alzheimer's Disease (AD)

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As long as no foreign agent was able to raise reactions in AD organism a Skin Test was not conceivable. The recent discovery of cross immunity of anti 480T to AD sera made this achievable.

Objectives: To reproduce in vivo the cross immunity or affinity reaction on which ELISA AD relies.

Methods: The rationale of this Skin Test is binding of a AD factor by a foreign Abs. For reasons of human security the test was reversed and carried out on a previously immunized laboratory model with a small amount(0,05 mll) of serum of the AD patient (or control) injected into superficial skin layers.

Results: on the sites of AD injection it appears an aseptic, dry,necrotic and well delimitated reaction without significant cellular infiltration of inflammatory immune process. The histological examination shows homngenous Amyloid in hypoderm and arteriolar and conjunctive tissue infiltration with Amyloid that extends much beyond the areas of injection

Conclusion: Unusual production and deposition of amyloid at the contact of AD serum with the model tissue. The extension and dynamics of Amyloid aggregation sugest a Domino pattern present in prion diseases.

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Tau-P301L Interferes With Calcium Homeostasis at the Golgi Apparatus Level

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Backgrounds And Aims: Mutations in tau, a microtubule binding protein, are causative for hereditary frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). The P301L tau mutation is the most frequently encountered tauopathy, making FTPD-17 of relevance to neuropathologists. The molecular mechanisms by which tau mutations impair brain function are not clear. Indeed, both functional and structural effects were found at the level of mitochondria and Golgi apparatus (GA) in transgenic mice expressing tau-P301L before the onset of tauopathy (1,2). Moreover, the FTPD-17 linked tau mutation V337M was correlated to an increased Ca2+ influx mediated by nifedipine-sensitive channels (3). In the present work, we aimed at understanding how cellular Ca2+ homeostasis is affected by tau-P301L.

Methods: As model we employed the cell line SH-SY5Y where the wild type (wt) or the P301L tau proteins were transiently expressed together with cytosolic or organelle-targeted recombinant aequorins. In addition, the effect of tau-P301L on voltage-gated Ca2+ channels was studied in rat cortical neurons at 5-7 DIVs by Fura-2, upon transient expression of the wt or mutant protein with the GFP reporter.

Results And Conclusions: In the SH-SY5Y model, tau-P301L alters Ca2+ homeostasis specifically at GA level without significantly modifying cytosol, mitochondria and endoplasmic reticulum Ca2+ dynamics. In rat cortical neurons, over-expression of tau-P301L increases the Ca2+ load induced by KCl depolarization.

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Impact of P301L Tau Mutation on Human Gene Expression Profiling

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Background and aims: Frontotemporal dementia (FTD) is the second most common form of early-onset dementia. Mutations in the microtubule-associated protein tau, including P301L, are genetically coupled to hereditary frontotemporal dementia with parkinsonism linked to chromosome 17. Aim of this study is to define human gene expression profiling associated with wild type or P301L tau proteins.

Methods: Gene expression analysis was done by the Genopolis consortium by using Affymetrix technology (GeneChip Array). Differentially expressed genes (DEG) were selected according to a significance level and clustered to group genes with similar expression profiles across the provided samples.

Results: Over-expression of wild type tau protein resulted in a down-regulation of 138 genes and an up-regulation of 135 genes; tau P301L determined the down regulation of a single gene and the up-regulation of 3 genes. We identified 3 clusters of genes with similar expression profiles over all conditions. Genes that failed to be down-regulated in the presence of the mutation (when compared with wild type protein) belonged to the following pathways: amyotrophic lateral sclerosis, proteasome, ribosome, oxidative phosphorylation, pyrimidine metabolism.

Conclusions: Genes that failed to be up-regulated in the presence of the mutation belonged to the following pathways: focal adhesion, tight junction and calcium signalling pathway. Thus, the mutated tau protein is associated with a loss of wild type protein function in regulating expression of several genes essential for cell metabolism and transmission.

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Effects of Alzheimer's Amyloid-Beta and Tau Proteins on Mitochondrial Dysfucntion in the Brain of Transgenic Mice

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Alzheimer's disease (AD) is the most frequent form of dementia among the elderly and is characterized by neuropathological hallmarks of extracellular amyloid plaques and intracellular neurofibrillary tangles in the brain of AD patients. Amyloid plaques are composed of the amyloid-beta (Aβ) protein, derived from its precursor protein APP. Neurofibrillary lesions are formed from paired helical filaments composed of hyperphosphorylated tau protein, a microtubule-associated-protein. Importantly, current data indicate a complex relation between the amyloid pathology and pathology involving microtubule-associated protein tau during disease.

Based on our previous findings, we hypothesize a direct impact of abnormally phosphorylated tau and Aβ on proteins/enzymes involved in energy metabolism and mitochondrial respiratory chain. For this approach we are currently investigating the brains of double (APP (KM670/671NL) / PS2 (N141l)) and triple (APP (KM670/671NL) / PS2 (N141l) / Tau (P301L)) transgenic mice. Mitochondrial respiration is studied by the measurement of the oxygen consumption with substrates and inhibitors specific for the mitochondrial chain complexes I and IV. In addition, enzyme activities of these complexes will be determined.

Our preliminary findings indicate that at the level of mitochondria, the two defining neuropathological AD proteins, tau and Aβ, seem to act in a synergistic or additive way finally leading to/accelerating neurodegenerative mechanisms and cell death.

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Parkinsonism in Tau Transgenic Mice

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Frontotemporal dementia (FTD) is a neurodegenerative disease characterized by tau aggregation. It is often associated with Parkinsonism which generally precedes cognitive decline. FTD tau transgenic mouse models exhibit memory impairment before massive tau aggregation, suggesting that abnormal tau function rather than its aggregation underlies FTD. However these mouse models lack Parkinsonism. We report an early-onset phenotype in a novel mutant tau transgenic mouse model (Syd1) reminiscent of Parkinsonism in humans. The Syd1

mice present similar symptoms to FTD patients with Parkinsonism, including tremor, bradykinesia, muscle rigidity and postural instability. They also show poor motor-coordination that is partially reversed by L-Dopa treatment, and enhanced by low doses of the dopamine receptor antagonist haloperidol. Syd1 neurons showed impaired axonal transport of the dopamine synthesizing enzyme tyrosine hydroxylase (TH) in the nigro-striatal system due to increased tau levels. The results suggest that defective axonal transport of TH is an underlying pathomechanism for Parkinsonism in FTD.

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Insulin-Regulated Amino Peptidase (IRAP) in the Pathology of Alzheimer's Disease

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Background and aims: Angiotensin-converting enzyme (ACE) gene variation has been implicated as a risk factor for Alzheimer's disease (AD). ACE inhibitors and angiotensin II (AngII) (a product of ACE activity) receptor antagonists, have been reported to positively effect cognitive function. Animal studies show that angiotensin IV (AngIV), a metabolite of AngII, can also mediate improved cognitive performance when it binds to insulin-regulated membrane aminopeptidase (IRAP). A proposed mechanism for these cognitive effects is interaction with the release of acetylcholine, a greatly depleted neurotransmitter in AD. Our aim was to investigate the relationship between IRAP and AD-associated pathology.

Methods: We analysed IRAP distribution and levels immunohistochemically, in paraffin sections of temporal lobe from 15 AD and 15 control brains matched for gender, age, post-mortem delay and ACE genotype. In a subset of subjects, frontal and parietal lobe sections were also analysed.

Results: Neuronal staining was observed in both cases and controls but no significant quantitative differences in the levels of total IRAP staining were demonstrable on computer assisted image analysis. However, whereas in controls labelling for IRAP was almost exclusively neuronal, in AD cases there was also labelling of small non-neuronal cells of astrocytic or microglial appearance. There was also initial evidence of reduced IRAP levels in frontal lobe sections from AD cases compared with controls. Double immunofluorescent staining of IRAP and phospho-tau in 6 AD cases and 3 controls revealed reduced IRAP in neurofibrillary tangle-bearing neurons.

Conclusion: Reduced IRAP in tangle-bearing neurons may affect their responsiveness to Ang IV.

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Structural Study of the Mapt Locus and Genetic Association With Alzheimer's Disease in Taiwan

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Background: The microtubule associated protein, tau (MAPT) locus is unusual in Caucasians for having complete linkage disequilibrium (LD) over a region of ~1.6 million bases (Mb), forming two distinct haplotype clades, H1 and H2. In Caucasians, the H1 clade and variants of it have shown association with numerous neurodegenerative diseases. We are interested in the linkage disequilibrium pattern of this region in Asian population where H2 haplotype is absent. An association between an H1 haplotype variant (H1c) and Alzheimer disease (AD) in Caucasian populations has also been reported. Thus, we sought to investigate the haplotype structure of MAPT in an Asian population (Taiwanese) and test it for association with AD.

Methods: 110 probable AD Patients from the dementia outpatient clinic of Chang Gung Medical Center and 117 controls were recruited. We analyzed the LD architecture of MAPT in a Taiwanese population with 21 SNPs. We genotyped the MAPT htSNPs in our Taiwanese AD case-control cohorts with 10 tagged SNPs.

Results & Conclusions: We show here that the Taiwanese MAPT gene has four blocks of LD, instead of the single large block found in the Caucasians. We have confirmed an association between genetic variability at a SNP in intron 0 of MAPT and AD in the Taiwanese population. This suggests that genetic variability in the haplotype block centred on intron 0 of MAPT (p=0.015, OR =1.610, CI=1.098-2.361) is likely to contribute the risk of development of AD, and thus that genetic variability in tau expression contributes to the risk of AD.

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Phosphorylation Induced Structural Change of Presenilin 1 Cytoplasmic Loop Domain Influences Beta-Catenin Turnover and Signalling

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Background: Presenilin 1 (PS1) is an essential component of the gamma-secretase-complex involved in the intramembrane cleavage of type I membrane proteins. In particular, processing of C-terminal stubs of beta-amyloid precursor protein leads to the formation of amyloid beta-peptide. Within the cytoplasmic loop domain of PS1, serines 353 and 357 have been identified as phosphorylation sites for glycogen-synthase-kinase-3-beta (GSK3beta).

Results: We show that PS1 is phosphorylated at Ser353 and Ser357 in stably transfected Hek293 cells and by GSK3beta in vitro. We demonstrate that phosphorylation at these sites induces a structural change of the hydrophilic loop domain. This leads to a strong decrease in the binding to beta-

catenin. Cells stably transfected with a Ser353/357Asp mutant, which mimics phosphorylated PS1, show a marked increase in the cytoplasmic pool of beta-catenin as compared to cells expressing PS1wt. Cells transfected with PS1 Ser353/357Asp have a decreased turnover of beta-catenin. Furthermore, in reporter assays, the transcription of beta-catenin target genes was reduced after PS1-wt transfection. Notably, this reduction was suppressed in PS1-S353/357Asp mutant cells.

Conclusion: The interaction of beta-catenin and PS1 is regulated by GSK3beta-dependent phosphorylation of the large hydrophilic loop resulting in a structural change of this domain. The phosphorylation of both PS1 and beta-catenin by GSK3beta suggests a synergistic mechanism to regulate beta-catenin turnover and signalling.

Support Contributed By: The Deutsche Forschungsgemeinschaft

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Tau Expression in Yeast Reveals C-Terminal Phosphorylation, Oxidative Stress and Mitochondrial Dysfunction as Major Determinants for Tau Aggregation

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Unraveling the biochemical and genetic alterations that enhance aggregation of protein tau is crucial to understand the etiology of tau-related neurodegenerative disorders. We expressed and compared wild-type and six clinical FTDP-mutants of human protein tau in S. cerevisiae. All six mutants yielded more sarkosyl-insoluble tau (SinT) than wild-type tau-2N/4R in wild-type yeast strains. Deficiency of Pho85, the orthologue of cdk5, enhanced significantly the phosphorylation of C-terminal epitopes in tau, which coincided with marked increases in the amount of SinT. Exception were the FTDP-mutants tau-P301L and tau-R406W, which were less phosphorylated at S396, S404 and S409 and produced less of the hyper-phosphorylated isoform that appeared important for SinT formation. This finding was substantiated by the observation that the synthetic tau-S409A mutant failed to produce significant amounts of SinT, while its pseudo-phosphorylated counterpart tau-S409E yielded more or comparable levels of SinT as wild-type tau-2N/4R. The highest levels of SinT were formed in strains subjected to oxidative stress and strains with mitochondrial dysfunctions. Even when under these conditions the SinT levels were increased more than 100-fold, this did not result in growth-inhibition, indicating that aggregation of tau is not toxic per se. These results validate yeast as cell-biological model, allowing definition of genetic and biochemical factors important for the physiology of tau and its role in tauopathies.

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Expression of Full-Length Tau in An Inducible Cell Model of Tauopathy

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For the purpose of developing drugs that prevent tau protein aggregation and degeneration of neurons in tauopathies, we generated several cell models of tauopathy. They are based on the N2a neuroblastoma cell line and express different variants of the repeat domain of tau (tauRD) in an inducible fashion (Tet-on): the wild-type sequence, a pro-aggregation mutant (ΔK280), and an anti-aggregation mutant, containing additional proline residues. The cells expressing the K18ΔK280 mutant show robust aggregation of tau. The aggregates are toxic to cells and their removal is beneficial. We also found that fragmentation of tauRD is important for initiation of aggregation and that phosphorylation in the repeat domain cannot be considered as a precursor of aggregation. We have now obtained a significantly improved N2a cell model generating tau aggregates composed of full length tau molecules. In order to evaluate the influence of the phosphorylation at SP or TP motifs on aggregation (in the flanking region of the repeats, targets of proline-directed kinases), we generated a new cell model expressing the full-length isoform htau40 K280 and observed its aggregation in the N2a cell model. The aggregation was characterized by three methods: 1) On the biochemical level, the presence of aggregates was demonstrated by sarcosyl extraction of the cells and analysis of soluble and insoluble components. 2) Fluorescence microscopy using ThS fluorescence reports on the propensity of the protein to form beta-structure. 3) We visualized PHFs by electron microscopy after density gradient enrichment and gold labeling. - Supported by DFG.

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Axotrophin a Ring-Variant Domain Protein Acts as E3-Ubiquitin-Ligase and Ubiquitinates the Microtubule-Associated Protein Tau

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A number of neurodegenerative diseases among others Alzheimer´s disease (AD) are characterized by aggregation and accumulation of misfolded proteins. These protein aggregates are partly ubiquitinated. Tau protein, which aggregates during AD, is poly-ubiquitinated in AD brain to some extent.

In order to find modifiers of tau aggregation and posttranslational modification we screened for proteins, which interact with tau protein. In previous experiments we showed that the tau protein interacts with the protein axotrophin of unkown function.

Affinity-purified antibodies against axotrophin C-terminus labeled tau protein aggregates in AD brains.

Axotrophin harbours a C4HC3 zinc-finger-like motif in the C-terminus, which is referred to as Ring-variant domain and has been implicated in protein ubiquitination. Recombinant expression and refolding of the C-terminus of axotrophin allowed us to test the E3-ubiquitin-ligase activity. We found that axotrophin shows E3-ubiquitin-ligase activity in combination with several E2 enzymes and becomes autoubiquitinated. Ubiquitination of tau protein but not KLC1,

another axotrophin-interacting protein, was mediated by axotrophin.

Further investigation of ubiquitination effects on the protein tau will give more insights about the E3-ubiquitin-ligase axotrophin and especially its role in AD.

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A Platform for the Quantification of Alpha-Synuclein in Cerebrospinal Fluid:

Evidence for a Potential Biomarker for Synucleinopathies

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Background: Intracellular aggregation of alpha-synuclein (aS) represents a neuropathological hallmark of several neurodegenerative diseases. These 'synucleinopathy' disorders, which include Parkinson disease (PD) and dementia with Lewy bodies (DLB), are often difficult to distinguish from other conditions, such as Alzheimer disease (AD) and Creutzfeldt-Jakob disease (CJD).

Methods: After studying the constitutive release of aS by dopamine cells, the unexpected presence of aS in choroid plexus epithelium, which produces >75% of cerebrospinal fluid (CSF) and the ultimate identification of aS from cell-free CSF after purification by mass spectrometry, we developed a highly sensitive and aS-specific enzyme-linked immunoadsorbent assay (ELISA) for the direct quantification of lower picomolar amounts of aS using 96- and 384-well plates loading 200 or 50?l unconcentrated CSF.

Results: Based on this aS-specific ELISA, we carried out pilot studies quantifying CSF aS from patients with DLB, PD, AD, CJD and control subjects (n=100). We recorded lower mean aS levels in CSF from patients with PD and DLB (n=46) when compared with Alzheimer's and control subjects (n=26) ($p<0.0042$). In contrast, CJD patients (n=8) showed elevation in CSF aS, most likely because of widespread neurodegeneration.

Conclusions: We conclude that CSF S can be directly quantified using 96- and 384-well plate formats; and that CSF aS levels are significantly reduced in patients with synucleinopathies and markedly increased in subjects with CJD.

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Regulation of Tau Phosphorylation and Toxicity: Insights From a Drosophila Model

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Neurofibrillary tangles (NFT) containing tau are a hallmark of neurodegenerative diseases including Alzheimer's disease (AD). NFT burden correlates with cognitive decline and neurodegeneration in AD. However, little is known about mechanisms that protect against tau-induced neurodegeneration. Although a number of kinases have been implicated in tau phosphorylation, including GSK-3beta, CDK5, and MARK, the relationship between tau phosphorylation and toxicity is not fully understood. We have used a Drosophila model expressing the longest isoform of human tau to explore the relationship between tau phosphorylation and toxicity. Coexpression of human GSK-3beta or its fly homolog, Shaggy, increases tau phosphorylation and toxicity. Mutation of eleven important proline-directed phosphorylation sites abolishes the Shaggy-induced increase in tau phosphorylation; however, this S11A form of tau retains toxicity. The fly homolog of MARK is PAR-1, and phosphorylation of tau by MARK is thought to promote subsequent phosphorylation by proline-directed kinases. We find that mutation of sites phosphorylated by MARK abolishes PAR-1-induced phosphorylation and diminishes tau toxicity, but does not impair the ability of tau to be phosphorylated by GSK-3beta/Shaggy. Our work suggests that although phosphorylation of tau is an important determinant of its neurotoxicity, other factors such as the activity of puromycin-sensitive aminopeptidase are likely to play additional roles in the development of tauopathy.

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Increases in Filopodia and Spine Degeneration Precede Cell Death in a Mutant Tauopathy Model

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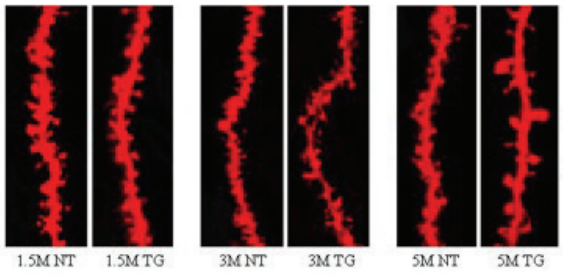
The cellular basis of early memory loss in neurodegenerative tauopathies is not well understood. The rTg4510 conditional mouse model of P301L tauopathy provides a valuable system to study early events underlying cognitive impairment. We utilized DiOlistics (ballistic delivery of lipophilic dye) to label the entire contents of neuronal cells including the dendritic spines in rTg4510 mice (TG) and control mice (NT). The density of neurons stained was controlled so that individual neurons could be imaged using confocal microscopy, thus allowing us to measure differences in spine density between the rTg4510 mice and controls. Ages examined spanned previously determined

(Santa Cruz et al 2005) pathological time points with regard to cell death: pre-pathologic (1.5 months (M)), early-stage (3M) and late-stage (5M). We found a decrease in dendritic spine density that occurred before the major cell loss events in this model. Interestingly we also observed an increase in precursor spine filopodia within the early-stage transgenic mice (3M) that did not persist in the late-stage mice suggesting that affected neurons attempt to reestablish synaptic connectivity, but ultimately fail. Similar dendritic changes are associated with learning and memory deficits in a number of human conditions including mental retardation and fragile X and are a likely cause of early memory loss in rTg4510 mice. Our findings have important implications for the genesis of early memory loss and potential treatment in human tauopathies.



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MEM 1003 is An L-Type Ca2+ Channel Modulator Targeted Towards Alzheimer's Disease Therapy

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Ca2+ serves a critical role in coordinating signaling events within neurons. Age or disease may impact the ability of cells to appropriately regulate intracellular Ca2+ levels and cause inappropriate Ca2+ signals to be generated. Abnormal Ca2+ signaling has been implicated in a number of neurological and psychiatric illnesses, including bipolar disorder, schizophrenia, and Alzheimer's disease. MEM1003, a novel dihydropyridine L-type voltage gated Ca2+ channel modulator, is being developed and is currently in clinical trials for Alzheimer's disease and Bipolar disorder. MEM 1003 is novel because of its preferential CNS activity versus peripheral effects on cardiovascular functions. MEM1003 is equipotent in blocking Ca2+ currents in CA1 hippocampal neurons, but 4 to 15-fold less potent in relaxing human (coronary artery) smooth muscle, suggesting a superior cardiovascular safety profile. MEM 1003 reduces the size of the slow afterhyperpolarization (sAHP) in hippocampal neurons and is also protective in cell culture models of Abeta toxicity. This profile of MEM 1003 suggests it would be of great therapeutic value in treating Alzheimer's disease. MEM1003 was effective in improving cognitive performance in multiple preclinical behavior models including attention, spatial memory and executive function. The pharmacokinetic and safety profiles of MEM1003 were evaluated in double-blind, randomized, placebo-controlled Phase1A and Phase1B clinical trials. MEM1003 was well tolerated up to the highest dose tested of 180mg twice daily. The safety data and pharmacokinetics of MEM1003 in these studies will be presented and the relevance of the exposure levels to preclinical animal efficacy models and toxicology studies will be discussed.

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Development of Novel, ADDL-Targeting, Disease Modifying Therapeutics for the Treatment of Alzheimer's Disease

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The discovery by Acumen's founders of Amyloid beta-derived diffusible ligands (ADDLs) and their potent ability to block memory related long term potentiation (LTP) led to a new hypothesis for Alzheimer's disease. In this new paradigm, ADDLs disrupt synaptic function and initiate aberrant signaling leading to failure of LTP and loss of memory function. The central role of ADDLs in the etiology of Alzheimer's disease is now widely accepted. Acumen has exploited the discovery of ADDLs for the development of a screening cascade to identify small molecule modulators of ADDL-mediated neurotoxicity. We will describe preclinical development of first-in-class small molecule therapeutics for Alzheimer's disease based on inhibiting ADDL formation and binding to mature neuronal cells. Screening a selected, diverse library of over 100,000 lead-like compounds resulted in the discovery of two proprietary chemical series that display low to mid nM potency in blocking the formation and toxicity of ADDLs. Both chemical series are non-aggregating, rule-of-five compliant, chemically tractable, and display good solubility and stability profiles. ADME studies show both series are suitable for advanced preclinical development. Biochemical and functional efficacy studies have been used to assess the mode of action and establish structure-activity relationships for each series.

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Role of Transthyretin in the Prevention of Alzheimer Phenotype in TgCRND8 Mouse Model

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Accumulation of amyloid β (Aβ) peptide has been suggested to be central in the pathogenesis of Alzheimer's disease (AD). Therefore, increasing the sequestration and clearance of Aβ may be effective in blocking this pathogenic cascade. Transthyretin (TTR) was shown to be the major Aβ sequestering protein in human CSF. Binding of TTR to Aβ prevents amyloid fibril formation and is suggested to be involved in the transport and clearance of Aβ. We have previously reported that age-related memory deficits in 24-months old rats were associated with decreased TTR gene expression and that TTR knock-out (TTR-/-) mice show memory impairments during aging. Here, treatment with TTR

and genetic deletion of TTR gene were done in TgCRND8 mouse model of AD to investigate the impact of TTR expression on Aβ deposition, amyloid plaque formation, neurofibrillary tangles, neuronal loss, and memory capacities in this mouse model at the onset (3-months old) of Alzheimer disease-like phenotype and when the phenotype is already well established (6-months old) in these mice. Since low endogenous expression level of TTR may be sufficient to prevent the development of neuropathological hallmarks of AD in aged rats, we determined if complete deletion of TTR could reveal AD hallmarks in 24-months old TTR-/- mice and if treatment with TTR could abolish Alzheimer phenotype. We expect that deletion of TTR in TgCRND8 mice will exacerbate and accelerate the onset of AD-like phenotype and that treatment with TTR will counteract the pathogenesis of AD. This work is supported by the CIHR.

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Regulation of APP Maturation: Key Mechanism by Which Cerebrolysin Decreases Amyloid Beta Production in a Transgenic Model of AD

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Cerebrolysin is a neuroprotective peptide mixture which might reduce the neurodegenerative pathology in Alzheimer's disease (AD). In a transgenic AD mouse model (mThy1-hAPP751) Cerebrolysin promotes synaptic repair, decreases amyloid burden and ameliorates behavioral deficits. However, the mechanisms involved are not completely clear. These studies aimed to investigate whether the reduction of amyloid deposition occurs via regulating Ab degradation or via modulating APP expression, maturation or processing. Mutant mice were treated for six months with Cerebrolysin and analyzed in the water maze, followed by RNA, immunoblot and confocal microscopy analysis of full-length (FL) APP and its fragments, b-secretase (BACE1) and Ab-degrading enzymes (Nep, IDE). Consistent with previous studies, Cerebrolysin ameliorated the performance deficits in the water maze and reduced the synaptic pathology and amyloid burden in the brains of transgenic animals. These effects were associated with reduced levels of FL APP and its fragments, however levels of BACE1, Notch1 (substrate for presenilin), Nep and IDE were unchanged. In contrast, levels of active CDK5 and GSK3b, kinases that phosphorylate APP, were reduced. Furthermore, Cerebrolysin reduced the levels of phosphorylated APP and the accumulation of APP in neuritic processes. Taken together, these results suggest that Cerebrolysin might reduce AD-like pathology by regulating APP maturation and transport to sites where Ab protein is generated. This study clarifies the mechanisms through which Cerebrolysin might reduce Ab production and deposition in AD and further supports the importance of this compound in the potential treatment of early AD.

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EHT 0202, a Neuroprotective Drug Active Against Amyloid-Beta Accumulation and Neurotoxicity That Improves Attention and Cognition of Old Rats

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We have identified a repertoire of genes abnormally spliced in brain samples from patients suffering from Alzheimer disease (AD). Among a library of such alteration is the epsilon subunit of the GABA(A) receptor. EHT 0202 was selected as a potential drug in the treatment of AD, because it exerts a specific regulation on epsilon subunit-containing GABA(A) receptors. EHT 0202 has shown in in vitro and in vivo (oral administration), neuroprotective properties against excitotoxicity and oxidative stress. Here we show that EHT 0202 reduces Aβ toxicity in cellular AD models by a mechanism involving the GABA(A) receptor. EHT 0202 redirects APP processing towards the α-secretase pathway, increasing the production of neurotrophic sAPPα. sAPPα induction is associated with the neuroprotection afforded by EHT 0202 since its blockade prevents EHT 0202 effect. sAPPα stimulation may also reduce Aβ production in vivo, potentially reducing amyloid plaque formation, since EHT 0202 lowers Aβ levels in vitro. In cognition models using old rats (Morris water-maze and Barnes tests), EHT 0202 improves attention, learning capabilities and cognitive behaviour. Phase I clinical trials indicate that EHT 0202 is a well tolerated drug in human with no sedative, emetic or adverse effects observed. Furthermore, blood exposure in human is fully compatible with the observed cognitive enhancement in rats. Therefore, EHT 0202 appears to be a promising drug for Aβ-related neurodegenerative disorders with cognitive impairment. In addition, these data confirm the power of ExonHit Therapeutics' DATAS technology to rapidly identify pharmacologically relevant targets in complex diseases such as AD.

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Vitamin E Facilitates Memory Retention in Rats: Possible Interaction With Alpha-Adrenergic System

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Vitamin E is an essential nutrient in humans. Vitamin E may be an important factor in maintaining neuronal integrity and preventing cell loss. Vitamin E and other antioxidants can prevent free radical-mediated cell death and diminish cognitive deterioration. Furthermore, respectable number of studies has indicated that alpha-adrenergic system has been associated with learning and memory. Norepinephrine (NE)-containing neurons of the brain are target-related changes. Regional changes in NE content, turnover and interaction with receptors have been reported for aging rodents, monkeys, and

humans, but the distribution and magnitude of the changes reported is variable. In the present study, the effects of vitamin E and alpha-adrenergic system on memory retention of passive avoidance learning in adult male rats were investigated. Vitamin E (50 microgram/rat) or phenylephrine (0.5, 1 microgram/rat), the alpha1-adrenoceptor agonist, increased, while prazosin (0.5,1 microgram/rat), the alpha1-adrenoceptor antagonist, decreased memory retention. The combination of different doses of vitamin E with a low dose of phenylephrine (0.1 microgram/rat) potentiated their effects on memory retention. Effect of prazosin was attenuated by vitamin E. Administration of clonidine (0.5, 1 microgram/rat), the alpha2-adrenoceptor agonist, decreased while yohimbine (0.5, 0.75 microgram/rat), the alpha2-adrenoceptor antagonist, increased memory retention. The combination of different doses of yohimbine with a low dose of vitamin E (10 microgram/rat) potentiated their effects on memory retention. Also, effect of vitamin E was attenuated by clonidine. It is concluded that vitamin E has a close interaction with the adrenergic system that is involved on memory retention process.

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Effects of TRH and Opioid Systems on Memory Retention of Passive Avoidance Learninig in Rats

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Thyrotropin-releasing hormone (TRH) as a neurotransmitter and neuromodulator is distributed widely in areas other than the hypothalamus. There exist a number of studies that report on the ability of TRH and its analogs to enhance learning and memory in a variety of drug and lesion paradigms. On the other hand, there is considerable evidence that the endogenous opioids are involved in memory storage, since opioid receptor antagonists given after training, enhance memory retention, whereas opioid receptor agonists impair it. The possible interaction between TRH and opioidergic system on memory retention was examined in passive avoidance learning. Bilateral microinjections into the dorsal hippocampus were carried out in all the experiments. Post training injections of TRH (2.5, 5 microgram/microliter), naloxone (10 microgram/microliter) and pentazocine (10 microgram/microliter) potentiated, while morphine (10 microgram/microliter) attenuated memory retention in the dose dependent manner. On the other hand, morphine decreased TRH-induced effects, while naloxone and pentazocine increased the TRH-induced response. It is suggested that an interaction between TRH and opioidergic system might exist on memory retention.

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Mechanisms of Dietary Docosahexaenoic Acid-Induced Protection and Amelioration on Impairment of Memory Learning in Alzheimer's Disease Model Rats

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We reported that dietary docosahexaenoic acid (DHA) protects against and ameliorates the impairment of memory learning induced by the infusion of amyloid β (Aβ) into the rat cerebral ventricle. However, these mechanisms have remained largely unknown. We, thus, investigated to clarify the mechanisms of dietary DHA-induced these effects in vivo and in vitro study.

Methods: In vivo: After administration of DHA for 12 weeks to an animal model of Alzheimer's disease (AD) rats infused Aβ into the cerebral ventricle, the cognition learning ability of AD rats was estimated and the detergent Triton X-100-insoluble membrane fractions (DIFs) of the neural plasma membranes were isolated from the cerebral cortex of the rats. In vivo: Effect of DHA on aggregation of Aβ protein was investigated by an aggregation-formation assay using thioflavin T.

Results: In vivo: Aβ infusion increased the levels of Aβ and cholesterol in the DIFs compared with those of vehicle rats. Conversely, the dietary DHA decreased the levels of Aβ and cholesterol in the DIFs, with the decrease being more prominent in the DHA-administered rats. Regression analysis revealed a positive correlation between Aβ and cholesterol. In vitro: The pre-treatment of DHA decreased fluorescence derived from thioflavin T in Aβ peptide, and the formed Aβ fibrils were attenuated by co-incubation with DHA, indicating an antiamyloidgenic and fibril-destabilizing effects of DHA.

Conclusion: DHA not only inhibits the deposition of Aβ in vivo but also destabilizes the preformed Aβ fibrils in vitro. DHA is effective in prevention and radical cure of AD.

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Abeta Upregulates BDNF Production From Astrocytes: Rescue From Abeta-Related Neuritic Degeneration

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Astrocytes, the most abundant type of glia in the brain, are considered to play a key role in Alzheimer's disease (AD) pathologies. In a cell culture study, we have previously shown that astroglial responses against Abeta occur before obvious neuronal damage could be detected, suggesting the possibility that astrocytes could be an attractive therapeutic target for treating AD. In the present study, we investigated astroglial gene expression changes in response to Abeta to further elucidate the role of astrocytes in Abeta toxicity. Using real-time PCR and ELISA analyses, we found that Abeta rapidly induced astrocytes to produce brain-derived neurotrophic factor (BDNF). Abeta42 was more effective than Abeta40 in increasing astroglial BDNF production. Moreover, BDNF treatment rescued the neuronally differentiated human

neuroblastoma cells from neuritic degeneration caused by Abeta toxicity. This is the first study to demonstrate that astrocytes are capable of increasing the production of a particular neurotrophic factor in response to Abeta. Our findings also identify BDNF as a potential therapeutic agent for preventing Abeta-related neuritic degeneration.

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Cognition Enhancing Properties of a Novel Ncam Derived Peptide, Vebv

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NCAM is abundantly expressed on the surface of neurons. It plays an important role in brain development, synaptic plasticity, and neuroregeneration. NCAM homophilic binding results in interaction of the fibronectin type III (F3,I and F3,II) modules of NCAM with the FGF receptor (FGFR). This interaction leads to activation of intracellular signaling cascades mediating cell differentiation and survival. We have already shown that a synthetic 15 amino acid peptide motif located in F3,II, termed FGL, is an FGFR agonist able to significantly enhance cognitive functions both in intact and impaired animals. FGL had positive effects in a number of in-vivo models of neurodegeneration, such as beta-amyloid induced toxicity and global ischemia. We have now developed a novel 11 aminoacid peptide, VEBv, derived from the same motif on the F3, II module as FGL. We have studied the cognition enhancing properties of VEBv using the Social Recognition Test (SRT) in intact adult rats. VEBv, administered subcutaneously, lead to a significant enhancement of social memory as shown in the SRT. Further we also tested the effects of VEBv against scopolamine induced amnesia. VEBv administration significantly counteracted scopolamine induced loss of social memory. We are currently testing the effects of VEBv against impaired cognition and neurodegeneration induced by beta-amyloid neurotoxicity. Our results show that VEBv is an NCAM derived peptide with significant activity on cognition in both intact and impaired animals. Its physico-chemical characteristics allow for flexible administration making it a potential drug candidate for the treatment of neurodegenerative disorders leading to cognitive impairment.

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Ribozyme Against Amyloid Precursor Protein Mrna

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Alzheimer's disease (AD) is a degenerative disorder of the human central nervous system (CNS) that destroys the brain and leaves its victims unable to function on their own. Etiological studies suggest that an elevation in amyloid-β peptides (Aβ) level contributes to its aggregation and leads to the development of the disease. The major constituent of these amyloid peptides, which occurs largely in the brain areas important for memory and cognition, is the 1 to 40-42 residues peptide (Aβ40-42) derived from the amyloid protein precursor (APP). Consequently, Aβ is a highly worthy therapeutic target, and reducing its level in the brain may block both aggregation and neurotoxicity. Most likely an approach that reduces either the concentration of Aβ, or the rate of amyloid aggregation and deposition in the brain would be beneficial for patients with ad. Among the several possible ways to lower the Aβ accumulation in the cells, we have selectively chosen to target the primary step in the Aβ cascade, in other words, the reduction of app gene expression. To this end, we engineered specific sofa hdv ribozymes, a new generation of catalytic rna tools, to decrease app expression. We demonstrated that APP-ribozymes are effective at decreasing app mrna as well as protein levels in neuronal cells. Our results have the potential of setting the ground work for a new therapeutic approach as a protective treatment for AD.

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Neuroprotective Effect of Pramipexole Against Beta Amyloid Toxicity Depends by Peptide Aggregated States

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Here we demonstrate that pramipexole, a dopaminergic agonist drug currently used for the treatment of Parkinsons's disease, exerts neuroprotective effects against beta amyloid neurotoxicity. It is well established that beta-amyloid may exert different effects depending by its state of aggregation. Using a peculiar protocol to test individually oligomers, fibrils, or unaggregated beta-amyloid 1-42, we demonstrated that pramipexole was able to protect cells against oligomers and fibrils, but not against unaggreagted state of beta-amyloid 1-42. The mechanism by which pramipexole protects against beta-amyloid neurotoxicity involved its antioxidant properties. Fibrils and oligomers were found to produce elevated amount of free radicals, while unaggregated state did not. Furthermore pramipexole prevented free radicals generation induced by oligomers and fibrils.

Thus, we propose pramipexole may become in the future a coadiuvant in the treatment of neuropathologies, beside

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Promotion of Neurogenesis by Docosahexaenoic Acid in Vivo and in Vitro

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The level of docosahexaenoic acid (DHA), one of the main structural lipids in mammalian brain, is low in the hippocampus of Alzheimer's disease (AD) patients. We reported that dietary administration of DHA protects against (2002) and ameliorates (2005) the impairment of learning ability in AD model rats produced by the infusion of amyloid β protein into the cerebral ventricle. In this study, we

examined the effect of DHA on neuronal differentiation of neural stem cells (NSCs) in vitro and in vivo.

[Design] In vitro: NSCs obtained from 15.5-day-old rat embryos were propagated as neurospheres and cultured under differential conditions with or without DHA. In vivo: adult Wistar rats were perorally administered DHA at 300 mg/kg/day for 7 weeks. [Results] DHA significantly increased the number of Tuj1-positive neurons on culture days, and the newborn neurons in the DHA group were morphologically more mature. DHA significantly decreased the incorporation ratio of BrdU, the mitotic division marker, during the first 24 hr period. DHA promotes the differentiation of NSCs into neurons by promoting cell cycle exit and suppressing cell death. DHA promoted the protein expression in neurogenesis and suppressed the protein expression in proliferation. Furthermore, dietary administration of DHA significantly increased the number of BrdU(+)/NeuN(+) newborn neurons in the granule cell layer of the dentate gyrus in adult rats.

[Conclusion] DHA effectively promotes neurogenesis both in vitro and in vivo, suggesting the new property of DHA modulating hippocampal function regulated by neurogenesis.

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Baseline Demographics of Dementia Patients Seen at Australian Clinics: Preliminary Data From Prime

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Aims: To analyse the epidemiology and treatment outcomes of dementia under conditions of routine clinical practice in Australian patients. Methods: Patients with a diagnosis of “early” to “mild” dementia who are living in the community are eligible for this ongoing observational cohort study. Each subject will be followed for a period of 36 months, with visits after 3, 6, 12, 24 and then 36 months as part of routine clinical practice. Results: Baseline data are available for 123 patients recruited from 4 clinics around Australia. The mean age at enrolment was 76.7±7.54(SD) years and mean age at diagnosis was 74.5±8.02(SD) years. The most common diagnoses were dementia of the Alzheimer’s type with late onset (42.3%), vascular dementia (13.0%), mixed Alzheimer’s and vascular dementia (13.0%) and dementia of the Alzheimer’s type with early onset (12.2%). The MMSE scores for these patients indicated they had mild dementia (mean score 22.6±5.16(SD)). The most commonly used medications were cholinesterase inhibitors (32.5% of patients were treated with donepezil, 30.9% with galantamine, 2.4% with rivastigmine), folic acid (13.0% of patients) or tocepherol (8.1% of patients). The large majority of patients were living at home without additional outside care (94.3%) and were cared for by a spouse (71.5%). The mean NPI Caregiver Distress scores (6.9±8.4(SD)) and mean Zarit Burden Interview scores (22.5±16.1(SD)) indicated a low level of caregiver burden at baseline. Conclusions: This rapidly growing disease cohort will provide valuable information on treatment patterns, outcomes and resource use in Australian patients with dementia.

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Effects of 6-Months Treatment With Donepezil and Rivastigmine on Results of Neuropsychological Tests in Patients With Alzheimer's Disease

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Alzheimer’s disease is the most common degenerative diseases of brain. Nowadays, the most common treatment used to slow down disease progression, includes Donepezil, Rivastigmine and Galantamine, as inhibitors of acetyl cholinesterase. General purpose of our study was to show effects of treatment with Donepezil and Rivastigmine, on results of four neuropsychological tests including MMSE (mini mental state examination), NPI (neuro psychiatry inventory), Clock and Bender; and to compare these effects between two drugs. We started a before&after type interventional study. Samples were selected from patients who had Alzheimer’s disease with DSM IV criteria and were candidate of receiving Donepezil or Rivastigmin, as treatment of AD, for the first time. We used these four tests to assess patients’ cognitive and behavioral changes during treatment with two drugs. Patients were divided to two groups (each group=35 cases); one taking Donepezil and the other taking Rivastigmin. The four tests were performed before starting treatment and 1, 3 and 6 months after treatment with Donepezil and Rivastigmine. Conclusion: MMSE, improved significantly 6 months after treatment both with Donepezil($p=0.04$) and Rivastigmin($p=0.007$). About Clock test, there was a significant improvement after 6 months of treatment with Rivastigmin; while this significant improvement was not seen in patients receiving Donepezil. In two other tests, no significant difference was seen before and after treatment. Also, No significant difference was detected between two groups and so no different effects on these tests between Donepezil and Rivastigmin

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A New Screening for Conformational Diseases With a Novel Mechano-Chemical Sensor

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Several neurodegenerative diseases are associated with the misfolding, aggregation and accumulation of proteins. We describe a novel screening method by detection of the intra- and inter- molecular forces that arise from the conformational changes of amyloid b peptide and a-synuclein films, using a new mechano-chemical (MC) sensor. Very thin (ca 1 mm in thickness) and porous films were made by an electrospray deposition technique. Some metal ions and low pH, which induce aggregation physiologically, increased the tensile force and stiffness of the films, and several metal chelators and high pH reversed them to a basal level. Accompanied with the

increase of the tensile force and stiffness of amyloid b films induced by zinc, the films became rich in b-sheet structure bound to 1-fluoro-2,5-bis(3-carboxy-4-hydroxystyryl)benzene (FSB). TEM images showed that the amyloid b film predominantly consisted of protofibrils including spherical amyloid and oligomers. Taken together, the conformational change of the protofibril, one of central players in neurodegeneration, to the b-sheet structure could be detected by the mechano-chemical sensor. BSB, a styrylbenzene derivative, inhibited the increase of tensile force of amyloid b and a-synuclein films by zinc, showing that the compound reduces b-sheet structure of amyloid intermediates to inhibit the fibril formation. We thus establish a new method to find drug candidates for conformational diseases with MC method to detecet protein conformational change to b-sheet structure with short time, non-label, and small amount of protein.

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Caprospinol Improves Brain Histopathology and Recovers Memory Function in a Rat Model of Alzheimer's Disease

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Backgound: Alzheimer's disease is a progressive neurodegenerative disease that associates brain tissue histological modifications like amyloid plaques deposition, neurofibrillary tangle formation, inflammatory processes and neuronal death to cognitive impairment. The disease, socially debilitating, ultimately leads to the death of the patient. Unfortunately, currently available treatments are not able to significantly slow down the progression of the disease or to stop it. We previously reported that the naturally occurring spirostenol (22R,25R)-20α-spirost-5-en-3β-yl hexanoate (caprospinol) protected in vitro neuronal cells against Abeta1-42 neurotoxicity by binding to the peptide, and inhibiting the formation of the neurotoxic oligomeric amyloid species ADDLs. We further showed that caprospinol protected neuronal cell mitochondrial respiratory chain function against Abeta1-42-induced impairment.

Aim: In vivo assessment of caprospinol "anti-Alzheimer" properties in a Alzheimer's disease rat model.

Methods: Infusion of Fe2+, Abeta1-42, and buthionine-sulfoximine (FAB), using an osmotic pump, into the left cerebral ventricle of Long-Evans rats for eight weeks induced memory impairment. The rats were treated with caprospinol (daily i.p., 10 mg/kg) for the four last weeks of the FAB solution infusion.

Results: Brains from FAB-infused animals displayed thioflavin-S-positive amyloid deposits, hyperphosphorylated Tau protein, neuronal loss, and gliosis. We report herein that caprospinol treatment of the FAB rats resulted in the reduction of both the amount of amyloid plaques and the number of degenerating neurons in the brains of the treated animals. These histopathological changes were associated with a dramatic recovery of memory.

Conclusion: Taken together, these data suggest that caprospinol is a promising drug candidate for Alzheimer's disease treatment.

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Omental Transposition to the Brain for Treating Alzheimer's Disease: A Preliminary Study

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Background and aims: The purpose of this study was to learn the effect of omemtum transposition to the brain of patients who exhibited serious long-standing Alzheimer's disease. Methods: Ten consecutive patients, aged 58-81, male 5, female 5, underwent graft of their elongated pedicled omentum onto their left frontal-temperal-parietal cerebral cortex. Those patients, who had more than five years of dementia with extremely low MMSE scores of 2-15, were diagnosed by a neurologist. All subjects underwent SPECT pre- and post-operatively. SPECT results were analyzed semi-quantitatively by calculation of the left/right radioactivity counts symmetry index (Si). The patients were followed up to one year. The outcome was evaluated by the neurologist with a modified scale of activities of daily living (mADL) as well as the MMSE. The commentary of patient's caretaker was also adopted. Results: Three months following the surgery, the Si of SPECT increased from 98.7±1.9% to 103.9±2.3% (P=0.0307). The neurological and neuropsychological testing scores augmented gradually during the follow-up period. By the one year, the MMSE and mADL score rose by 29.6±19.3% and 35.6±17.4%, respectively. According to the caretaker's assessment, some subjective and objective improvements, especially in terms of their functional status, were demonstrated in 5 patients. Conclusions: We believe that omental transposition to the brain increases cerebral blood flow and might be helpful to decelerate the processing of Alzheimer's disease

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Cerebrolysin Promotes Neurogenesis in An APP Transgenic Mouse Model of Alzheimer's Disease

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The neurotrophic effect of Cerebrolysin might reduce the neurodegenerative alterations in Alzheimer's disease. It has previously been shown that Cerebrolysin improves synaptic plasticity and behavioural performance of a APP tg mouse model of Alzheimer's disease. The underlying mechanism might be the ability of Cerebrolysin to induce neurogenesis in the hippocampal dentate gyrus. To further investigate this mechanism, tg mice expressing mutant APP under the Thy-1 promotor were loaded with BrdU and treated with Cerebrolysin for 1 and 3 months. Markers for newly generated neurons (BrdU), proliferation (PCNA), migrating neuroblasts (doublecortin), and apoptosis (TUNEL) were analysed in the subgranular zone of the hippocampus. The vehicle-treated APP tg mice showed decreased numbers of BrdU+ and

doublecortin+ neural stem cells in the dentate gyrus compared to non tg controls. Treating APP tg mice with Cerebolyisn resulted in an significant increase of BrdU+ cells, doublecourting+ neuroblasts and a decrease in TUNEL+ neural stem cells compared to vehicle treated APP tg mice. Cerebrolysin did not change the number of PCNA+ proliferating neural stem cells and the ratio of BrdU+ cells converting to neurons and astroglia in the dentate gyrus cells in the APP tg mice. These findings support that Cerebrolysin rescues the defects in neurogenesis in APP tg mice by enhancing the survival and reducing the rate of apoptosis of the neural stem cells in the hippocampus. The effect of Cerebrolysin on neurogenesis might contribute to alleviating the synaptic and cognitive deficits in patiens with Alzheimer's disease.

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Cerebrolysin Protects Granule Neurons Against Serum Deprivation But Not Against Apoptosis Induced by Staurosporine

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Background and aims: Alzheimer's disease (AD) is characterized by a complex pathogenic scenario where decreased levels of neurotrophic factors constitute a significant trigger of neuronal degeneration. Cerebrolysin (CL) is a peptide mixture with neurotrophic effects which is neuroprotective in various experimental paradigms, including AD models. It has been shown that CL decreases beta-amyloid production by regulating maturation of amyloid protein precursor and counteracts inhibition of neurogenesis induced by elevated fibroblast growth factor levels. However, the precise cellular mechanisms responsible for the neurotrophic and neuroprotective effects of CL are not well described yet. In order to explore these mechanisms, we induced apoptosis in rat cerebellar granule cells by serum deprivation and staurosporine, which activate different cell death pathways.

Methods: We estimated cell loss by cell density in culture and apoptosis by chromatin staining with propidium iodide and measurement of cell body volume.

Results: CL significantly improved cell density, increased cell volume and reduced the percentage of nuclei with apoptotic morphology in granule cells deprived of serum for 48 hours. In contrast, CL did not change significantly the cell volume or the percentage of apoptotic nuclei in granule cell cultures exposed to staurosporine for 6 or 24 hours. However, CL did significantly increased cell density in cells exposed to staurosporine.

Conclusions: Based on these findings we conclude that CL counteracts activation of upstream pathways of apoptosis and we currently investigate the effects of this drug at the level of Trk receptors, PI3 kinase, Akt and MAP-kinase.

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Beta-Amyloids Interact With the Nogo66 Receptor

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b-amyloid peptides have been demonstrated to interact with multiple protein targets such as the a7 nicotinic acetylcholine receptors, p75, receptor for glycation end products, apolipoproteins and other serum proteins, and are believed to be central to the pathogenesis of Alzheimer's disease. The neuronal Nogo66 receptor that binds multiple myelin proteins and mediates the inhibition of axon/neurite growth in the CNS has been shown to interact with the amyloid precursor protein, primarily through the b-amyloid sequences. Administration of a recombinant form of the Nogo66 receptor to the APPswe-PS1 transgenic AD mice reduces amyloid plaques and improves memory function as measured by the water maze test. Here we characterize the biochemical interactions between b-amyloids and the Nogo66 receptor. We demonstrate that the b-amyloids specifically bind in vitro to immobilized recombinant Nogo66 receptor protein and to cells expressing the Nogo66 receptor. The binding can be inhibited by b-amyloid peptide fragments or other forms of the recombinant Nogo66 receptor. Our data suggests the potential application of recombinant Nogo66 receptor protein for treating Alzheimer's disease by modulating the b-amyloid pathway.

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Treatment of Cognitive Impairments in Cerebrovascular Diseases

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Background: Vascular diseases is actual reason of dementia.

Objectives: To study the character of cognitive impairments in cerebrovascular diseases and estimation of treatment efficiency.

Methods: Forty patients with cognitive impairments were examined with clinical and neuropsyphological methods.

Results: Moderate cognitive impairments were revealed in 24 patients, dementia – in 16. Treatment with Memantine demonstrated positive clinical and neuropsyphological dynamics.

Conclusion: Cognitive impairments in vascular patients are connected with lesions of both frontal and mediobasal structures. In cases of minimal focal symptoms degree of dementia doesn't depend on neurological deficit. Memantine can be used in both moderate and severe cognitive impairments.

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Beta-Amyloid Induced Neuronal Cell Death and Protection by Metallothionein

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Alzheimer disease (AD) is characterized by senile plaques, neurofibrillary tangles and neuronal loss. Deposits of beta-amyloid (Ab) are responsible for neurodegenerative changes and eventually neuronal death and dementia. Metallothionein (MT) research in the brain has shown that this family of proteins plays a major role in brain physiology. There is compelling evidence that MT-I&II are involved in the response of the brain to damage, and indeed there is a significant upregulation of these proteins in AD. To our knowledge there are no studies that might provide insight into the putative role of MT in the development of AD and we investigated the effect of MT.

We studied toxic effect of Ab on primarily cultured neurons or mouse neuroblastoma cell line. By treating oligomeric Ab, cellular viability of cells was dramatically decreased in a time and dose dependent manner. Protection by MT was evaluated by adding recombinant MT-I&II into culture media. Cellular injury was attenuated significantly by MT. Oxidative stress to Ab was measured by detecting production of reactive nitrogen and oxygen species and other products of oxidation. Ab induced production of oxidative stress products. When MT was treated with Ab, production of oxidative stress products was significantly reduced.

In this study, we suggest that Ab toxicity can be protected by MT and MT can be a potential therapeutic target of Alzheimer's disease.

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Selective Estrogen Receptor Modulators and Inflammatory Response of N9 Microglia

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Increased levels of cytokines IL-6, IL-1beta and TNF-alpha can contribute to the progression of neuronal damage in Alzheimer's disease (AD). Some studies have indicated that the use of as anti-inflammatory considered estrogen after menopause may reduce the risk of developing AD. We analysed the influence of estrogen and selective estrogen receptor modulators (SERMs) Ospemifene, Tamoxifen, and Raloxifene, on the expression of pro-inflammatory cytokines in mouse N9 microglial cell line. The cells were pretreated 48 h with estrogen or SERMs. Media were replaced to fresh compounds containing, and 20 ug/ml LPS was added for 24 h. The effects were examined as Northern blots or ELISAs. RNA level of IL-6 and TNF-alpha reduced 20%, whereas IL-1beta was unaffected after 10 nM estrogen treatment, compared to the untreated control. Treatment with 1 uM Ospemifene decreased RNA of TNF-alpha 25% and IL-1beta slightly. Tamoxifen and Raloxifene increased markedly RNA of IL-6 and TNF-alpha, whereas IL-1beta decreased them. Estrogen as 0.1-5 uM exposure reduced also protein levels of IL-6 by 5-20%, and Ospemifene about 15% in culture medium. Low concentration estrogen treatments had minor effect on TNF-alpha protein level, but 5 uM treatment increased its level by 40%. All SERMs increased TNF-alpha protein levels. As a conclusion, the results showed that Tamoxifen and Raloxifene increased expression of pro-inflammatory cytokines. Ospemifene is a promising new SERM, since its effects resembled those of estrogen, and the cells tolerated it even at high concentrations.

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Inhibition of TNF **A**lpha Production Induces Decrease of Amyloid Peptide and Nuclear Phosphorylated PKR Expressions

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Alzheimer's disease is a complex neurodegenerative disorder pathologically identified by the presence of extracellular senile plaques composed of aggregates of the amyloid peptide and intracellular aggregates of protein tau as neurofibrillary tangles. Neuronal degeneration usually involves concomitant changes in other cells, such as inflammatory response of astrocytes and microglial cells, which induce release of inflammatory cytokines like TNFα for example. Moreover, it has been shown that extracellular amyloid peptide induced a phosphorylation and activation of double-stranded RNA-dependent protein kinase (PKR), which is then translocated in nucleus and plays a significant role in neuronal death.

Objective of our study was to investigate the role of an inhibition of the TNFα production on amyloid peptide and nuclear phosphorylated PKR expressions.

For this, SH-SY5Y APP695 cells, which overexpress the amyloid peptide, were treated by two molecules, described as inhibiting TNFα production, and were studied concerning their amyloid peptide expression by immunofluorescence and their nuclear phosphorylated PKR expression by immunoblotting.

SH-SY5Y APP695 significantly more express nuclear phosphorylated PKR than mock control cells. After cells treatment by imipramine 20 and 40 µM for 2 hours and 10 and 20 µM for 24 hours, and by yohimbine 100 and 500 µM for 2 hours, we observed a decrease of the amyloid peptide expression by these cells. This decrease of the amyloid peptide expression seems to be correlated with a decrease of nuclear phosphorylated PKR expression and it would be confirmed by complementary studies in neuron-microglial cocultures derived from Alzheimer transgenic mice and then in vivo.

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The Relationship Between Level of Homocysteine and Severity of Alzheimer' Disease

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Background and aims: Alzheimer's disease (AD) is the common causes of dementia. Mild cognitive impairment (MCI) refers to the clinical condition between normal aging and dementia. Recent studies have appraised the possibility that homocysteine might play a role in the pathogeneses of AD. The purpose of this study was to investigate the relations of AD and MCI with homocysteine.

Methods: The study population consisted of 147 consecutive subjects, 108 of whom were eligible for analyses. The plasma total homocysteine was measured in 25 non-demented elderly control subjects, 28 MCI patients, and 65 AD patinets.

Results: Homocysteine was significantly increased in patients with AD and MCI as compared to controls. Subjects in the highest homocysteine tertile had significantly higher adjusted odds ratio for AD (AOR, 5.4) and MCI (AOR, 2.4). In addition, homocysteine was correlated with folate, vitamin B12, age, depression, and MMSE scores, but not with schooling years.

Conclusions: In this study, significantly elevated homocysteine levels were found in patients with AD and MCI. These findings suggest that hyperhomocysteinemia might be a risk factor for cognitive decline in the elderly.

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Effects of AAD-2004, a Neuroprotective and Anti-Inflammatory Drug, on Plaque Pathology and Behavior in Appswe/Ps1deltae9 Transgenic Mice

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We examined the effects of AAD-2004, an anti-oxidant and anti-inflammatory drug derived from salicylate, in double transgenic model of AD, APPswe/PS1deltaE9 (APP/PS1). Levels of beta amyloid, amyloid plaque, and 8-OHdG are increased in the cortex and hippocampal formation at 10.5 months of age in APP/PS1 transgenic mice compared to the wild type control. Increased amyloid burden and oxidative stress was attenuated by daily administration of AAD-2004 in the diet, beginning from 3 months of age. In elevated plus maze, APP/PS1 transgenic mice spent more time in open arms and had higher number of open arm entries than the control. The behavioral deficit was significantly attenuated by administration of AAD-2004 in the diet. In cultured CHO cells overexpressing wild type APP+PS1Δ9, AAD-2004 treatment significantly lowered Aß42 production. These finding suggest that AAD-2004 ameliorates amyloid burden, oxidative stress, and cognitive deficit in APP/PS1 transgenic mice. In ameliorate antioxidant and amyloid lowering properties of AAD-2004 might have a therapeutic potential for treatment of AD.

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Neuroprotective and Anti-Inflammatory Action of 2-Hydroxy-5-[4-(Trifluoromethyl) Phenethyl Amino] Benzoic Acid (AAD-2004) in Vitro

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Evidence has accumulated suggesting that oxidative stress and inflammation are key mediators of neuronal death and amyloid plaque formation. However, clinical trails of anti-oxidants and anti-inflammatory drugs have failed to show beneficial effects in AD primarily due to lack of efficacy and drug-induced side effects. We have developed AAD-2004, a cell protective drug with anti-inflammatory action derived from salicylate. AAD-2004 protected cultured cortical neurons and glia from free radical toxicity induced by 50 µM Fe2+, 10 mM DL-buthionine-[S,R]-sulfoximine, 50 µM 1-methyl-4-phenylpyridinium (MPP+) or 5 µM sodium nitroprusside. AAD-2004 reduced levels of a stable free radical, 1,1-diphenyl-2-picrylhydrazyl in test tube and Fe2+-induced free radical production in cultured neurons, suggesting that the neuroprotective effects of AAD-2004 are attributable to its free radical scavenging action. In addition, AAD-2004 showed anti-inflammatory action in BV-2 microglial cells. AAD-2004 attenuated nitric oxide production and expression of inflammatory cytokines such as TNF-α, IL-1β, and IL-6 induced by lipopolysaccharides. The present study suggests that AAD-2004 can be applied to prevent neuronal death and inflammation in AD and other neurodegenerative diseases.

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Effects of AAD-2004 on Plaque Pathology and Behavior in Appswe Transgenic Mice

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We examined the possibility that AAD-2004, an anti-oxidant and anti-inflammatory drug derived from salicylate, would modify pathological process in APPswe (Tg2576) mice expressing the Swedish mutation of the human amyloid precursor protein. Tg2576 mice developed age-related AD-like pathology such as Aβ deposits, amyloid plaque formation, and cognitive impairment. Daily administration of AAD-2004 in the diet, beginning from 6 months of age, markedly attenuated levels of Aβ42 produced at 12 months of age in Tg2576 mice compared to the wild type control. Daily administration of AAD-2004, beginning from 9 months of age, reduced escape latency in Morris water maze test and Aβ42 levels at 17 months of age in Tg2576 mice. Tg2576 mice treated with AAD-2004 for 3 days at 7 months of age markedly reduced levels of SDS-soluble Aβ42. These data indicate that AAD-2004 can prevent plaque pathology by inhibition of Aβ42 production and memory deficits in Tg2576 mice.

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Anti – Inflammation Action of AAD-2004 as A Potential Disease – Modifying Drug for Alzheimer's Disease

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Two lines of evidence support that inflammation contributes to pathological process in Alzheimer's disease (AD). First, activation of microglia and expression of inflammatory cytokines are increased in AD and associated with beta amyloid deposit. Second, progression and incidence of AD is reduced in patients with rheumatoid arthritis (RA) taking nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs are expected to exert their effects by reducing inflammation and beta amyloid burden in brain. However, clinical trials of NSAIDs including celecoxib and rofecoxib have failed to show beneficial effects in AD primarily due to drug-induced gastric and cardiovascular damage. We have developed AAD-2004, an anti-inflammatory drug with anti-oxidant effect derived from salicylate. Anti-inflammatory action of AAD-2004 was verified in vitro and also in animal models of inflammatory bowel disease and RA. AAD-2004 did not cause gastric damage but rather prevented ethanol/HCl-induced gastric lesion. Furthermore, AAD-2004 protected the hippocampal neurons from transient forebrain ischemia, an animal model of cardiac arrest (Our companion study, Park et al). The present study supports that AAD-2004 can be applied for the prevention of two pathological changes in AD, inflammation and oxidative stress, presumably without causing gastric and cardiovascular damage.

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Intervention of Oxidative Stress and Inflammation by AAD-2004 in A Transgenic Mouse Model of Amyotrophic Lateral Sclerosis

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Evidence has accumulated suggesting that oxidative stress and inflammation contribute to neuronal injury in neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). We have developed AAD-2004, an anti-oxidant and anti-inflammatory drug that is derived from salicylate and aimed to halt nerurodegenerative process in AD and related neurodegenerative diseases. We examined if AAD-2004 would prevent oxidative stress, inflammation, and neuronal death in transgenic mice model of ALS (G93A mice). Daily administration of AAD-2004 significantly enhanced life span and motor function of G93A mice. AAD-2004 prevented oxidative stress and inflammatory response that were markedly increased in the lumbar spinal cord from G93A mice. The present study suggests that dual pharmacological action of AAD-2004 with anti-oxidant and anti-inflammation can be applied for therapeutic intervention of neuropathological process in AD and PD as well as ALS.

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Ibuprofen Attenuates Nadph Oxidase-Derived Ros Production Through a Cyclooxygenase-Dependent Mechanism in Fibrillar Beta-Amyloid Stimulated Monocytes and Microglia

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Epidemiological studies have demonstrated long-term use of nonsteroid anti-inflammatory drugs (NSAIDs) reduces the risk for Alzheimer's disease (AD). Considerable evidence points to inflammatory processes being involved with the etiology of AD. Many inflammatory events center around the fibrillar beta-amyloid (Abeta) containing plaques. These plaques are surrounded by activated microglia, the principle immune effector cells in the brain. Microglia interact with Abeta through a cell surface receptor complex, which initiates a tyrosine kinase based signaling cascade leading to proinflammatory signaling and reactive oxygen species (ROS) production through NADPH oxidase activation (Bamberger et al. 2002). We recently reported a mechanistic link between the Abeta receptor complex and downstream signaling events leading to NADPH oxidase assembly. Vav, a guanine

nucleotide exchange factor (GEF) for Rac1 GTPase, is an essential component in this signaling cascade. Vav-/- microglia fail to elaborate Abeta-induced ROS (Wilkinson et al. 2006). The present study examines ibuprofen's effect on signaling cascades related to microglial NADPH oxidase activation. Pretreatment of THP-1 monocytes with racemic ibuprofen (R and S enantiomers) attenuated Abeta-stimulated Vav phosphorylation. Cyclooxygenase (COX)-dependent and –independent effects were addressed by comparing the contribution of either the R or S enantiomer. The S enantiomer is the active enantiomer with respect to COX inhibition. S enantiomer pretreatment reduced the Abeta-stimulated Vav phosphorylation, whereas the R enantiomer was ineffective. The S enantiomer also attenuated Abeta-induced ROS production in primary murine microglia. These findings suggest that COX can regulate intracellular signaling cascades leading to NADPH oxidase activation.

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Effects of Ginkgo Biloba Extract EGB 761® on Neuropsychiatric Symptoms of Dementia: Findings From a Randomised Controlled Trial

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Background: Neuropsychiatric symptoms are common in dementia and often determine the degree of patients' and caregivers' disease-related distress.

Methods: We enrolled 400 patients with mild to moderate dementia associated with neuropsychiatric features in a clinical trial of Ginkgo biloba extract EGb 761®. Patients scoring 9 to 23 on the SKT cognitive test battery were eligible if they met the diagnostic criteria of the National Institute of Neurological and Communicative Disorders and Stroke / Alzheimer's Disease and Related Disorders Association for probable Alzheimer's disease, those of the National Institute of Neurological Disorders and Stroke / Association Internationale pour la Recherche et l'Enseignement en Neurosciences for vascular dementia or relevant criteria of both sets for possible Alzheimer's disease with cerebrovascular disease. Patients were randomly assigned to receive either 240 mg EGb 761® per day or placebo in a double-blind manner for 22 weeks.

Results: EGb 761® was significantly superior to placebo with respect to the primary (SKT test battery) and all secondary outcome variables. The mean composite score and the mean caregiver distress score of the Neuropsychiatric Inventory (NPI) dropped from 21.3 ± 9.5 to 14.7 ± 9.5 and 13.5 ± 6.7 to 8.7 ± 5.5, respectively, in the EGb 761®-treated patients, whereas increases from 21.6 ± 9.9 to 24.1 ± 12.8 and 13.4 ± 6.4 to 13.9 ± 7.2, respectively, were found in those who received placebo ($p < 0.001$). Item-by-item analyses revealed the largest drug-placebo differences in favour of EGb 761® for apathy/indifference, anxiety, irritability/lability, depression/dysphoria and sleep/nighttime behaviour.

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Statin Protects From Abeta-Induced Episodic Memory Loss Through Up-Regulating Clearance of Abeta in Mice

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(Background and aims) Dementia is caused by cerebrovascular disorder and neurodegeneration. Hypertention, hyperlipidemia,and diabetes are risk factors of dementia. Recently, a number of studies have demonstrated that statins may exert anti-inflammatory effect and inhibit the formation of beta-amyloid. Alzheimer disease(AD) is a chronic neurodegenerative disorder that is manifested by cognitive decline, neuropsychiatric symptoms, and diffuse structural abnormalities in the brain. So we investigated whether statin therapy is effective in cognitive function in patients with AD. (Methods) We examined the effect of fluvastatin on amnesia induced by Abeta1-40 in mice. Six-week-old male ddY mice received fluvastatin 5mg/kg/day or vehicle . After 2weeks, mice were treated(i.c.v.) with abeta1-40 and took orally fluvastatin or vehicle more 3weeks. Then Y-maze test and water-finding task were carried out. Within 2weeks after the tests, mice were sacrificed for westernblotting and immunohistchemical analysis. (Results) Fluvastatin-treated mice showed improvement of latent learning in a water-finding task. (control,62.0Â±21.4sec;n=7; Abeta1-40+vehicle, 212.67Â±31.48sec;n=3; Abeta1-40+fluvastatin,52.75Â±24.08sec; n=4). Brain abeta1-40 was significantly lower with fluvastatin treated mice. Total choresterol level did not differ significantly among these groups. (Conclusion) These results suggest that statin therapy is effective in the treatment of Alzheimer disease.

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Early Disruption of Blood-Brain Barrier in Doubly Transgenic Mice of Mutant Betaapp and Presenilin-1: Role of Beta Amyloid and MMP-9

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Blood-brain barrier (BBB) disruption has been reported in AD and also in Tg2576 Alzheimer disease (AD) model mice. We studied temporal profile of BBB disruption in transgenic mice expressing both mutant betaAPP and presenilin-1 (APP-PS1). BBB permeability was increased in the cortex and hippocampal formation before amyloid plaque was formed in APP-PS1 mice. The intracerebroventricular injection of Aβ1–42 also resulted in increased BBB

permeability as evident by penetration of evans blue and albumin into brain. This suggests a causative role of beta amyloid in BBB disruption. Expression of MMP-9 known to mediate BBB disruption following ischemic brain injury was increased in endothelial cells in APP-PS1 transgenic mice and following administration of Aβ1–42. Administration of AAD-2004, an anti-inflammatory and anti-oxidant drug derived from salicylate, protected BBB breakage from Aβ1–42. Taken together, beta amyloid produced in AD may cause MMP-9-mediated BBB disruption possibly through mechanisms involving inflammation and oxidative stress.

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Mechanisms Underlying Reversal of Memory Deficits by Ciproxifan, An H3 Histamine Receptor Antagonist, in a Mouse Model of Alzheimer's Disease

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We have investigated the effect of ciproxifan on transgenic mice (Tg2576) over expressing the Swedish mutation of human amyloid precursor protein. This model displays biochemical, pathological, and behavioral markers consistent with many aspects of Alzheimer's disease including impaired memory. Using 18 week old mice, we have shown that ciproxifan (3 and 10 mg/kg i.p.) reversed cognitive deficits in a contextual fear conditioning model. Transgenic animals exhibit significantly reduced CREB phosphorylation levels that could be recovered by ciproxifan treatment. H3 receptor mRNA and protein levels were significantly decreased in the cortex of transgenic animals, compared to wild type mice, suggesting that elevated beta amyloid may lower H3 receptor expression. Histamine and acetylcholine (ACh) levels were measured in the cortex of transgenic and wild type littermates by in vivo microdialysis. Basal neurotransmitter levels were not significantly different in wild type and transgenic mice. However, following acute ciproxifan treatment (10 mg/kg i.p.), ACh levels were increased in both groups with a significantly greater increase in the transgenic compared to wild type (68% and 31% above basal, respectively). In contrast, histamine levels were significantly increased by 80% following ciproxifan treatment in the wild type mice but no increase was seen in the transgenic mice. Our data demonstrate that the reversal of the cognitive deficits in a mouse model of AD by ciproxifan correlates with an increase in ACh levels and restoration of normal CREB phosphorylation, supporting the use for H3 receptor antagonists in AD.

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Glycogen Systhase Kinase-3b Activity Plays Very Important Roles in Determining the Fate of Oxidative Stress-Inflicted Neuronal Cells

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Background and aims: Glycogen synthase kinase-3, especially the beta form (GSK-3β), plays key roles in oxidative stress-induced neuronal cell death, an important pathogenic mechanism of various neurodegenerative diseases including Alzheimer's disease. Although the neuroprotective effects of GSK-3β inhibitors have been described, the optimal level of GSK-3β inhibition for neuronal cell survival has not yet been determined.

Methods: We investigated the effect of varying GSK-3β activity on the viability of oxidative stress-injured neuronally-differentiated PC12 (nPC12) cells and intracellular signals related with the GSK-3β and caspase-3 pathways.

Results: Compared to the nPC12 control cells treated with only 100 μM H2O2, treatment of 50-200 nM GSK-3β inhibitor II or 25-500 nM GSK-3β inhibitor VIII reduced the increased enzyme activity by about 50% and protected the cells against H2O2-induced oxidative stress. The optimal concentration of GSK-3β inhibitor II enhanced heat shock transcription factor-1 levels, decreased levels of phosphorylated tau (Ser202) and cytosolic cytochrome c, activated caspase-3, and cleaved poly (ADP-ribose) polymerase. In contrast, higher concentrations of GSK-3β inhibitor II (more than 500 nM) induced neuronal cell death, and showed opposite effects relative to the above described intracellular signals.

Conclusion: These results suggest that optimized inhibitor levels for modulating GSK-3β activity may prevent apoptosis induced by oxidative stress associated with neurodegenerative diseases.

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P25/Cdk5 Inhibitors for AD Therapeutics

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Background And Aims: Alzheimer's disease (AD) is an irreversible, progressive brain disorder that is characterized by dementia. Elevated levels of p25 and cdk5 kinase activity have been demonstrated to be specifically upregulated in AD patient's brain samples. Considerable evidence now points importance of p25/cdk5 in increase of BACE activity, generation of Abeta peptides and hyperphosphorylation of tau linking amyloid plaques and neurofibrillary tangles, the pathological hallmarks of AD.

Methods: To develop an assay system to screen for p25/cdk5 inhibitors which reduce the secretion of Abeta we generated a PC12 cell line in which expression of p25 is regulated by tet-off system and constitutive expression of p25 in the absence of doxycyclin stimulated Abeta secretion.

Results: A library of 3.4 million chemicals has been screened in silico for their interaction with P25 and 108 compounds were selected for in vitro cell-free and cell-based assays. Five different chemotypes were selected from the assays. Among the compounds which were derived from a selected chemotype, IDRS-35 showed strong inhibition of Abetąsecretion in P25-expressiong PC12 cell line.

Conclusion: We believe that P25 inhibitors should have therapeutic potential in the treatment of sporadic AD.

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Effects of Filamentous Phage Treatment on Brain Pathology of Alpha Synuclein Tg Mice

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Background: C57BL6 mice, over-expressing human wild-type alpha-synuclein under the regulatory control of the human PDGF-ß promoter, display high alpha-synuclein RNA and protein levels. These mice are a suitable model to study alpha-synuclein production, sequestration and deposition, as well as the possible effects of various drugs on these parameters. They develop Lewy body-like alpha-synuclein positive neuronal inclusion bodies at an early age, a pathological relevant situation as described in Parkinson's disease or Lewy body dementia. Severity of the brain pathology correlates with increasing age. Recently it has been shown that the linear structure of filamentous phages exhibits disaggregating and brain penetrating abilities.

Method: In the present study, 2.5 month-old mice over-expressing alpha-synuclein were treated intranasally once a week for eight weeks with 10µl of filamentous phage or aqua bidest. At the end of the treatment, brains were removed and hemisected; the right hemisphere was postfixed and frozen for evaluation of alpha-synuclein immunoreactivity in cortex and hippocampus. Additionally, astrocytosis, activated microglia, and other brain markers were immunohistochemically investigated.

Results: Alpha-synuclein expression in cortex and hippocampus was significantly decreased by approximately 50% due to treatment with filamentous phage, compared to PBS treated controls. Evaluation of inflammation markers is currently under investigation.

Conclusion: Filamentous phages have anti-aggregating abilities, are able to enter nerve cells, dissolving Lewy body-like inclusions in mice over-expressing alpha-synuclein, suggesting a novel therapeutic approach for treatment of diseases related to intracellular aggregates.

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in Vivo Reversal of Aged Abeta1-40-Induced Memory Impairment and Neuronal Changes by Breaker Peptides Bilaterally Injected Into Rat Amygdala

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Amyloid plaques are the pathological hallmark of Alzheimer's disease. No treatment targeting the peptides is available. However the breaker peptides, forming part of the sequence of the ABeta, are being investigated for Alzheimer's disease therapy. This study examined the effect of bilateral amygdala injection of breaker peptide ABeta15-22 (15 and 30 nM/side) on memory impairment induced by ABeta1-40(3 nM/side) using 8-armed radial maze. Moreover the neuronal changes were examined in brain sections stained by H&E, Cresyl Violet, Congo Red and immunostaining using rabbit polyclonal anti- ABeta unconjugated antibody. ABeta decreased the correct choices by 20% ($P<0.04$), whereas increased the error choices by 138 % ($P<0.004$) after 14 days. These effects were accompanied with nuclear shrinkage, condensation and/or chromatin fragmentation. The breaker peptide decreased memory impairment (increased correct choices by 33%, $P<0.001$, decreased error choices by 79%, $P<0.001$), and reversed the pathological neuronal changes induced by ABeta. These results suggest that breaker peptides could be a promising therapeutic approach of ABeta -induced neurodegeneration and memory impairment including Alzheimer's disease.

This study is supported by The Scientific and Technological Research Council of Turkey (TUBITAK-SBAG-K-50-104S262).

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Long-Term Treatment With Donepezil Differently Influences Acetylcholinesterase Variants From Cerebrospinal Fluid in Patients With Alzheimer's Disease

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We analysed whether donepezil differently influences acetylcholinesterase (AChE) variants from cerebrospinal (CSF) in patients with Alzheimer's disease (AD) after long-term treatment. Overall CSF-AChE activity in AD patients before treatment was not different from controls, but the ratio between the major tetrameric form, G4, and the minor light species, G1 and G2, was significantly lower. When patients were re-examined after 12 months treatment with donepezil, however, there was a remarkable increase in CSF AChE activity in both the G4 and the lighter molecular forms. We also compared AChE species in terms of their ability to bind the lectin, Concanavalin A, and the antibody, AE1. These experiments revealed differential effects on a small subset of AChE forms. In particular, as compared with placebo, donepezil caused decreases in the percentage of AChE activity, primarily from G1 and G2 forms, that failed to bind to the lectin and the antibody. SDS-PAGE analysis showed that the major 77-kDa immunoreactive AChE band, attributed in part to inactive AChE, was lower in AD patients than in controls. Unlike enzyme activity, the intensity of this band did not increase after donepezil treatment. The varying responses of different AChE species to ChE-I treatment suggest different modes of regulation, which may have important therapeutic implications.

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Plasmalogens and APP Processing

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Background and aims: In our previous studies we could show that extensive regulatory cycles between cholesterol, sphingolipid-homeostasis and APP processing exist. Beside cholesterol and (glycol-)sphingolipids plasmalogens play an important role in raft biology, which suggests that beside cholesterol plasmalogens may contribute equally or even stronger to AD pathology. Therefore we investigated a potential connection between APP processing and plasmalogen metabolism.

Methods: In order to elucidate the effect of plasmalogens on AD pathology several plasmalogens, which differ in length and saturation of the fatty acid, were analyzed on their potential to alter amyloid production. In return the effect of APP processing on the plasmalogen homeostasis was investigated. amyloid production was analyzed in COS7 SPC99 transfected cells reflecting gamma-secretase activity. The plasmalogen pattern was studied by mass spectroscopy and thin layer chromatography. In addition the activities of important enzymes in the plasmalogen pathway were determined by new established enzymatical assays.

Results: Suprisingly the effect on amyloid production differs depending on the plasmalogen. In return plasmalogen pattern appeared to be APP processing dependent. In line with these experiments the enzymes of the plasmalogen pathway seem to be changed.

Conclusion: Beside the regulatory cycles of cholesterol, and amyloid production plasmalogen metabolism is affected by APP processing. In return plasmalogens drastically influence amyloid production. Treating Alzheimer patients with drugs, that modulate plasmalogen metabolism could reveal beneficial effects in the progression of dementia comparable to the drugs lowering cholesterol levels.

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Docosahexaenoic Acid Stimulates Non-Amyloidogenic APP Processing Resulting in Reduced Abeta Levels in Cell Models of Alzheimer's Disease

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Background and aim: Epidemiological studies suggest that high intake of polyunsaturated fatty acids is associated with a reduced risk of Alzheimer's disease (AD). Recently, a diet enriched in the polyunsaturated fatty acid docosahexaenoic acid (DHA) was found to dramatically reduce amyloid burden in aged Tg2576 mice with biochemical studies demonstrating reduced Aβ levels together with some effects on amyloid precursor protein (APP) processing. Here, we used a simplified system to examine the effects of DHA on APP processing more in detail.

Methods: Human neuroblastoma SH-SY5Y cells expressing APP with the Swedish familiar AD mutation or APP with both the Swedish and the Arctic familiar AD mutations were cultured in media supplemented with DHA where after APP processing was studied.

Results: DHA administration increased the C83/C99 ratio and reduced Aβ levels in both cell models, indicating increased α-secretase and/or decreased β-secretase cleavage. Also the αAPPs/βAPPs ratio was increased in APPSwe cells treated with DHA, but unaffected in APPArc-Swe cells, probably due to the reduced α-cleavage associated with the Arctic mutation itself. Interestingly AICD, the product from γ-secretase cleavage at ε-site of C83 and C99, also increased in cells treated with DHA. This increase was abolished in presence of γ-secretase inhibitor, indicating a γ-secretase dependent mechanism.

Conclusion: In conclusion, our results demonstrate that DHA shifts APP processing towards the non-amyloidogenic pathway, resulting in reduced Aβ levels. In addition, γ-secretase is affected resulting in increased AICD levels. These results suggest a protective role of DHA in AD pathogenesis on a molecular basis.

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Nuclear Liver X Receptors in the Brain: Effect on Inflammatory Response Induced by A-Beta

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Microglial activation is a normal protective response to CNS injury, however, it is also believed to contribute to, or initiate neurodegeneration by releasing proinflammatory and cytotoxic factors, including NO and IL-1þIn peripheral macrophages, in response to LPS stimulation, treatments with LXR ligands inhibit the expression of inflammatory mediators.

We used primary microglial cells, induced inflammatory response by LPS or fibrillar A-beta treatments and examined the effect of synthetic LXR ligands T0901317 and GW3965 on NO and interleukins. We found that iNOS expression in response to LPS was increased more than 5 fold and T0 suppressed this effect in a dose-dependent manner. The effect of T0 and GW on NO production and IL-1b secretion was similar. A-beta increased the expression of iNOS also, but the response was less pronounced. Similarly, LXR ligand treatment decreased the expression of inflammatory mediators, upregulated in response to A-beta. Moreover, we demonstrate that the detrimental effect of A-beta induced cytokine release on neuronal survival was inhibited by LXR ligand treatment.

Finally, aged APP23 transgenic mice were treated with T0 or vehicle for 25 days and then we evaluated the expression level of >20,000 genes in their brains. Quantitative real time PCR was performed to verify the results of gene array assays. The final results support the hypothesis that the expression level of genes, thus functions of their related proteins, involved in the response to different inflammatory stimuli in the brain can be modulated by LXR ligands, which opens a new direction for treatment of neurodegenerative disorders.

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Nuclear Liver X Receptors in the Brain-Effect on A-Beta Amyloidosis

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Recent studies demonstrate that in vitro treatment with natural and synthetic LXR ligands decrease the amyloidogenic processing of APP and A-beta secretion. We have also demonstrated that short time treatment of young APP23 mice with the synthetic LXR ligand T0901317 caused an increase in ABCA1 expression and significant reduction in the levels of soluble A-beta40 and A-beta42.

To further determine the effect of LXR ligands on AD phenotype we administered T0 to 6-months-old APP23 mice for 25 days. We found the levels of soluble ApoE and ApoA-I extracted from cortices and hippocampi substantially increased. Next, we examined the level of insoluble A-beta and found that T0 treatment caused a decrease, with a negative correlation between soluble ApoE level and insoluble A-beta.

To assess changes in gene expression associated with T0 treatment, the Affymetrix 430A 2.0 chips were used to survey gene expression in the brains. Altered genes were identified by comparing T0-treated with vehicle-treated control groups.

Based on these results we propose a model for coordinated effect of upregulated ABCA1 and ApoE on A-beta aggregation, amyloid deposition, and clearance. We speculate that the increased level of ApoE after T0 treatment is a combination of (1) increased amount of ApoE mRNA, and therefore increased amount of ApoE protein in the brain, and (2) transcriptional upregulation of ABCA1 that leads to an increased cholesterol efflux and lipidation of ApoE and ApoA-I in brain. The final outcome of increased levels of lipidated ApoE and ApoA-I is a decreased A-beta aggregation and amyloid deposition.

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LXR Agonists Can Directly Modulate Gamma-Secretase Activity

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Recent studies have suggested that regulating brain cholesterol metabolism may have beneficial effects on processing of APP to Abeta peptide. Agonists of liver X receptor (LXR) transcriptionally induce genes involved in cellular lipid efflux and transport. Multiple studies have focused on the effects of LXR activation on metabolism of Abeta in-vitro, but with discrepant findings; Fukumoto et al showed that LXR agonist, TO901317, increased Abeta secretion, while Koldamova et al and Sun et al demonstrated that the same compound decreased Abeta secretion.

As a first step in clarifying the role that LXR plays in Abeta metabolism, we treated Abeta-secreting H4 neuroglioma cells with a range of structurally diverse LXR agonists. It was found that whereas TO901317 stimulated Abeta42, GW3965 decreased Abeta42, while, native ligand 22(R) hydroxycholesterol had no effect on Abeta42 secretion. A possible explanation for the observed contradictory effects of LXR agonists on Abeta is that these compounds have pleiotrophic actions on the gamma-secretase complex, as was recently reported for a range of NSAID-like structures. We therefore determined whether TO901317 or GW3965 have direct effects on broken cell preparations of the gamma-secretase complex. Importantly, these membrane preparations are devoid of nuclear material and therefore should not be responsive to LXR stimulation. Under these conditions, TO901317 still stimulated Abeta42 production and GW3965 inhibited Abeta42 production, suggesting that the effects of these compounds in vitro are not related to their binding affinity towards LXR, but rather via a direct interaction with the gamma-secretase complex.

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Delayed Treatment With Fluvastatin Improved Learning and Memory Deficit After Stroke, Associated With Reduction of Abeta Deposition in Rats

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Background & Purpose: Recent clinical evidence suggests that the commencement of statins within 4 weeks of stroke results in a favorable 90-day outcome. In the present study, we evaluated the effects of delayed post-ischemic treatment with fluvastatin on functional recovery after ischemic stroke in rats.

Methods: Thirty-two rats were exposed to permanent middle cerebral artery occlusion (DAY 1). Based on the sensori-motor deficits and body weight evaluated at DAY 7, the rats were divided equally into saline or fluvastatin-treated rats. At DAY 56, the cognitive function was studied in survival rats.

Results: Rats treated with fluvastatin showed significant recovery of learning and memory as assessed by Morris water maze tes. Microangiography showed a significant increase in capillary density in the peri-infarct cortex and basal ganglia of fluvastatin-treated rats, after 3 months of treatment. In addition, rats treated with fluvastatin also showed a reduction of superoxide anion after 7 days of treatment and a reduction of Abeta deposits in the thalamic nuclei after 3 months of treatment.

Conclusion: Thus, delayed post-ischemic administration of fluvastatin had beneficial effects on the recovery of cognitive function without worsening the infarction after ischemic stroke. Pleiotropic effects of fluvastatin, such as angiogenesis and neuritogenesis, through the inhibition of

superoxide production and Abeta deposition, might be associated with a favorable outcome.

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Accumulation of Sphingolipids Increases Secretion of the Amyloid ß-Peptide by Stabilization of the ß-Amyloid Precursor Protein

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Alzheimer's disease is associated with extracellular deposits in the brain of amyloid ß-peptides (Aß) that are generated by proteolytic processing of the ß-amyloid precursor protein (APP). It has been shown that membrane lipids, including cholesterol and sphingolipids affect the subcellular transport of APP in the secretory pathway and its proteolytic processing. Previously, we also showed that the inhibition of glycosphingolipid (GSL) biosynthesis reduces the secretion of APP and Aß.

The proteolytic processing of APP and its derivatives was studied by the detection of full length and soluble APP, APP C-terminal fragments (CTFs) as well Aß and by pulse chase experiments. Distribution of APP and its processing products in different endocytic organells was analysed by iodixanol density gradient.

The accumulation of GSLs markedly increased the secretion of endogenous APP and Aß. A strong increase in total APP-CTF levels upon addition of GSLs was also observed. Addition of sphingomyelin showed similar effects. However gamma-secretase activity was not affected by GSLs in in vitro assays. By biochemical and cell biological experiments, we demonstrate that the increased levels of cellular GSLs altered the distribution and stability APP-CTFs. Similar results were also obtained in independent genetic cellular models of GSL storage. On the other hand, GSL deficient cells showed decreased levels of APP-CTFs.

Together, these data demonstrate that GSLs enhance the secretion Aß likely by stabilising APP-CTFs, thereby providing more substrate for gamma-secretases. Our studies suggest a novel role of GSLs in regulation of APP trafficking and Aß generation along the endocytic pathway.

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Quantitative Analysis of Neuronal ATP-Binding Cassette Transporters and Their Role in Regulation of Cholesterol Efflux and Amyloid Precursor Protein Processing

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Background And Aims: Increasing evidence suggests that neuronal membrane cholesterol levels regulate amyloid precursor protein (APP) processing to generate amyloid-beta (A-beta) peptides. Neuronal cholesterol levels are increased via de novo synthesis and receptor-mediated lipoprotein uptake and decreased via efflux pathways that are incompletely characterised.

Methods: Here we examined the efflux pathways contributing to the removal of [3H]-cholesterol from primary human neurons and SK-N-SH neuroblastoma cells. Partially lipidated apolipoprotein-E (apoE) discs resembling nascent CSF lipoprotein particles were used as extracellular cholesterol acceptors.

Results: ApoE discs potently stimulated cholesterol efflux from neurons via a pathway that was saturable and APOE genotype independent. HPLC analysis of cholesterol effluxed from neurons indicated that at least 75% of the released cholesterol was recovered in a native state (not modified to polar products such as oxysterols); thereby implicating the involvement of a transporter pathway. Real-time PCR studies revealed expression of ATP-binding cassette transporters ABCA1, A2, A3, and G1 and low but clearly detectable levels of ABCA7 and A8. Transient expression of either ABCA1 or G1 cDNAs in HEK293 cells stimulated cholesterol efflux to apoE discs whereas ABCA2 or A3 did not. In addition, ABCA1 or G1 expression in CHO cells that stably express human wild-type APP695 significantly reduced A-beta generation whereas ABCA2 had no impact.

Conclusions: Of the ABC transporters we have detected in neurons, ABCA1 and ABCG1 appear to play a significant role in the regulation of neuronal cholesterol efflux to apoE discs and in the regulation of APP processing to generate A-beta peptides.

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BACE-1 Relocates to Lipid Rafts in Human Neuroblastoma SH-SY5Y Cells After Exposure to Chronic Hypoxia

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Alzheimer's disease (AD) is characterised by the presence of senile plaques in the brain, formed from the neurotoxic amyloid–beta (Abeta) peptide. Abeta is produced from the transmembrane amyloid precursor protein (APP). In the amyloidogenic pathway, APP is cleaved sequentially by the beta-secretase BACE-1 and then by gamma-secretase. Cholesterol and sphingolipid-rich lipid rafts have been shown to be important in regulating the amyloidogenic processing of APP. After an ischemic episode, hypoxic conditions induced within the brain significantly increase the possibility of developing AD. The aim of this study was to address whether hypoxic conditions may alter lipid raft integrity thereby promoting the amyloidogenic processing of APP. Human SH-SY5Y neuroblastoma cells stably transfected with BACE-1 were exposed to chronic hypoxia (1% O2, 24 h). Lipid rafts were isolated by buoyant sucrose gradient centrifugation in the presence of Triton X-100 and the distribution of APP and its cleavage products, as well as BACE-1, were determined by western blotting. After 24 h of chronic hypoxia BACE-1 was present in both the detergent soluble non-raft and detergent resistant raft fractions. In cells exposed to normal cell culture conditions BACE-1 remained exclusively in the detergent soluble non-raft fractions of the sucrose gradient. This movement of BACE-1 from the non-raft to the raft fractions may promote proteolysis of APP thereby bringing about a

corresponding increase in Abeta production. This possible link between chronic hypoxia and the increase in Abeta production alongside the relocation of BACE-1 within the cell membrane could provide insight into the pathogenesis of AD.

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Changes in Cholesterol Metabolism Are Associated With Psen1 and Psen2 Gene Regulation in SK-N-BE

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Background and aims: Several lines of evidence suggest that the cholesterol content of neuronal membranes influences amyloid precursor protein (APP) processing; however, its role in transcriptional regulation of the cofactors for gamma-secretase, the key enzyme for the production of the Abeta peptide, is poorly understood. Our research investigates whether the changes in cellular cholesterol metabolism modulate the expression of genes involved in the gamma-secretase complex function.

Methods: The abundance of mRNA transcripts for presenilin 1 and 2 (PSEN1 and PSEN2), APP and nicastrin were evaluated in SK-N-BE cells exposed either to serum-depleted medium, to low density lipoproteins (LDL) and to cholesterol metabolism modulating drugs.

Results: We observed that cholesterol esterification was markedly inhibited by mevinolin and U18666A, but was not significantly affected by any other of the test treatments. Moreover, gamma-secretase genes and cofactors were not co-regulated and were not influenced by statin inhibition of cholesterol synthesis. Nicastrin and the APP isoforms showed constitutive expression. In the absence of exogenous lipids, cell PSEN1 and PSEN2 expression was induced by LDL and by lysosomal sequestration of cholesterol. However, a different pattern of induction of presenilin gene expression was observed in the latter condition, suggesting that lysosomal cholesterol levels are strong inducers of PSEN2 transcription.

Conclusions: Taken together, these results indicate that lipid metabolism has a complex influence on gamma-secretase transcriptional pathways and, in particular, exogenous cholesterol and compartmentalization in neuroblastoma cells play a relevant role in regulating the transcription of presenilins.

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The Role of Rafts and Rabs in Alzheimer's Disease

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The endocytic system has been implicated in the amyloidogenic cleavages leading to the formation of Aβ. However, the identity of the intracellular compartment where the amyloidogenic secretases cleave and the mechanism by which the intracellularly generated Aβ is released into the extracellular milieu are not clear. Our results show that β-cleavage occurs in rab5-positive early endosomes followed by routing of Aβ to multivesicular bodies (MVBs) in HeLa and N2a cells. Subsequently, a small fraction of Aβ peptides can be secreted from the cells in association with exosomes, intraluminal vesicles of MVBs that are released into the extracellular space as a result of fusion of MVBs with the plasma membrane. Exosomes are enriched in raft lipids, such as cholesterol and sphingomyelin and raft asscoiated proteins. Exosomal proteins were found to accumulate in the plaques of AD patient brains suggesting a role in the pathogenesis of AD. The role of raft lipids in plaque formation will be discussed.

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Lipids Induce Release of Neurotoxic Oligomers From Inert Amyloid Fibers

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Alzheimer's disease is associated with the aggregation of Amyloid-beta peptide (A-beta). It is established that neurotoxicity is caused during the A-beta aggregation process, by soluble A-beta species, and not by the A-beta fibers themselves that are considered as inert end-products of the aggregation process. Nevertheless, A-beta fibers may serve as a large reservoir of potentially neurotoxic material. We found that inert A-beta fibers can be reversed to toxic oligomers in the presence of synthetic phospholipids and lipid rafts components as gangliosids, sphingomyelin and cholesterol. Association of mature A-beta fibers with lipid vesicles results in immediate neurotoxicity to primary neurons followed by apoptotic cell death. The process is triggered by lipid-induced disassembly of amyloid fibers leading to the release of soluble toxic A-beta oligomers, containing among other oligomers subspecies the toxic p56-kDa soluble A-beta. Finally, we observe that release of toxic oligomers and subsequent neurotoxicity may be caused by other disease-associated amyloid peptides as TAU, Prion 1 and synthetic amyloidogenic peptide in the presence of lipids. We propose that lipid-induced fiber disassembly and release of soluble oligomers is a common generic mechanism of amyloids. An important implication of our work is that amyloid plaques are not inert and should be considered as potential large reservoirs of neurotoxic oligomers that can rapidly be mobilized by lipids. Although lipid metabolism has been implicated in neurodegenerative diseases the precise involvement of lipids in basic toxicity mechanisms in AD is a major question. Our data could help to understand this A-beta/lipid relationship in more detail.

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Mature BACE Localises Exclusively to Lipid Rafts in Rat Brain Synaptosomes

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The amyloid β-peptide (Aβ) is formed through the sequential cleavage of the amyloid precursor protein (APP) by β- and γ-secretases. Epidemiological, cell-based and transgenic data suggest a critical role for cholesterol in Aβ production. These observations propose that the amyloidogenic processing of APP may take place in cholesterol-enriched membrane domains referred to as lipid rafts. However, these studies have largely been performed in non-neuronal cell lines, transformed cells or cells over-expressing individual components of the amyloidogenic pathway. We have isolated lipid rafts from rat brain synaptosomal preparations and SH-SY5Y cells using Triton X-100 and differential centrifugation. Validation of the efficient separation was achieved by analysing the localisation of known raft (flotillin) and non-raft (γ-adaptin) proteins by immunoblotting. The rafts isolated from SH-SY5Y cells contained <5% of the β-secretase (BACE) whereas those isolated from the synaptosomal P2 preparation contained more than 30%. APP and Presenilin also co-localised with BACE in these domains. Interestingly in purified synaptosomes mature BACE (~75 kDa) was confined to the rafts whereas the immature form (~70 kDa) was localised predominantly to the non-raft fraction. The mature form of the proposed α-secretase ADAM 10 was both raft and non-raft associated whereas, like BACE, the immature form was predominantly non-raft localised. These data suggest that synaptosomes may provide a useful, physiologically relevant system with which to study amyloidogenic processing in a native membrane environment.

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Oxidative Stress Induces Cell Death Independent of Abeta Aggregation in GT1-7 Hypothalamic Neurons Exposed to Cholesterol Secoaldehyde: Relevance for Alzheimer's Disease

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Alzheimer's disease (AD) is a neurodegenerative disease resulting in progressive loss of neurons and memory. The events leading to neuronal loss are poorly understood, however a strong association with abnormalities in cholesterol metabolism has been reported. Recent studies indicate that oxidation products of cholesterol are more critical than cholesterol alone in the pathogenesis of AD. An oxidation product of cholesterol, 3β-hydroxy-5-oxo-5,6-secocholestan-6-al (ChSeco), has been shown to be present in the arterial and senile plaques. Here we show that ChSeco (1-20 microM) induces Aβ aggregation, oxidative stress, and cell death in murine GT1-7 hypothalamic neurons. The intraneuronal Aβ aggregation (immunofluorescence) was supported by corresponding decrease(s) in soluble Aβ (western blot). The ChSeco-induced effects resulted in mitochondrial damage and caspase-3/7 activation causing apoptotic cell death (phosphatidylserine translocation and DNA fragmentation). Pretreatment with Aβ fibrillogenesis inhibitors did not rescue the cells, whereas antioxidants, N-acetyl-L-cysteine and Trolox, markedly facilitated the survival of neurons, without affecting Aβ aggregation in situ. Thus, it appears that neuronal loss is mediated by oxidative stress and is independent of Aβ aggregation. Agents that caused depletion or efflux of cellular oxysterols (e.g. methyl-β-cyclodextrin) inhibited both Aβ aggregation and cell death indicating that ChSeco induces Aβ aggregation and cell death through distinct mechanisms. Development of treatment strategies that target depletion or reduced formation of oxysterols, especially, ChSeco may provide avenues that would lead to better treatment options and patient care. [Corresponding author (e-mail): rao_uppu@subr.edu; Funding from NIH ARCH program of NIEHS (ES10018) and BRIN program of NCRR (P20 RR6456) is acknowledged.]

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Simvastatin-Treatment Prolongs Survival Times in a Murine Prion Model

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Prion diseases are fatal and at present there are neither cures nor palliative therapies known/available, which delay disease onset or progression. Cholesterol-lowering drugs have been reported to inhibit prion replication in infected cell-cultures and to modulate inflammatory reactions. We aimed to determine whether simvastatin-treatment could delay disease onset in a murine prion model. Groups of mice were intracerebrally infected with two doses of scrapie strain 139A. Simvastatin treatment commenced 100 days post infection. The treatment did not affect deposition of misfolded prion protein PrPres. However, expression of marker proteins for glia activation like major histocompatibility class II and galectin-3 was found to be altered. Analysis of brain cholesterol synthesis and metabolism revealed a mild reduction in cholesterol precursor levels, whereas levels of cholesterol and cholesterol metabolites were unchanged. Simvastatin treatment significantly delayed disease progression and prolonged survival times in established prion infections of the CNS ($p \leq 0.0003$). The results suggest that modulation of glial responses and the therapeutic benefit observed in our murine prion model of simvastatin is not due to the cholesterol lowering effect of this drug.

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Decreased Mibg Uptake and Impaired Recognition of Smell and Facial Expression in a Patient With Rem Sleep Behaviour Disorder

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[Background] Neuropathological and neurological studies have shown that REM sleep behaviour disorder (RBD) can be an early symptom of Parkinsonâ€™s disease (PD). We examined whether early symptoms of PD could be observed in a patient with apparent idiopathic RBD. [Patient] The patient was a 66-year-old man. Since the beginning of 2005, he began sleep-talking and moving his upper and lower limbs while sleeping. In October 2005, he exhibited these symptoms almost every night. An all-night polysomnography was performed in June 2006, in which he demonstrated jerky limb movements related to REM sleep without atonia and periodic leg movements while he was asleep. Based on ICSD-2, he was diagnosed with RBD. Although the symptoms were infrequent, he also fulfilled the NIH criteria for restless legs syndrome. He had normal intelligence and showed no signs of dementia. He had no history of heart disease, and he had not taken any medications that affect the heart. [Methods] 123I-MIBG myocardial scintigraphy, an olfactory test with T&T olfactometer (Masaoka et al. in press), and a facial expression recognition test (Kan et al. 2002) were performed. [Results] MIBG uptake was abnormally low in this patient. The patient also exhibited impairments in olfactory perception and recognition of facial expressions. [Discussion] Early symptoms of PD were observed in a patient with RBD. Assessment of 123I-MIBG uptake, olfactory perception, and facial expression recognition in RBD patients may contribute to early prediction in the diagnosis of PD.

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Effects of Nicotine and Selective Nicotinic Agonists Against 6-Ohda and MPTP-Induced Neurotoxicity in Rats and Mice

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Epidemiological evidence shows a reduced incidence of PD amongst smokers, suggesting that some component of tobacco smoke may impart neuroprotective effects. Experimental data obtained so far points towards nicotine, the principal pharmacologically-active alkaloid in tobacco smoke, being the likely candidate. It has also been reported that nicotine is neuroprotective in vitro and that the effects are mediated via alpha4beta2 and alpha7 nicotinic receptors. However, data to date in rodent models of PD is conflicting.

In the current studies we evaluated the behavioural, histological and neurochemical effects of nicotine, alpha4beta2 and alpha7 selective nAChR agonists in a rat 6-OHDA median forebrain bundle (MFB) lesion model and in a mouse MPTP model.

We found a modest protective action of nicotine on striatal TH-immunoreactivity in the 6-OHDA model. The effect of nicotine does not appear to be mediated by alpha4beta2 or alpha7 receptors alone since subtype selective compounds failed to show any efficacy. In addition, this histological effect of nicotine was not translated into an improvement in basal extracellular dopamine levels, or in the response to potassium or d-amphetamine in the caudate putamen. Nicotine and subtype selective compounds failed to provide any histological improvement in the mouse MPTP model. Overall the data suggest that nicotine may have a modest effect on some end-points and in some models. However, the data further highlight the need to look in more than one model and the need to optimise the dose, dosing protocol, route of administration and end-points when assessing putative neuroprotective agents.

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Antiparkinson's Drugs and Motor Fluctuations in a Movement Disorder Clinic

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Background & Aims: here has been wide ranging studies of effectiveness and use of various anti-Parkinsons drugs. Motor complications of Levodopa use are widely reported. We looked at our usage of these drugs in a Movement disorder clinic serving a population of 100000 in South Wales, UK and incidence of motor fluctuations as noted in Case records over a period of 3 years.

Methods: andom case notes of 103 patients with confirmed Parkinson's disease (PD), were screened through our electronic database (Welsh movement disorder e-network) and analysed retrospectively. Demography, severity of PD (Hoen & Yahr), drug prescribed were recorded. Any Motor fluctuations were noted over a period of 3 years through Clinic records.

Results: 61 males and 42 females, mean age males 71 yrs & females 75 yrs. Mean duration of PD at the time of analysis was 5 yrs. (males) and 3.9 yrs (females) with 41% and 31% > H&Y 2.0 respectively. 78 Patients were taking Levodopa with mean dose of Levodopa 300mg / day, 40 patients were Dopamine agonists, 30 on Levodopa + Entacapone combinations. 39 (38%) patients were on one agent. 40% were on > 1 agent, while 20% were on >2 agents. Only 15 (15.5%) patients with mean dose of Levodopa 450mg/day had major motor fluctuations after mean of 9 years.

Conclusion: In this study prevalence of motor fluctuations were low (than quoted in most literature), possibly due to keeping the dose of total Levodopa down and combinations of therapeutic agents (60%).

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Effect of Melatonin, Amk and Some Synthetic Analogs in a Model of MPTP - Induced Parkinson's Disease

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Objective: we evaluated the neuroprotective potential in vivo of melatonin, its metabolite (amk) and some structurally related synthetic compounds on substantia nigra (sn) and striatum (st) in the mptp model of pd.

Methods: mice were divided into the following groups (93 animals per group): control; vehicle; mptp; mptp + compound. Seven doses of mptp (15 mg/kg b.w.) Were injected as follows: four doses every two hours; the three other doses has been inoculated at the same time that before day. Moreover, two doses of compound(20 mg/kg b.w.) Were too injected one hour before the administration of mptp. Thirty-one hours after treatment, animals were sacrificed for biochemical analysis.

Results: we founded that both inos and nnos may be implicated in the development of pd and that no• produced by these enzymes can be responsible for the oxidative damage founded. Mptp also induced an increase in mtnos (specifically i-mtnos) and mitochondrial no• production, also responsible for the mitochondrial damage. In addition, we have shown that exist an abnormal function in the etc (c-i), as well as an oxidative insult to lipids. We saw that compounds are capable to counteract these effects, being it more important in mitochondria, specifically in the sn.

Conclusions: mptp induces the mitochondrial increase in mtnos activity and no• production, leading to oxidative stress and mitochondrial dysfunction. Melatonin and amk behave as endogenous neuroprotective agents susceptible to be used as pharmacological tools. Is fundamental found selective constitutive and inducible nos ihibitors, but is more important synthesize mitochondria selective inhibitors.

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Neuroprotection and Reduction of Neuroinflammation Provided by Human Interleukin-10 Gene Transfer in Rats With Unilateral 6-Hydroxydopamine Induced Nigro-Striatal Degeneration

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Neuroinflammation occurs in the substantia nigra (SNc) of PD patients concurrently with dopaminergic neurodegeneration and depigmentation. It is unknown whether neuroinflammation is an active process that contributes to dopaminergic neuron death or a response to ongoing cell death. In neurotoxin-induced experimental models of PD, microglial activation precedes neurodegeneration in the SNc. These models show that innate immunity, involving glial inflammatory factors such as pro-inflammatory cytokines as well as reactive oxygen or nitrogen species, lead to the demise of dopaminergic neurons. In PD, adaptive immunity may also play a role in disease progression. We have investigated the neuroprotective effect of an adeno-associated viral (AAV2) vector platform containing the gene for human interleukin-10 (hIL-10) in the unilateral 6-hydroxydopamine (6-OHDA) rat model of PD. Infusion of AAV2-hIL-10, but not a control vector, reduced the 6-OHDA-induced loss of tyrosine hydroxylase (TH) positive neurons of the SNc. Since sparing of SNc dopaminergic cell bodies does not always correlate with protection of their corresponding striatal nerve fibers, we demonstrated attenuation of the loss of dopamine and its metabolites (DOPAC, HVA) in the striatum. Furthermore, pretreatment with AAV2-hIL-10 reduced microglial activation in the SNc, with fewer "amoeboid" microglia observed. Assessment of rotational behavior in response to apomorphine challenge showed absence of asymmetry, confirming increased dopaminergic innervation of the lesioned striatum. At baseline, 6-OHDA lesioned animals displayed a deficit in contralateral forelimb use and pretreatment with AAV2-hIL-10 reduced forelimb akinesia. These results suggest a role for neuroinflammation in the etiology of PD and other neurodegenerative disorders.

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Correction of Superior Brain Functions at Vascular Encephalopathy With Slight Cognitive Disorders

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Research objective: The estimation of Akatinol effectiveness in treatment of patients with vascular encephalopathy followed slight cognitive disorders.

Patients and Methods: The research included 10 patients aged between 48 and 82 years with vascular encephalopathy, suffering from cerebrovascular disorders attending by affection of carotid system. All patients had slight cognitive disorders. Superior brain function disorders were revealed by enhanced neuropsychological test based on classic A.R. Luria??s method and Global Deterioration Rating.

The patients were tested by CES-D scale, estimating the depression status. Thus, the presence of the depressive disorders and the nootropic drugs intake were excluded. All selected patients were treated with Akatinol (10 mg per day).

The test survey was performed after 30 days, and the final ?? after 60 days from the treatment beginning.

Results: The treatment was completed without any complications and side effects. All the patients noticed the significant subjective amelioration, total activity and efficiency increase. The final neuropsychological test results fixed: attention increase on 20-50%, memorization increase on 20-30%, elocution velocity and visual gnosis improvement on 20-40%.

Conclusion: Preliminary research of Akatinol influence on superior brain functions at cerebrovascular disorders followed slight cognitive disorders confirms the efficiency of Akatinol in correction of attention and memory disorders, elocution velocity and visual gnosis pathology.

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Low Drug Drug Interaction Potential of Rotigotine

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Rotigotine (Neupro®), a non-ergolinic D3/D2/D1 agonist, is being developed as a once-daily transdermal delivery system for the therapy of Parkinson´s Disease. Potential pharmacokinetic drug drug interactions (DDI) have to be considered for this chronic therapy since the patient population consists of older patients. Rotigotine metabolism was studied in human liver microsomes and hepatocytes. Several cytochrome P450 (CYP) isoforms are involved in N-dealkylation in vitro, indicating that there is no rate-limiting enzyme. This was confirmed by a clinical trial in healthy male subjects: No change in steady-state pharmacokinetics of rotigotine (4mg/24h) was observed when the non-specific CYP inhibitor cimetidine (400mg, bid) was coadministered. The inhibitory and induction potential of rotigotine was studied in heterogeneously expressed human CYP isoforms and human hepatocytes. Based on the calculated inhibitory concentration (I) and Ki values, there is no risk for DDI with substrates of CYP1A2, CYP2C9, CYP2C19, CYP3A4 and a low risk for CYP2D6 considering maximal plasma levels at the highest therapeutic dose (8mg/24h). Induction assays with rotigotine in vitro did not show any significant change in enzymatic activity of CYP1A2, CYP2B6, CYP2C9, CYP2C19 and CYP3A4. These data were confirmed for CYP1A1, CYP1A2, CYP2C19 and CYP3A4 in vivo in monkeys (Western Blotting).

In vitro assays in Caco-2 cells showed that rotigotine is not a substrate of the drug efflux transporter P-glycoprotein and does not modulate digoxin transport.

These data indicate a low drug drug interaction potential of the dopamine agonist rotigotine related to cytochrome P450 dependent drug metabolism and P-glycoprotein-dependent drug transport.

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Rasagiline Provides Significant Benefits as Adjunct Therapy in Patients With Moderate Parkinson's Disease: Subgroup Analyses

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Background and aims: Rasagiline has demonstrated efficacy as adjunct to levodopa, significantly reducing OFF time and improving UPDRS-Motor scores during ON time. These characteristics, along with a favourable safety profile and ease of dosing, prompted our analysis of rasagiline in moderate patient subgroups.

Methods: Data were pooled from patients with motor fluctuations receiving rasagiline 1.0 mg/day as adjunct to levodopa in the randomised, placebo-controlled PRESTO (n=472) and LARGO (n=687) studies. Other concomitant anti-PD medications were permitted. For this analysis, moderate PD patients were defined by two criteria: patients receiving only levodopa at baseline (i.e., no concomitant treatment with COMT inhibitors/dopamine agonists, n=253), and patients with relatively 'mild fluctuations' (baseline daily OFF time less than or equal to 4hr, irrespective of concomitant medication, n=217). These subgroups accounted for ~30% of patients; there was an overlap of ~40% (n=87) between the two subgroups. The primary endpoint was total daily OFF time, with secondary endpoints including CGI-examiner, UPDRS-Motor ON, and UPDRS-ADL OFF scores.

Results: In the 'levodopa only' subgroup, addition of rasagiline significantly reduced OFF time (treatment effect: -0.78hr; $p<0.01$), and improved CGI-examiner ($p<0.001$), UPDRS-Motor ON ($p<0.001$), and UPDRS-ADL OFF ($p<0.01$) scores, versus placebo. Results from the 'mild fluctuator' subgroup were similar, with rasagiline significantly reducing OFF time (treatment effect: -0.98hr; $p<0.001$), and improving CGI-examiner score versus placebo ($p<0.001$).

Conclusions: Thus, rasagiline provides efficacy benefits, both as adjunct to levodopa, and for patients with mild fluctuations.

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Modulation of Intracellular Aggregates Through Filamentous Phages in a Cellular Model of PD

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In recent years it has been recognized that bacteriophages have several potential applications in the field of biotechnology. They have been proposed as delivery vehicles for protein and DNA vaccines, as gene therapy delivery vehicles, as alternatives to antibiotics and as tools for screening libraries of proteins, peptides or antibodies.

Previous study conducted in our lab had demonstrated that the filamentous phage fd, display anti aggregating properties including prevention and disaggregation of already formed A-beta aggregates. Based upon these findings, we propose a novel approach utilizing filamentous phages as anti aggregating agents of intracellular aggregates. This phage dependent system combines the effect of a RGD cyclic peptide and the intrinsic disaggregation properties of fd filamentous phage.

An internalization peptide with a cyclic presentation of a RGD motif was cloned into pVIII coat protein of the phage. This sequence was shown to mediate cell internalization through integrin binding.

We employed dopaminergic SH-SY5Y neurons overexpressing alpha-synuclein as a model of PD and analyzed the internalization of engineered RGD phages. Here we demonstrate that SH-SY5Y cells treated with the RGD phages can internalize these phages as detected in cells extract by ELISA. MTT viability assay showed no toxic effect on SH-SY5Y following phages treatment.

Due to the intrinsic anti aggregating properties of these filamentous phages, we aim to investigate Lewy body modulation in cells following treatment with RGD presenting phages.

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Long-Term Evaluation of Antitremor Effects of Pramipexole by Actiwatch-Neurologica

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Objective: The purpose of this study was to assess quantitatively whether pramipexole (PRX), nonergot DA agonist, affects tremor features in Parkinson's disease (PD).

Methods: The long-term quantitative measurement of tremor using Actiwatch-Neurologica was performed in 3 patients with PD and the effects of pramipexole on tremor score, intensity and duration as well as motor activity were compared before and after drug intake for more than 3 weeks.

Results: Under the smallest doses of pramipexole, 0.375 mg/day, the tremor score was remarkably improved by 60 %, before the sum of motor activity began to increase (Fig 1). Fig. 2 shows drug-response diurnal variations and would be useful in design and optimization of prampexole dosage.

Conclusions: Pramipexole revealed a more potent anti-tremor action while being effective against akinesia. Actiwatch-Neurologica provides a long-term quantitative measure of tremor that is intuitive and reflects the circadian variations of tremor. It should be useful in evaluating tremor fluctuation across patients in adjusting the dosage and measuring the efficacy of therapeutic interventions.

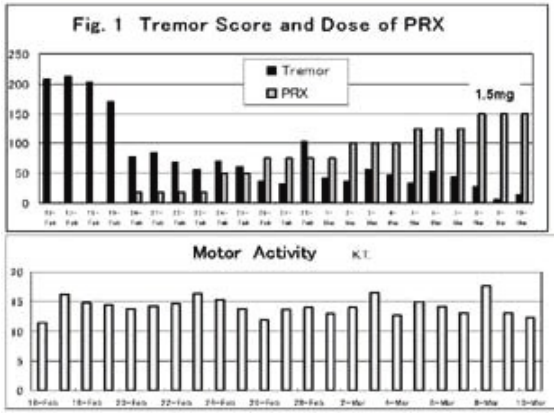
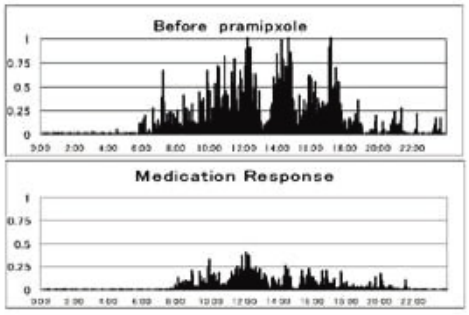


Fig. 2 24-Hour Tremor Profile



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Efficacy of Risperidon in the Psychotic Symptoms of Dementia

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Background and aims : About one third of patients with dementia of the Alzheimer type (AD) have psychotic symptoms such as hallucinations and delusions as well as agitation and bizarre behaviors which are especially disturbing for the families of affected patients. To assess the efficacy of risperidon for these symptoms, a survey was done.

Methods : Fifteen patients (7 male & 8 female) with dementia of the Alzheimer type (according to DSM-IV) who had psychotic symptoms, received risperidon (0.5 – 2 mg./day) for 4 weeks. The patients followed by the scale for assessment of positive symptoms (SAPS) and structured psychiatric interview.

Results : The mean age of patients was 69 ± 8.1 year and the most common hallucinations and delusions were auditory and persecutory, respectively.

Thirteen out of 15 patients showed significant improvement in their psychotic symptoms after the trial. No disturbing drug adverse effect was observed in this group.

Conclusions : Risperidon , a serotonin-dopamine antagonist , has minimal extrapyramidal side effects in low dosage and seems to be an effective approach to the AD patients with psychosis

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Effect of Subthalamic Deep Brain Stimulation on Pain in Parkinson Disease

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Background: It is well recognized that pain is a feature of Parkinson disease (PD). Pain in PD may fluctuate as 'non-motor fluctuations' like motor fluctuations. Subthalamic deep brain stimulation (STN DBS) is an established treatment for motor fluctuations in PD. However, the effect of STN DBS on pain in PD is not well known.

Methods: PD patients who were considered for STN DBS were asked if they had pain. The severity of pain was scored in each body part. In patients with motor fluctuation, pain in 'on' and 'off' state were recorded separately. Patients were evaluated preoperatively and 3 months after surgery. Some patients were followed for 6 months.

Results: Twenty-three of 29 patients had pain preoperatively. Of 24 with motor fluctuation, 21 had pain, and 18 had fluctuating pain. Pain improved in 20 out of 23 with preoperative pain at 3 months postoperatively. Of 18 with fluctuating pain, 12 reported decrease in, and 5 complete disappearance of 'off' pain. Of 4 with nonfluctuating preoperative pain, 2 reported improvement. Pain was severe in some. STN DBS improved pain to a tolerable degree. In 7 of 29, new pain developed during 3 months follow-up. Sixteen

patients were followed for 6 months. All 11 patients who had improvement at 3 months continued to get benefit from STN DBS. Two additional patients who had no improvement at 3 months reported improvement at 6 months.

Conclusions: Pain is frequent in PD and STN DBS improves pain, especially 'off' pain in PD.

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New Concept of Essential Tremor Treatment -Two Case Report

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Tremor usually begins in early adults life and has a tendensy to become manifest during volontial movdement and to disappear when the limb is in repose.

Essential tremor is a 4 to 12 Hz postural and kinetic tremor that is among the most common adult neurologic conditions.

The basic patophysiology of essentiale tremor in not well anderstend.

Because of that essential tremor is difficult to treat condition that may be related to dysfunction in gamma-aminobutyric acid (GABA) neurotransmission.

Standard therapeutic approches incloude primidone as welo as beta-adrenergic blockers such as propranolol,but these drugs are often found to be ineffective or intorerable or are contrindicated because of comorbic disorders.

The multiple action if topiramat including activity on GABA receptors may contribute to its potential efficacy.

I describe two patients (one man and one woman) with typical unilateral hand tremor, began 3-5 years ago and with no effect after thypical treatment.

Topiramat was initiated with gradual titration to 200mg daily with good therapeutic effect and with good toleration.

Conclusion: This report of two patients with essential tremor suggest that maybe topiramat may be useful in the treatment of essential tremor, when the traditional treatment is not effective.

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Sleep Architecture During Sustained Administration of Rotigotine in Normal Rats

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Background and Aim: Rotigotine (Neupro ®) is a non-ergolinic D3, D2, D1 dopamine agonist approved in EU for the treatment of early Parkinson's Disease using a transdermal administration system. Experimentally, an oily crystal suspension of rotigotine was formulated which provides a continuous administration for more than 48 h thus mimicking the pharmacokinetics of a patch. In order to evaluate whether the continuous receptor stimulation affected the sleep and diurnal rhythms, the present study was conducted.

Methods: Rats were chronically instrumented with EEG and EMG electrodes. EEG and motor activity pattern were recorded continuously over six days. REM sleep periods could be clearly separated from periods of slow-wave-sleep (SWS) and awake states.

Results: Rotigotine (applied s.c. at a dose of 0.5 or 5 mg/kg every second day for 6 days) did not affect the sleep architecture even at the high dose (5 mg/kg): rotigotine did not affect the time to onset of REM or of slow wave sleep; it did not affect the duration, the number or distribution of REM sleep episodes, of slow wave sleep nor of wakefulness. For comparison, nomifensine given daily (16 mg/kg p.o.) markedly influenced the sleep pattern.

Conclusion: The continuous administration of rotigotine, although providing sustained dopamine receptor stimulation, does not disturb the sleep architecture and diurnal activity pattern in rats.

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Effects of Dopamine Agonists Pramipexole on Dyskinesias in Parkinson's Disease

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Introduction: The effects of dopaminergic treatment on different types of dyskinesias in Parkinsons disease (PD) are various. Dyskinesias are the most troublesome motor complications of dopaminergic therapy in PD.

Objective: To evaluate the effect of the dopamine agonist pramipexole therapy on the dyskinesias in PD.

Methods: The effect of pramipexole therapy was assessed in 215 PD patients during at least the one year therapy. 86 patients with early PD had a pramipexole monotherapy and 129 patients with advanced PD pramipexole was being added to the levodopa. Clinical assessments were made before and monthly during the period of therapy.

Results: Treatment was associated with a statistically significant improvement of night, early morning and day «off» period dystonia. Therapy was associated with appearance of «on» phases dyskinesias in 3 patients with early PD and in 19 patients with advanced PD who never had it before. Dyskinesias consist of choreic movements and more rare types of dyskinesias like myoclonus (3) and dystonia-camptocormia (4). Dyskinesias slightly increased in the 28 patients who had a hyperkinesis before the pramipexole treatment. Most dyskinesias were resolved after the pramipexole or levodopa doses decreasing. Camptocormia was discontinued 1-4 months later the pramipexole withdrawal. Predictors of the appearance of dyskinesias «on» phases were higher severity of PD and dose of levodopa, female sex.

Conclusion: Pramipexole therapy is very effective in respect of dystonia «off» period. Patients with advanced PD therapy needs more attention due to the possible appearance of some rare types of dyskinesias.

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Availability of Vesicle Fistula for Neurogenic Bladder for Parkinson Disease and Multiple System Atrophy

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Parkinson disease (PD) and multiple system atrophy (MSA) usually exhibit urinary disturbance due to neurogenic bladder. These patients need management of urination. We experienced four cases of cystostomy for neurogenic bladder in PD and MSA, and discussed availability of vesicle fistula.

Subjects were 66- to 74-year-old bedridden male, consisted of one patients with PD (disease duration of 22 years) and three with MSA (disease duration of 5 to 9 years). All patients used urethral catheter intermittently or continuously because of neurogenic bladder.

Cystostomy was performed percutaneously in supine position under local anesthesia. After cystostomy, frequency of urinary tract infection, insertion pain of catheter, and caregiver burdens were reduced.

Because keeping vesicle fistula clean and in easy, urinary tract infection less frequency occurred than using urethral catheter. Since a change of vesicle fistula catheter is easy and safe without pain or bleeding, burdens of caregivers are decreased. Cystostomy is also safe and easy. Therefore, vesicle fistula is very useful for neurogenic bladder of PD and MSA patients.

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Two Cases of Parkinson Disease With Severe Hallucination and Paranoia Who Needed a Treatment in the Psychatry Ward

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We report 2 cases of Parkinson disease with severe hallucination and paranoia, who needed a treatment in the psychiatry ward.

A 72-year-old woman suffered from tremor of her hands and gait disturbance for 3 years. Two hundred mg a day of levodopa was effective for gait disturbance. For tremor, she was administered 4mg of trihexyphenidyl-hydrochloride. It was effective for tremor, however 4 days later, hallucination and paranoia occurred. Although it was stopped 9 days later, hallucination and paranoia were not improved. After her husband was hospitalized, those symptoms worsened. She needed a treatment in the psychiatry ward by psychiatrist.

A 64-year-old woman suffered from resting tremor of her hands for 5 years. Because of wearing-off, she was administered 500mg of levodopa, divided into five times a day. After the death of her first son, hallucination and paranoia occurred. She was hospitalized to the general ward. Though she began to be administered of quetiapine fumerate, the hallucination and paranoia was not diminished. She needed a treatment in the psychiatry ward by psychiatrist.

Once hallucination and paranoia occur, medication is very difficult and psychosis is not improved immediately. Before those symptoms occur, we should be careful of psychosis. It is necessary to cooperate with psychiatrist immediately after accompanied with psychosis.

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Rapid Management Strategy for Acutely Ill Patients With Akinetic-Rigid Syndromes and Dementia

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Background: Exacerbation of bradykinesia and/or cognitive decline frequently complicates parkinsonian syndromes with dementia during acute toxic-metabolic challenges. We describe a pharmacological regimen that can be rapidly titrated and appears useful for acutely ill patients suffering akinetic-rigid syndromes with dementia.

Methods: An 88 year old male with a 12 year history of carbidopa/levodopa (C/L) responsive parkinsonism and a 5 year history of cognitive decline with rare hallucinations was admitted to the University Medical Center. He had a week history of dysphagia, fever, cough and progressive unresponsiveness interspersed with combativeness. Intravenous levofloxacin was administered with improvement in his aspiration pneumonia. Nasogastric tube was placed, continuous enteral nutrition was begun along with previous doses of C/L without improvement in his akinetic-rigid and unresponsive state. Hospice placement and withdrawal of supportive measures was considered.

Neurology recommended the following therapeutic interventions: Enteral nutrition stopped around each dose of C/L; Liquid rivastigmine mixed with the enteral nutrition given as boluses every 8 hours and titrated over 72 hours from 2mg per day to 8mg per day.

Results: Within 72 hours after the initiation of the above regimen, the patient was oriented to person, place and situation, speech was fluent and spontaneous with marked improvement in his akinetic-rigid state. Similar cases were subsequently managed, initiating and titrating rivastigmine up to a maximum of 12mg per day over a 72 hour period.

Conclusions: Rapid restoration of dopaminergic and cholinergic tone in patients with Parkinson's and dementia may positively impact outcome and reduce hospital stay.

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Effect of Pulsed Electro Magnetic Fields in Patients With Idiopathic Parkinson´S Disease

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The Pulsed Electro Magnetic Fields (PEMF) treatment is a new non-invasive technique for direct cerebral cellular stimulation. The treatment is comparable with the Transcranial Magnetic Stimulation (TMS), but TMS uses strong magnetic fields while PEMF is using quite weak magnetic fields.

The head applicator, anchored in a helmet, inducing the therapeutic pulsed electromagnetic fields consists in seven coils placed in the bilateral temporal and parietal cerebral regions, and in the occipital region.

We investigated the effect of PEMF treatment in 8 patients with the diagnosis idiopathic Parkinson, to represent a reduction of the symptoms of Parkinson´s disease (PD). Each treatment session was 30 min / day and were given 7 times pr. week in 5 weeks at all. The pulse generator works with a frequency of 50 Hz and single pulse duration of 3 msec. Each pulse generates by a potential change from +50 to –50 V.

During the 5 weeks of PEMF treatment the patients were examinated with the Unified Parkinson´s Disease Rating Scale (UPDRS), the Major Depression Inventory (MDI) and the Parkinson´s Disease Questionnaire (PDQ 8).

This treatment results in an indicative decrease in UPDRS p=0,12, a significant increase in PDQ 8 amounted p=0,0087 and a significant decrease in MDI p=0,011.

The PEMF could have a therapeutic role for PD patients. Further trials are necessary to reveal the therapeutic effect of PEMF in PD. Further studies should be performed aiming to reveal the optimal stimulation parameters.

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Idiopathic Pleurothotonus in a Patient With Parkinson's Disease

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One of essential criteria to diagnosis as “Pisa syndrome” is a history of medication (mainly with psychotropic drugs) preceding or concurrent with the onset of dystonia. So “drug induced” pleurothotonus is a synonym for “Pisa syndrome.” A 73 year-old-man with right side tremor and rigidity came to our clinic on January 2005, and we started treatment with L-dopa and ropinerole for relieving his parkinosonian symptoms. His symptoms were improved after treatment. On November 2006, he developed a marked tonic deviation on the right side of head, neck, and trunk. His dystonic postures remained unchanged even after dopaminergic medications were stopped for 2 weeks. He never took any other psychotic medication or antiemetic drugs and no psychiatric disturbances such as hysteria or catatonia. With regard to terminology in this patient, we confused the term “Pisa syndrome” and “pleurothotonus,” and “Pisa syndrome” designates a more specific entity. According to definition as existence a history of psychotropic medication for “Pisa syndrome.” we prefer to diagnosis this patient as an “idiopathic pleurothotonus” rather than “Pisa syndrome.”

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Antidepressive Treatment of Major Depression in Parkinson’s Disease

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Introduction: Major depression is a common sign in Parkinson’s disease (PD), but mostly underdiagnosed and undertreated. Although patients suffer a lot there are little studies on antidepressive treatment in PD.

Methods and Patients: Venlafaxine, a serotonine and noradrenaline reuptake-inhibitor, was assessed in 22 in-patients at the Hospital BHB Eggenberg. All patients suffered from idiopathic PD and major depression (mean age 70.11 a). Other antidepressants were withdrawn at least 7 days before beginning of the study, if required patients were prescribed a benzodiazepine for insomnia. 11 patients received the extended release preparation of venlafaxine, 11 received the non-retard preparation. Patients were assessed at week 0 and after 3 weeks of treatment with the Beck’s Depressions Inventar (BDI) assessing major depression.

Results: Three patients in the non-retard group were withdrawn from the study because of nausea and emesis. Patients receiving the extended release preparation tolerated a higher dose of venlafaxine than the non-retard group (156.4 mg [SD 76] vs. 84.4 mg [SD 30]). The mean BDI at week 0 for both groups was 31.0 (SD 7.92), at week three the mean BDI was 12.42 points (SD 6.78). In the non-retard group one patient met the criteria for remission (minimal symptomatic depression or no depression) and in the extended release group 6 patients.

Conclusion: Venlafaxine is an effective antidepressant in major depression in PD, especially the extended release preparation shows good tolerability. To objectify results a larger, double-blind, placebo-controlled study is needed.

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Organic Depressive Disorder in Huntington’s Disease: Effective Anti Depressive Treatment

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Introduction: A prominent sign in Huntington’s disease (HD) is major depression, frequently seen in a prodromal stage.In advanced stages of HD nearly every patient suffers from depression, nevertheless only 16 studies on depression in HD, assessing a total of 40 patients, have been published until now (Bonelli and Wenning 2006).

Methods: 25 in-patients of the Department of Psychiatry of the Graz Medical University suffering from HD and organic depression (9 women, 16 men) were treated with a mean daily dose of 147 mg of venlafaxine. A Hamilton Rating Scale for Depression (HAM-D), a Hamilton Anxiety Scale (HAM-A) and a Beck-Depressions-Inventar (BDI) were done at baseline and after 4 weeks (±1 week). 3 HD-patients were asymptomatic (Shoulson's clinical stages 1), 9 in stage 3, 6 in stage 4, and 7 HD-patients were in stage 5 (Shoulson 1979).

Results: At week 0 all tests showed significant worse results than at week 4±1: HAM-D 18.04(SD6.64) vs. 8.96(SD4.40); HAM-A 18.60(SD9.95) vs. 7.04(SD5.15); BDI 15.12(SD5.50) vs. 7.56(SD3.28). Clinical signs and symptoms of depression decreased remarkable. Venlafaxine was as effective in those 52% of HD-patients in stage 4 and 5 who were already severely handicapped as in the group of asymptomatic patients.

Conclusion: Venlafaxine is an effective treatment in HD and is well tolerated in those patients. Still suicide and depression are a common cause of death in HD, therefore right treatment for these patients and more studies on antidepressive treatment in HD are urgently needed.

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Sertindole Therapy in a Case of Huntington´S Disease

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HD is a neurodegenerative disorder which is autosomal dominantly inherted. HD clinically shows a trias of symptoms. Hyperkinesia, psychiatric symptoms like depression and psychosis, and ultimately dementia. It is wellknown and documented that neuroleptic drugs reduce hyperkinetic movement in HD. We describe the case of a 36 year old woman with genetically proven HD. When she was referred to our department she showed severe hyperkinetic movements which were evaluated with the UHDRS. Chorea scored to 25 points which is severe. Sertindole 16 mg/d was given for a one week periode. The effects of the treatment were evaluated by another UHDRS after five days. The score for chorea improved significantly to 3 points but only due to highly increasing rigidity (parkinsonism from 6 to 9 points) which worsened life-quality for our patient. She was no longer able to get out of bed without assistance and activities of daily life such as cutting food by herself were no longer possible. Choreatic hyperkinesias are commonly treated with atypical neuroleptics. There are some reports on olanzapine, quetiapine, clozapine and zotepine, but, to our knowledge, sertindole has not been described before. As an atypical neuroleptic drug sertindole has good antipsychotic effects and very few EPS-side-effects, but, as we could see in our case, it can induce rigidity. This is only an anecdotal case report and further study is needed. Nevertheless the authors suggest that sertindole should not be the treatment of choice for choreatic movement in HD.

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Apoe Deficiency Induces Age-Dependent Brain Hypoperfusion and Neuronal-Glial and Microvascular Damage in Mice

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The E4 allele of apolipoprotein E (ApoE) is involved in cardiovascular and cerebrovascular disorders and is a major predisposing risk factor for late-onset Alzheimer's disease (AD). We investigated the role of age-dependent ApoE deficiency (ApoED) on cerebral blood flow (CBF) as an initiator of brain hypoperfusion using ApoE knockout mice compared to age-matched wild-type (WT) mice. ApoED and WT mice were divided into three age groups: 6-weeks, 6-months, and 12-months old. CBF was measured using [14C] iodoantipyrene autoradiography. Transmission electron microscopy (TEM) was used to examine neuronal, glial, and microvascular changes. Compared to WT mice, CBF (mean ± SD, ml/g/min) was lowered significantly ($p < 0.05$) in ApoED in 6-month (0.68 ± 0.21 vs. 0.98 ± 0.23) and 12-month (1.06 ± 0.09 vs. 1.54 ± 0.12) groups. ApoED 6-week mice had lower CBF (0.63 ± 0.15) compared to WT mice (0.82 ± 0.15) but the difference was not significant. Structural damage in young and aged endothelial microvessels of ApoED extended to the matrix of perivascular cells, perivascular nerve terminals and to neurons and glial cells. Our findings indicate for the first time that ApoED reduces CBF gradually to create brain hypoperfusion when compared to WT and that differences in CBF reduction are greatest as animals age from 6-weeks to 12-months. Chronic ApoE deficiency may explain age-dependent pathology seen in AD.

Supported by NIH and Alzheimer's Association (IIRG) .

Neuro–degenerative Diseases

Neurodegenerative Dis 2007; 4(suppl 1):1-350

Saturday, March 17, 2007

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Translational Research and the Development of Targeted Drugs for Alzheimer's Disease

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Several approaches have been taken to model the features of Alzheimer's Disease in mice. Good models now exist that develop plaques and tangles, and some show degenerative features of the disease. These models are now being used to test a wide range of possible therapeutic approaches, and several possible treatments have gone into clinical trials. An overview of the models, and the translational research showing greatest promise will be presented.

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Alzheimer's Disease: Models, Mechanisms and Experimental Therapeutics

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Alzheimer's Disease, associated with genetic causes/risk factors, is characterized by extracellular toxic Aβ42 peptides (oligomers), neuritic plaques, and aggregates of tau in tangles. Transgenic models of the disease and knockout approaches have been used to: clarify the mechanisms of amyloidogenesis, identify potential therapeutic targets, and test novel treatments. I will discuss the opportunities and challenges associated with the manipulation of β- and γ-secretase activities.

Mutant genes encoding the amyloid precursor protein or presenilins (PS1 and 2) influence the levels and/or size of Aβ peptides, generated via APP cleavages by the sequential activities of BACE1 and multi-protein γ-secretase complex (PS, Nct, pen2, Aph-1). Mutant APP/PS1 mice show age-associated increases in levels of Aβ42, neuritic plaques, and deficits in working memory. Wong et al have targeted genes encoding proteins hypothesized to be critical in disease. BACE1-/- mice are viable and do not produce Aβ. APPswe;PS1ΔE9;BACE1-/- mice do not form Aβ deposits or plaques, nor do they show memory deficits. However, BACE1-/- mice show cognitive and emotional alterations, with the former phenotype rescued by overexpression of APP. Knockouts of PS1, Nct, and Aph-1 lower γ-secretase activity and reduce production of Aβ. Nct-/-APPswe;PS1ΔE9 mice have decreased Aβ in the CNS, but, they also develop skin tumors (reflecting the importance of the Notch1 signaling pathway in suppression of neoplasms of the skin). In conclusion, inhibition of pro-amyloidogenic secretases provide outstanding opportunities to reduce Aβ burden in patients.

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Molecular Links by Which Abeta Facilitates the Development of Tau Pathology

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Alzheimer disease is a proteinopathy in which aggregates of two proteins, Aß and tau, accumulate in selective brain regions. Using a transgenic approach, we generated mice, referred to as 3xTg-AD, that develop aggregates of these two proteins in a progressive and age-related fashion. Using both genetic and pharmacological approaches, the 3xTg-AD mice can be used to elucidate the molecular pathways by which Aß and tau influence the onset and progression of each other. Our findings indicate that modulating Aß directly impacts the development of the tau pathology, whereas the converse is not true. Moreover, we note that Aß facilitates the development of tau pathology through multiple pathways. Overall, our findings are consistent with the Aß cascade hypothesis, which stipulates that Aß lies upstream of tau in the neurodegenerative cascade. This would suggest that targeting Aß alone should be sufficient to improve the clinical situation in mice. Using immunotherapy, however, we find that in aged mice, a reduction in both soluble Aß and soluble tau levels is required to improve the cognitive phenotype in mice with both plaques and tangles. This finding suggests that combination therapies, i.e., anti-Aß therapies in combination with approaches that mitigate other high value targets, such as tau pathology, may lead to even greater cognitive improvements than just targeting Aß alone. Combination therapies may be particularly needed for the treatment of individuals with severe AD.

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The Physiologic Origins of Age-Related Abeta Deposition

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Brain Abeta deposition is regarded as the central pathogenic event in Alzheimer's disease (AD). However, brain Abeta deposition is extremely common in non-demented

elderly humans (87% in those over 90 at our center) as well as in a wide range of mammalian species. This high prevalence would suggest that rather than being abnormal, brain Abeta deposition is part of normal aging. Individuals who develop AD-related dementia may just represent one end of the normal distribution of this process. As most investigators would agree that the chances for preventing AD are better than the chances for curing it, we should attempt to determine the causes of normal age-related Abeta deposition, as it seems intuitive that preventing this process would prevent AD. We have modeled age-related physiological changes in the rabbit to determine whether they influence brain Abeta concentration and/or deposition. These changes are all well documented as part of normal human aging. Immunotoxin-induced cortical cholinergic deafferentation resulted in 8-fold increases in cortical Abeta concentrations as well as vascular Abeta deposition. Ovariectomy-induced estrogen deprivation resulted in 1.7-fold increases in cortical Abeta. A high cholesterol diet resulted in a 4.3-fold elevation in cortical Abeta. However, a 40% reduction in cortical perfusion, produced by bilateral carotid artery ligation, did not alter cortical Abeta levels. Reversal of all of the induced cortical Abeta increases has been achieved with specific pharmacologic therapies. These results suggest that a rational strategy for preventing AD would include the use of one or more of these approaches.

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Impact of Abnormal Intracellular Abeta Accumulation in Transgenic and Cell Models

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The central pathological cause of Alzheimer's disease (AD) is hypothesized to be an excess of β-amyloid (Aβ) which accumulates in the extracellular space of the brain in the form of toxic polymeric forms and ultimately in amyloid plaques. These deposits disrupt neural and synaptic function and ultimately lead to neuronal degeneration and dementia. In addition, recent investigations in human brain material, transgenic animal models, and cell systems would indicate that the intracellular accumulation of Aβ peptides could significantly contribute to neuronal dysfunction, at early stages of Alzheimer's disease and Down syndrome. Our investigations in a transgenic rat model with a phenotype of accumulation of intracellular Aβ, prominently in pyramidal neurons of areas CA2/3 of the hippocampus, shows that this abnormal accumulation of Aβ induces changes in subcellular organelles, cell signaling pathways, alterations in the proteome and impaired behavioral performance. To better understand the impact of intracellular Aβ in cell function we recently developed vectors achieving moderate, intermedium and high levels of expression of the peptide. We investigated how these different levels of expression, from nearly "physiological" to abnormal and microscopically visible Aβ affected the activity-dependent stimulation of the CRE-regulated gene expression. The results obtained would favor the hypothesis that low levels of intracellular Aβ would facilitate the CRE-regulated gene expression while the intracellular abnormal accumulation of Aβ would block this fundamental mechanism for eliciting synaptic plasticity.

Acknowledgements: This research was supported by grants from the CIHR (MOP 67170) and the NIH (R01 AG20529-01A2).

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PD, LBD and MSA: Animal Models and Their Use in Therapeutic Strategies

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Alpha-synucleinopathies are the most common neurodegenerative movement disorders affecting about 2% of the general population over the age of 65. These include Parkinson's disease (PD), Lewy bodies dementia (LBD) and multiple system atrophy (MSA) due to the presence of alpha-synuclein (aSYN) positive cytoplasmatic inclusions in either neurons (Lewy bodies, LB) or glial cells (glial cytoplasmatic inclusions, GCIs in MSA). Ideally, animal models should reproduce the clinical manifestation of the disease and the neuropathological phenotype characterised with selective neurodegeneration and aSYN inclusion pathology. There are several ways to develop animal models of a-synucleinopathies. One approach is based on the application of selective neurotoxins which may induce neuronal loss in targeted brain regions. Second approach is based on the reproduction of specific mutations or overexpression of disease-specific proteins in transgenic animal models. Finally, combined models which reproduce the possible genetic predisposition and the role of environmental factors in the pathogenesis of a-synucleinopathies have been developed.

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Parkinsonism in Pathologically Confirmed FtId-U With Progranulin Gene (PGRN) Mutations

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Background: Frontotemporal dementia is clinically and pathologically heterogeneous. The most common pathology is frontotemporal lobar degeneration with ubiquitin-immunoreactive neuronal inclusions (FTLD-U). The most common cause of familial FTLD-U is mutation in the progranulin gene (PGRN) on chromosome 17. While most patients with FTLD-U have a behavioral and language variant of frontotemporal dementia, parkinsonism is also frequent.

Methods: In an autopsy series of 18 cases of FTLD-U with PGRN mutations, some degree of parkinsonism was detected in over 70% of the cases, and in 4 of the cases the presenting syndrome was that of Parkinson's disease, dementia with parkinsonism, parkinsonism-plus syndrome or corticobasal syndrome. Three additional cases were evaluated under the auspices of the PSP Brain Bank and thus considered

to have had, at least at some point in time, consideration of a Parkinsonian disorder.

Results: Comparison of these 7 cases to the rest of the cases revealed more severe Parkinsonism, shorter disease duration (5 vs 8 years) and less severe cortical and hippocampal degeneration and a trend for fewer ubiquitin immunoreactive neuronal lesions in cortex. They did not differ with respect to severity of striatal or nigral degeneration.

Conclusions: The results emphasize the importance of extrapyramidal pathology in PGRN-related FTLD-U. Like mutations in the tau gene, which cause frontotemporal dementia and parkinsonism, PGRN needs to be considered as a genetic basis of parkinsonism.

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Tau and Alpha-Synuclein Interaction in Neurodegenerative Diseases

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Intracellular protein aggregates are present in several neurodegenerative diseases. Microtubule-associated protein tau forms filamentous intracellular deposits in Alzheimer's disease. The identification of genetic mutations in the tau gene as the cause of frontotemporal dementia and parkinsonism linked to chromosome 17, has proved the link between tau and neurodegeneration and diseases with tau pathology are now known as tauopathies.

Alpha-synuclein has been identified as the major component of the Lewy body, the filamentous inclusion characteristic of Parkinson's disease and diseases with alpha-synuclein deposits are now known as alpha-synucleinopathies.

Although tau and alpha-synuclein pathologies were believed to segregate in different diseases, it is now clear that they can be present in the same brain in various disorders. The reason for this co-localization is not clear. Tau and alpha-synuclein are both present in axons where alpha-synuclein is transported. We have identified the interaction of tau with a protein involved in axonal transport and tau dysfunction can affect axonal transport. On the other hand, it is possible that alpha-synuclein aggregates constitute a seed for tau aggregation.

In order to determine whether alpha-synuclein and tau can interfere with each other aggregation, we have produced double transgenic mice expressing human mutant A30P alpha-synuclein and human mutant P301S tau both under the control of the Thy 1 promoter. The single transgenic A30P alpha-synuclein mice present only accumulation of alpha-synuclein and the P301S tau mice only tau deposits. Preliminary results indicate that under certain conditions, tau and alpha-synuclein can interact in the double transgenic mice.

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New Targets for Drug Discovery in Alzheimer's Disease, Parkinson's Disease and Frontotemporal Dementia

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Alzheimer's disease (AD), Parkinson's disease (PD) and frontotemporal dementia are neurodegenerative disorders characterized by CNS accumulations of amyloid that result from the fibrillization of normal proteins produced by neurons and glial cells. The discovery of mutations in genes encoding disease proteins in familial AD, familial PD and familial FTD tauopathies (i.e. frontotemporal dementia with parkinsonism linked to chromosome 17 or FTDP-17) have provided insights into the pathogenesis of hereditary forms of these disorders as well as into mechanisms underlying their sporadic counterparts. While considerable progress has been made in the development of therapies focused on Abeta in AD, new therapeutic targets now are being identified and validated in order to abrogate pathways of alpha-synuclein mediated neurodegeneration in PD and related synucleinopathies as well as pathways of tau mediated neurodegeneration in AD and other tauopathies that manifest as FTD. Efforts to target with drugs and thereby impede or reverse amyloidogenic processes and the toxic effects of brain amyloids formed by Abeta, tau and alpha-synuiclein will hasten efforts to develop more effective strategies for the treatment as well as the prevention of AD, PD and FTD.

Supported by grants from the National Institutes of Health

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Imaging Amyloid and Inflammation in Alzheimer's Disease, Parkinson's Disease Dementia (PDD) and Lewy Body Dementia (LBD)

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At post-mortem varying levels of amyloid pathology have been reported in PD with late dementia (PDD) and subjects who fulfilled concensus criteria for Lewy body dementia (LBD). The beta amyloid plaque load in PDD and LBD has not, to date, been assessed in vivo but this is now possible with 11C-PIB PET, a thioflavin marker which specifically binds to fibrillar amyloid. To date, 80% of our LBD and 15% of our PDD cases have shown a significantly raised cortical amyloid load compared with 90% of clinically probable AD cases. Alzheimer and Lewy body pathology are known to be associated with microglial activation. With 11C-PK11195 PET levels of microglial activation can be assessed and correlated with dementia and locomotor function. We have detected significant and similar levels of cortical microglial activation in AD, DLB and PDD cases irrespective of whether amyloid

pathology is present. To summarise, the majority of LBD cases also have concommitant Alzheimer pathology and PET can identify those cases where an anti-amyloid strategy may be relevant. The dementia in PDD is rarely related to amyloid but anti-inflammatory strategies may be relevant to this disorder.

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Neuropathological Aspects of AD, PD, and Frontotemporal Dementia

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Proteinopathies are a heterogenous group of dementias and movement disorders, characterized by intra/extracellular accumulations of abnormal amyloidogenic filamentous proteins. They include Alzheimer disease (AD) with deposition of ß-amyloid peptides (Aß) and hyperphosphorylated tau protein (3+4 repeat/R/tau), Parkinson disease (PD) with α-synuclein (αSyn) deposits (Lewy bodies and neurites), and frontotemporal dementia (FTD, sporadic and familial) with tau-positive/negative or ubiquitin-containing neuronal and glial inclusions. Overlap of clinical and morphological features in these disorders are related to molecular interactions between Aβ, αSyn and tau which may occur within the same brain in various distribution patterns, αSyn and tau even within the same neuron. In addition to "classical" morphologic phenotypes, e.g. "plaque and tangle AD", brainstem predominant Lewy body disease (PD), Pick disease (with 3R-tau deposits), dementia with Lewy bodies (DLB), sporadic and familial FTDs with and without tau and ubiquitin inclusions, many phenotypes show mixed pathologies, eg. AD with αSyn pathology in brainstem and/or amygdala, PD and DLB with AD lesions, and FTDs with a mixture of various proteinopathies, while others are featured by one principal pathology without other lesions (fibrillary tangle form of senile dementia, pure PD). Human and animal models documenting their co-occurrence and in vitro studies showing mutual promotion of fibrillation of these proteins indicate their synergistic interaction in the pathobiology of AD, PD and FTD. Their overlapping pathocascade illustrated by mixed clinical and morphological phenotypes that, at least partly, are genetically influenced, will be presented and discussed.

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Non-Motor Symptoms in Parkinsonism: Cross-Sectional Results From the Priamo (Parkinson and Non-Motor Symptoms) Study

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Objective: To describe the prevalence rate of non–motor symptoms (NMS) in a sample of Italian patients with parkinsonism, including Parkinson's disease (PD) and atypical Parkinsonism (AP) and to assess the impact of NMS on quality of life (QoL). Here we report data on the cross-sectional phase of the study.

Background: Non-Motor Symptoms (NMS) include various co-morbidities such as psychiatric, urinary and gastro-intestinal disorders. These symptoms are particularly important as they affect the QoL in parkinsonian patients.

Methods: PRIAMO is a 2-y ongoing longitudinal observational study aimed at enrolling patients with a diagnosis of parkinsonism in 58 centers widely distributed throughout Italy. Patients undergo a clinical examination, including a standardized evaluation of NMS and a battery test of self and hetero-evaluation scales: EQ-5D, PDQ-39, SHAPS, HDRS, MMSE, FAB.

Results: 1325 patients were recruited. IPD patients represent the 86.5% of the sample. The prevalence of NMS ranged between 14.6% for instability to 67.6% for psychiatric disturbances in IPD patients. Patients with AP most frequently show psychiatric disturbances (77.4%) and fatigue (77.4%). The mean PDQ-39 total score was statistically lower ($p<0.001$) in PD patients without Vs with NMS: mean (SD) score was 32.6 (20.5) in patients with psychiatric disturbance Vs 20.5 (13.9) in patients without psychiatric disturbance.

Conclusions: PRIAMO is the first large epidemiological Italian survey on parkinsonism using validated instruments to collect reliable data. Data show that the NMS are frequently associated to IPD and AP. Moreover NMS significantly affect QoL.

Study supported by an unrestricted grant by Boehringer Ingelheim, Italy.

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LRRK2 is a Component of Lewy Bodies

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Mutations in LRRK2 encoding leucine-rich repeat kinase 2 are thus far the most frequent genetic cause associated with autosomal dominant and idiopathic Parkinson's disease (PD). To examine whether LRRK2 is directly associated with neuropathology of PD, we analyzed LRRK2 in brains of patients affected by PD, dementia with Lewy bodies and other related disorders using highly specific antibodies to LRRK2. Here, we report that anti-LRRK2 antibodies strongly labelled brainstem and cortical Lewy bodies, the pathological hallmarks of PD and dementia with Lewy bodies, respectively. In addition, anti-LRRK2 also labelled brain vasculature, axons, and neuronal cell bodies. Interestingly, the immunocytochemical profile of LRRK2 varied with different antibodies depending upon specific antigenic sites along the LRRK2 protein. All anti-LRRK2 antibodies tested that were raised against various regions of LRRK2, were found to be immunoreactive to recombinant LRRK2 on Western blots. However, only the antibodies raised against the N-terminal and C-terminal regions of LRRK2, but not the regions containing folded protein domains, were positive in immunolabeling of Lewy bodies, suggesting a differential exposure of specific antigenic sites of LRRK2 on tissue sections. We conclude that LRRK2 is a component of Lewy bodies, and therefore plays an important role in the Lewy body

formation and disease pathogenesis. Information on the cellular localization of LRRK2 under normal and pathological conditions will deepen our understanding of its functions and molecular pathways relevant to the progression of PD and related disorders.

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Leucine-Rich Repeat Kinase 2 Gene (LRRK2): A Multi-Function Kinase and Gtpase Linked to Parkinsons Disease Pathogenesis

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LRRK2 mutations are the most common determinant of PD. We cloned LRRK2 from human substantia nigra (SN), raised polyclonal antibodies and conducted functional studies. LRRK2 is enriched in microsomal, synaptic vesicle-enriched and vesicle-associated fractions and it is heterogeneously localized in the brain including the SN and striatum. EM detects LRRK2 at membranous and vesicular structures, including lysosomes, endosomes, transport vesicles, mitochondria and microtubules. In vitro kinase assays indicate that LRRK2 is a mixed lineage kinase and familial-linked mutations increase its kinase activity leading to neuronal cell death that is attenuated by interference with its kinase and GTPase activity. The association of LRRK2 with a variety of vesicular and membrane structures suggests a prominent role for LRRK2 in the trafficking and recycling of vesicles and membranes. Understanding the molecular mechanisms by which LRRK2 regulates these processes and their role in neurodegeneration holds particular promise for undestanding the pathogenesis of PD.

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Quantification of Alpha-Synuclein in Dopamine Cells, Peripheral Blood, Genotyped Brain and CSF

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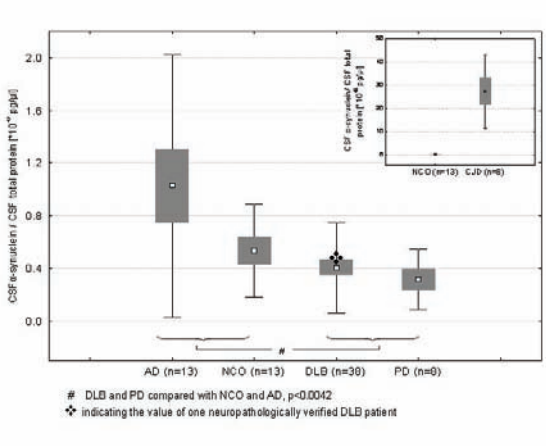
Background/Aim: Elevated alpha-synuclein (AS) expression plays a role in the development of 'synucleinopathies'; these disorders, which include Parkinson disease (PD) and dementia with Lewy bodies (DLB), are often difficult to distinguish from other neurodegenerative conditions.

Method: We report the development and validation of a third-generation ELISA for AS quantification. The assay was used to measure AS in dopaminergic cells, snca- and parkin-genotyped brain, blood products, and in cell-free cerebrospinal fluid (CSF).

Results: In two pilot studies measuring AS concentrations directly in cell-free CSF, we recorded lower CSF AS levels in patients with PD and DLB compared with Alzheimer's and control subjects. Patients with definite Creutzfeldt-Jakob disease showed >21-fold elevated CSF AS (Figure).

Conclusion: AS is physiologically present in extracellular biological fluids and can be specifically quantified by ELISA in the low picogram range.



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Phage Therapy in AD/PD

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Filamentous phages (Ff) are well understood both at structural and genetic levels. Unlike lytic phages Ff phages did not affect their E. coli host cells. They have a filamentous appearance, with a long (900 nm) and narrow (7 nm) Ff virion consisting of a singe-stranded (ss) DNA genome packaged with coating proteins. We recently found that the linear structure of filamentous phages confers permeability to the CNS and disaggregating properties towards amyloid plaques. Modifying phage's linear structure and rendering it spherical abolished its disaggregating as well as penetrating abilities. Here we describe the modulating effect of filamentous phage on aggregation of amyloid-β peptide and/or α-synuclein. Phage ability to disaggregate β-amyloid plaques was demonstrated by intracerebral injection and/or intranasal administration to transgenic mice (Tg) overexpressing the human amyloid precursor protein (hAPP). Intranasal administration of filamentous phages to Parkinson's disease (PD) Tg mice led to considerable reduction of Lewy bodies in the hippocampus and cortex. No toxic effects were observed in the challenged animal sections, which included the brain, liver, kidney, spleen and lung, following phage administration. The therapeutic potential of phage in AD/PD, as well as in other amyloidogenic diseases, stems from their unprecedented ability to access the central nervous system (CNS), without causing any adverse effects in the brain and periphery, to disaggregate extra- and intracellular amyloid deposits unrelated to their primary structures, and for their lack of natural tropism for mammalian cells.

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The Role of Alpha Synuclein in Neurodegenerative Diseases: A Potential Target for New Treatment Strategies?

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Background and aims: AS a main constituent of Lewy bodies, diagnostic hallmarks of PD, moved into the focus of interest after mutations, causing rare forms of familial PD, have been discovered. AS pathology can be detected also in other neurodegenerative diseases like AD, Lewy bodies (LB) dementia or multi system atrophy. Data from patients carrying a triplicate of AS gene and studies with tg-mice overexpressing AS suggested that any increased abundance is leading to formation of abnormal aggregates, but it remains still unclear what species of the protein, monomers, oligomers or mature aggregates are most toxic. Initial data supported a toxic gain of function of synuclein aggregates, finally triggering neurodegeneration. In the meanwhile new results support neuroprotective function. Beside the role in synaptic vehicle recycling, AS modulates anti-apoptotic signalling pathways, provides endogenous anti-oxidative activity and serves as chaperon. Based on these findings it can be speculated that a loss of function due to aggregation might be the cause for neurodegeneration.

Conclusions: The psychological function of AS remains still unclear, and it seems that other members of the synuclein gene family are supporting with redundant activity. Therefore different treatment strategies for alpha synucleinopathies are currently under development, some of them are addressing abnormal protein aggregation, others are focusing on replacement of neuroprotective activities attributed to AS. Results from investigations using small peptides and peptidomimetics derived from the N-terminus of beta synuclein indicate protective activity in hAPP-tg mice is caused by a complex interaction of anti-aggregatory activity combined with anti-apoptotic cell signalling.

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Dentate Gyrus Neurogenesis: A Therapeutic Approach to Alzheimer Disease and Related Disorders

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Background and Aims: A number of studies have shown that exercise and enriched environment increase dentate gyrus neurogenesis and associated learning in rodents.

Methods: Here we describe an approach to enhance pharmacologically both neurogenic and neurotrophic activities in rodents.

Results: Treatment of normal adult rats with Cerebrolysin, a peptidergic neurotrophic drug, increases both the number of newborn neurons and their maturation in the dentate gyrus. These animals show enhancement of both spatial learning and memory. Employing neutralizing antibodies to various neurotrophic factors, we found that Cerebrolysin contains CNTF, GDNF, IGF-1, IGF-2, BDNF and NT4 activities. We have generated an 11-mer peptide corresponding to the active sites of CNTF and found that treatment of normal adult C57BL6 mice with this peptide increases the dentate gyrus neurogenesis.

Conclusions: These studies suggest that neurotrophic peptide-based therapy is a promising approach for the treatment of Alzheimer disease and related disorders of cognitive impairment.

(Supported in part by the New York State Office of Mental Retardation and Developmental Disabilities and a grant from EBEWE Pharma, Austria.)

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The Diagnostic Significance of Amyloid Beta Peptides 42/40 Concentration Ratio

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The cerebrospinal fluid (CSF) concentrations of amyloid beta (Ab) peptides ending at the amino acid position of 42 (Abx-42, and Ab1-42) are widely accepted neurochemical dementia diagnostics (NDD) biomarkers of Alzheimer's disease (AD). However, in subjects with constitutively high- or low CSF concentrations of total Ab peptides (tAb), the NDD interpretation might lead to erroneous conclusions as these biomarkers seem to correlate better with the total Ab load than with the pathological status of a given patient in such cases. Non-demented individuals with relatively low tAb might be burdened with 'pathologically' low Abx-42 concentrations, and vice versa, AD subjects with high tAb might present Abx-42 in a normal range. To test this working hypothesis, in the current multicenter study we have measured corresponding biomarkers in carefully selected patients characterizing with high- or low CSF tAb, and correlated them to the CSF concentration of total Tau (tTau), and phosphorylated Tau (pTau181) as an independent, objective biomarkers. We concluded that the amyloid beta concentration ratio (Abx-42/x-40) should replace the 'raw' concentrations of corresponding Ab peptides to improve reliability of the neurochemical dementia diagnosis.

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New Immunosensitive Detection of Circulating Aggregated Ab: Validation Using a CSF Ab 42/40 Prediction Test

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Several studies provide evidence that plasma Aβ concentrations are associated with risk of dementia. Owing to present the limitations of CSF marker analysis, a reliable test measuring plasmatic markers will be very useful.

A new immunosensitive method employing specific antibodies is described which allows the detection of aggregated and protein bound Aβ isoforms in plasma and CSF from patients with highly probable Alzheimer's disease (AD). Beside the clinical diagnosis, to provide more accuracy in the validation of this new method, 74 patients were tested using three validated CSF ELISA tests: Aβ40, Aβ42 (Genetics©) and P-Tau (Innogenetics ©). The study included AD N=27, clinically established other dementia (OD) including possible Alzheimer'patients N=24, evaluated neurological diseases and controls(NA) N=23.

CSF P-Tau results were in agreement with literature findings. The most significant results were obtained for CSF Aβ 42/40 ratio statistically different for AD versus OD and NA (0.71pg.ml;1.10pg.ml; 1.21pg.ml; $p<0.001$).

We constructed a prediction model with sensitivity of 91% and specificity of 92%.

From our predictive model, our results suggest that circulating plasma Aβ aggregated forms are associated with risk of dementia. Moreover, the validation study enabled us to confirm that, using this model, the CSF Aβ 42/40 ratio could be useful in the diagnosis of Alzheimer's disease and may be used by clinicians as a predictive tool.

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Potential Diagnostic Relevance of Amino-Terminally Truncated Beta- Amyloid Species in Sporadic Alzheimer's Disease: A Prospective Brain Study

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Background: The presence of amino-terminally truncated beta-amyloid species in plaques has been for over two decades, but its potential relevance to the natural history of Alzheimer's disease, has only recently been explored.

Experimental approach: New highly specific antibodies against different amino-terminal beta- amyloid species, for instance amino-truncation 8, were generated and used in sandwich immunoassay and immunocapture mass spectrometry approaches to characterize amyloid peptide species in 52 specimens of well-characterized brain tissue from a prospective brain study. These comprised specimens from 25 elderly subjects clinically defined as controls or with other neurological diseases, 7 patients with mild cognitive impairment cases, 9 with dementia with Lewy bodies, and 11 with Alzheimer's disease).

Results and Conclusions: Amino-terminally truncated species were abundantly present, not only in all clinically defined AD cases and also in the preclinical stages, in brain regions with the earliest neuropathologically detectable signs of Alzheimer's disease. Based on the immunocapture massspectrometry data, the earliest species of amino-terminally truncated species are full-size, N-truncated 4 (a potential nephrylisin proteolytic cleavage site) and N-truncated 8 amyloid (a potential angiotensin-converting enzyme cleavage site). Amino-terminal truncation is closely associated with amyloid plaque deposition and with no obvious difference between plaque formation in Alzheimer's disease or in dementia with Lewy bodies. Ongoing studies on the presence of these N-truncated amyloid beta species in cerebrospinal fluid and blood plasma will be presented.

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Cerebrospinal Fluid Beta-Amyloid Fragment Signatures in Neurodegenerative Diseases

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Pathogenic events in Alzheimer's disease (AD) involve an imbalance between the production and clearance of the neurotoxic beta-amyloid peptide (Abeta), especially the 42 amino acid peptide Abeta1-42. While much is known about the production of Abeta1-42, many questions remain about how the peptide is degraded. To investigate the Abetaisoform pattern, we developed a method based on immunoprecipitation combined with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (IP-MS) that determines the Abeta fragment pattern in cerebrospinal fluid (CSF).

The peptides were immunoprecipitated from CSF using magnetic Dynabeads coated with Abeta specific antibodies. Different antibodies with different epitope, such as 6E10, 4G8 and 11A50-B10 (epitope 4-9, 18-22 and specific for Abeta1-40, respectively, Signet Laboratories Inc.), were used. After washing and eluting the extracted peptides using KingFisher mL (Thermo), the clear Abeta-containing solution was further analyzed using mass spectrometry.

We found in total 18 C-terminally and 2 N-terminally truncated Abeta peptides. We here provide direct evidence that an Abeta relative abundance fragment signature consisting of Abeta1-16, Abeta1-33, Abeta1-39, and Abeta1-42 in CSF distinguishes sporadic AD patients from non-demented controls with an overall accuracy of 86%. We have also extended the investigation to other dementia disorders, such as MCI, which will be presented.

The Abeta fragment signatures could potentially be used as a diagnostic test to distinguish AD patients from non-demented controls and other dementia disorders, a hypothesis that is currently under investigation. Further, the Abeta fragment pattern may give further information on the pathogenetic pathways for Abeta metabolism directly in patients with AD.

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Elevated Platelet Beta-Secretase Activity in Mild Cognitive Impairment

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Background: ß-secretase activity is the rate-limiting step in Aß peptide production from amyloid precursor protein (APP). Aβ peptides aggregate to form the amyloid plaques that characterise Alzheimer's disease (AD) neuropathology. Human platelets contain full-length and processed APP. We have reported increased platelet ß-secretase activity in established AD (Johnston et al, Neurobiology of Aging, in press) and now report our follow-up work in patients with Mild Cognitive Impairment (MCI).

Methods: Platelets were isolated from 46 patients (MMSE 25 ± 1.2) and 59 age-matched controls (MMSE=30), lysed (25 mM HEPES, pH 7.2) and fractionated into particulate and soluble fractions by centrifugation (100,000 g for 1 h). The membrane fraction pellet was suspended in 25 mM HEPES, 2% CHAPS and 5 µg protein from each sample was assayed for β-secretase activity in triplicate using the internally-quenched fluorogenic substrate (MCA)EVKMDAEFK(DNP)-NH2 (Calbiochem) in 50 mM sodium acetate, pH 4.5 at 37°C. Fluorescence was read continuously over 30 min, and initial rates calculated.

Results: We identified a significant 27% increase in β-secretase activity in platelet membrane fractions from patients vs controls ($p < 0.0006$, unpaired t-test). The range of ß-secretase activity detected in different individuals was wide raising important questions about in vivo regulation of this proteolytic activity.

Conclusions: MCI may be an early precursor of AD and elevated ß-secretase activity in an accessible peripheral tissue at very early stages of this disease is of considerable interest in relation to pathobiology and diagnosis.

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Biomarkers and Metabolomic Analysis in Parkinson`S Disease

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Background: Parkinson`s disease (PD) is considered a sporadic disease but recently various hereditary forms have been recognized, the most frequent one is the LRRK2-G2019S substitution.

At present, there are no established diagnostic biomarkers that can confirm Parkinson`s disease clinically or assess the disease progression. The aim of this study is to investigate biochemical markers.

Methods: This study included 15 patients with sporadic Parkinson`disease, 11 patients with LRRK2-G2019S substitutions, 11 family members without the mutation and 11 controls. Metabolomics were used for measuring small signalling molecules.

Plasma samples were performed at all these patients and prepared for analyses by liquid chromatography electrochemical array detection (LCECA) provided by a database representing about 2000 metabolites. Multivariative data analysis and frequency distributions analysis were used in order to determine the metabolites.

Results: Projections into three dimensions provided statistically significant separations between 3 subgroups: controls, family members without mutation and the LRRK2-G2019S PD patients. Analysis of the data from sporadic PD (n=15) also demonstrated separation from the G2019S patients (n=11).

Conclusions: Metabolomic analysis significantly separated PD patients from controls and even LRRK2 from sporadic PD.

This significant separation of metabolites may constitute a preliminary biochemical signature for the disease. These signatures should be confirmed in a larger population of patients. They could also enable better stratification of Parkinson`s disease subtypes and provide insight into pathogenesis of the disease.

Metabolomics may offer a potentially new tool for confirming PD diagnosis and monitoring disease progression in a non-invasive way.

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CSF Neurofilament Light Chain and Tau Differentiate Multiple System Atrophy From Parkinson Disease

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Background: In early disease stages it can be clinically difficult to differentiate idiopathic Parkinson's disease (IPD) from patients with multiple system atrophy predominated by parkinsonism (MSA-P). In the present study we investigated if the analysis of neurofilament (NF) proteins and neurotransmitter metabolites in cerebrospinal fluid (CSF) may discriminate between IPD and MSA-P.

Methods: In CSF of 31 patients with IPD and 19 patients with MSA-P, we analyzed tau, neurofilament light chain (NFL) and heavy chain (NFHp35) and the noradrenergic metabolite 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG).

Results: CSF levels of NFL, NFHp35, and tau were increased in MSA-P, whereas MHPG levels were decreased in MSA-P (all $p<0.0001$). Optimal discriminative cut-off values for each biomarker for the differentiation between MSA-P and IPD were calculated resulting in high sensitivity (76-94%) and specificity (83-97%) levels. Multivariate logistic regression analysis selected only NFL and tau according to the following model: $y = NFL + 0.15*Tau$. A cut-off of $y > 35$ resulted in a sensitivity of 88%, a specificity of 87% with an area under curve of 0.94.

Discussion: We demonstrated that CSF analysis can discriminate IPD from MSA-P with both sensitivity and specificity higher than 80%. Median CSF levels of the axonal proteins NFL, NFHp35, and tau were significantly increased in MSA-P compared to IPD, which may reflect advanced axonal degeneration in MSA-P. Furthermore, CSF levels of MHPG are decreased in MSA-P compared to IPD. Differentiating MSA-P from IPD could be accurately possible with CSF analysis of a combination of axonal and neurotransmitter biomarkers.

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Increasing CSF P-Tau Levels During Cognitive Decline and Progression to Dementia

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Background: Little is known about longitudinal changes of the CSF dementia biomarkers and neurodegenerative progression. We have previously shown that Rey Auditory Verbal Learning Test (RAVLT) is a useful test to predict which patients with memory complaints who 3 years later will decline cognitively (Andersson et al 2006). Using this concept we here here wanted to study longitudinal changes of the CSF dementia biomarkers – total-tau (T-tau), phospho-tau (P-tau) and B-amyloid (AB-42) in cognitive decline.

Aims: To use RAVLT to define memory function at baseline and follow longitudinal changes in CSF biomarkers during cognitive decline.

Methods: 40 memory clinic patients (47.5% females), aged 61.3±7.6 (SD) years, non-demented at baseline, were lumbar punctured and neuropsychologically examined at two occasions. Baseline mean MMSE-score was 28.3±1.8. Patients were divided into three groups based on baseline RAVLT results; severely impaired (SIM), moderately impaired (MIM) and no impairment (NIM).

Results: There was a significant increase in P-tau in the SIM-group during follow-up, while the levels in MIM and NIM did not change. 83% of SIM-patients converted to dementia (80% converted to AD) during follow-up, while most patients in the MIM and NIM groups did not convert to dementia. Conversion to AD was paralleled by significantly increasing P-tau- and relatively stable T-tau- and AB42-levels. MIM and NIM showed unchanging biomarker levels.

Conclusion: Increasing P-tau levels was shown during cognitive decline (assessed by neuropsychology tests) and in conversion to AD, suggesting that P-tau may be useful as a marker of severity of the neurodegenerative process.

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Immunotherapy Against Beta-Amyloid in Alzheimer's Disease

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Immunotherapies targeting brain beta-amyloid are currently tested for safety and efficacy in patients with Alzheimer's disease (AD). Initial attempts with active Abeta immunization were halted because of the development of meningoencephalitis in a subgroup of patients. Follow-up studies established that Abeta immunization induced chronic elevations of antibodies against beta-amyloid. These are transported across the blood brain barrier into brain tissue and cerebrospinal fluid. In patients who had developed such antibodies, cognitive decline was slowed, brain beta-amyloid load was greatly reduced, and shrinkage of hippocampus volume was prevented. These initial beneficial clinical effects were independent of the prior occurrence of meningoencephalitis. Together, these pilot data suggest that chronically elevated titers of antibodies against beta-amyloid in AD patients could be safe and potentially efficacious in reducing the decline of cognitive functions, hippocampus tissue loss and beta-amyloid-related pathology.

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Complement C3-Deficient APP Tg Mice Have Accelerated AD-Like Pathology and Neuron Loss But Still Respond to Abeta Immunotherapy

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Complement proteins, key mediators of inflammation, are associated with neuritic plaques in AD brain. To assess the role of complement in AD pathogenesis and clearance via immunotherapy, we crossbred complement C3-null mice with J20 APP tg mice. At 12 months, J20/C3-null mice had similar levels of soluble Abeta but slightly increased insoluble Abeta40 and Abeta42 in brain homogenates and elevated plasma Abeta compared to J20 complement-sufficient mice. Plaque deposition and gliosis were modestly elevated. At 17 months, J20/C3-null mice had less soluble Abeta (49% lower Abeta40, $p<0.05$) and more insoluble Abeta40 (23%) and Abeta42 (55%, $p<0.01$) in brain homogenates and 51% more plasma Abeta compared to J20 mice. Gliosis was increased while synaptophysin levels were reduced. The number of CA2/CA3 hippocampal neurons was reduced ($p<0.05$). APP and alphaAPPs levels were unchanged. Next, we intranasally immunized 6 mo-old APP/C3-/- mice weekly with Aβ1-40/42 and adjuvant LT(R192G) or vehicle. After 7 months, all Abeta immunized mice made Abeta antibodies (~80 µg/ml) of IgG2b and IgA isotypes that recognized the amino-terminus of Abeta. Immunized mice had significantly reduced insoluble Abetax-42 levels ($p<0.05$), increased plasma Abeta ($p<0.01$), reduced plaque burden in hippocampus (49%, $p<0.01$) and cortex (60%, $p<0.05$) compared to controls. Thioflavin S plaques were unchanged. Gliosis was reduced. T and B cells were absent in brain. Together, our results suggest that C3 has a beneficial, neuroprotective effect on Abeta clearance/deposition in APP tg mice, however, complement C3 is not required for the Abeta lowering in Abeta immunotherapy.

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Reduction of Abeta Protofibrils in Transgenic Mice Model of Alzheimer's Disease Using a Conformation-Specific Monoclonal Antibody

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Background and aims: There is an increasing interest in soluble Aβ oligomers as the pathogenic species in Alzheimer's disease (AD). Different types of Aβ oligomers have been described: low molecular weight Aβ oligomers, Aβ derived diffusible ligands (ADDLs), amylospheroids and large soluble Aβ oligomers, i. e. protofibrils. We are focusing on the protofibrils since the Arctic mutation (Aβ E22G) causes AD due to an enhanced propensity of Arctic Aβ peptides to form protofibrils. Thus, the strength of our approach is that it is based on a clinical observation.

Methods: We have raised conformation-specific monoclonal IgG antibodies against Aβ protofibrils. One of these, mAb158, had a thousand-fold higher affinity for protofibrils than for monomers. mAb158 did not react with other amyloids like IAPP, medin or alpha-synuclein, and did not react with APP.

Results: By using mAb158 in immunoassays Aβ protofibrils could be detected at low pM levels, which enabled measurement of protofibrils in brain homogenates from transgenic mice and cell-culture media. The ability of the antibody to inhibit protofibril-induced neurotoxicity was evaluated in vitro using PC12 cells, and the therapeutic efficacy was analyzed in vivo using our APP-ArcSwe transgenic mouse model. After four months of i.p. treatment with mAb158 a significant reduction in Aβ protofibril levels was found in treated mice.

Conclusions: The reduction of protofibril induced toxicity in PC12 cells and diminished levels of protofibrils in transgenic mice brain indicate that mAb158 is a good candidate for passive immunization in the treatment of AD.

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N-Terminal Structure of Amyloid-Beta Peptide Determines Its Metabolic Fate in Brain

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Amyloid-beta peptide (Abeta), a pathogenic agent of Alzheimer's disease (AD), is a physiological peptide constantly metabolized in vivo under normal conditions. We examined the structural determinants that influence its life span by analyzing the catabolic fate of radiolabeled AbetaX-42 variants in rat hippocampus. The metabolic stability was in the following order: Abeta3(pE: pyroglutamate)-42 >> Abeta1-42 > Abeta2(A)/3(E)/4(F)-42. The results, consistent with the selective pathological deposition of Abeta3(pE)-42 in Alzheimer brain, indicate that anti- and pro-catabolism signals reside in the N-terminal structure of Abeta. This "N-end rule" may be applicable to preventing Abeta deposition in human brain.

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Intraneuronal Abeta is a Major Risk Factor - Novel Evidence From the APP/PS1KI Mouse Model

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We investigated bigenic mice expressing human mutant amyloid precursor protein APP751 (KM670/671NL and V717I) with a knocked-in human mutant presenilin-1 (M233T/L235P), named APP751/PS1KI. The objective was to study the consequences of intraneuronal Abeta aggregation on neuron function on several levels: hippocampal synaptic plasticity; neuron loss; axonal degeneration in brain and spinal cord; behavioral deficits; general phenotype. Working memory and motoric performance, long-term potentiation (LTP), paired pulse facilitation (PPF), neuropathology of brain and spinal cord, stereology. At the age of six months we observed an age-dependent dramatic change in all of these properties and characteristics. There is a significant reduced ability to perform working memory and motoric tasks. The APP/PS1KI mice were smaller and showed development of a thoralumbar kyphosis, together with reduced body weight, and axonal degeneration in brain and spinal cord. CA1 neuron loss in these mice is likely to contribute to the working memory deficits, which correlates with almost complete loss of synaptic plasticity (LTP and PPF) after stimulation of the Schaffer collaterals. Intraneuronal oligomeric Abeta and peptides beginning with aspartate at position 1, and pyro-glutamate at position 3 were detected at two months of age. Accumulation increased significantly at the age of six months. Overall, this mouse model develops a robust neuron dysfunction and degeneration, which triggers AD-typical changes on different levels including synaptic plasticity and working memory. We conclude from these observations that intraneuronal Abeta X-42 accumulation is the major neurotoxic factor.

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Pyroglutamated Aß Peptides - N-Terminally Modified Aß Variants Generated by Glutam(in)Yl Cyclase

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pGlu-Aß peptides are highly abundant in sporadic and inherited Alzheimer's Disease, constituting up to 50% of total Aß. Formation of pGlu confers resistance against cleavage by aminopeptidases, increases the cytotoxicity of the peptides and speeds up Aß aggregate formation.

Our in vitro and in vivo studies provide strong evidence for a slow Glutaminyl cyclase (QC, EC 2.3.2.5) catalyzed cyclization of N-terminal glutamic acid, substantiating a crucial role for generation of pGlu-Aß peptides. Truncated Aß peptides generated in HEK293 cells displayed significant N-terminal pGlu formation following co-expression of QC.

Significant amounts of pGlu-Aß(3-40) were also found after injection of pharmacological doses of Aß(3-40) into the cortical region of rat brain, as revealed by immunohistochemistry and ELISA evaluation. Concentration of pGlu-Aß was significantly reduced after co-injection of a QC inhibitor, strongly implying a QC-catalyzed pGlu-Aß formation in vivo.

To evaluate the role of pGlu-Aß in an AD mouse model, a QC-inhibitor was applied orally to the familial AD Tg2576 mice for duration of 6 months. As evidenced by this and other studies, APP-mutant transgenic mice reveal only negligible pGlu-Aß formation in contrast to human sporadic AD brains. However, a dose-dependent effect of the QC-inhibitor on solubilized pGlu-Aß as well as Aß(x-42) was observed.

The unexpected catalytic feature of QC, i.e. its EC-activity, suggests a crucial role of the enzyme for the formation of N-terminally pyroglutamated Aß peptides, potentially influencing the neurodegenerative processes underlying Alzheimer's Disease.

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Pathways Leading to the Neurotoxic Pyroglutamated Aβ-Peptides Differ in Wildtype APP- and Mutant APP-Expressing Systems

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Brains of demented AD-patients and non-demented patients with pathological aging display almost equal plaque load. Moreover, neither BACE-1, Neprylisin, oxidative state nor Aß(1-42) correlate with the neuropsychiatric symptoms of the AD-patient group. Correspondingly, mice expressing human APP(WT) do not show plaque-like histopathology, but develop memory impairment similar to mutant APP-expressing mice, which primarily deposit Aß(1-40/42). Similar, newly designed animal models display N-terminal Aß-heterogeneity as found in human sporadic AD and memory deficits before extracellular deposits are detectable suggesting that intraneuronal toxicity of Aß-peptides initiates neurodegeneration.

In search of intracellular Aß-peptides most prone to form toxic oligomers, we found Aß-peptides possessing a N-terminal pyroglutamic acid (pGlu) aggregate two orders of magnitude faster as Aß(1-42). These pGlu-peptides are potent seeds of Aß(1-42) oligomerization. pGlu-peptides are formed by Glutaminyl Cyclase (QC, EC 2.3.2.5) requiring N-Glu-peptides as substrates. Thus, the generation of pGlu3-Aß is either preceded by single or stepwise proteolytic events near the APP ß-cleavage site.

Focusing on pathways responsible for the formation of the Aß(N3Glu) peptides we found in HEK293-cells expressing human APP(WT), APP(Swedish), APP(London) and APP(Swedish/London) different ß-secretase products as well as different ß-secretases releasing a distinct Aßx-42 peptide spectrum.

Moreover, we observed that the presence of isoaspartate instead of Asp672 within APP-sequence fragments and BACE-1 substrates leads to a different cleavage pattern by BACE-1 and other ß-secretase candidate enzymes.

Thus, we conclude that pathways and proteolytic enzymes responsible for the formation of Aß(N3Glu) inducing the QC-catalyzed formation of toxic Aß(N3pGlu) differ in sporadic Alzheimer and familial forms of the disorder.

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A Novel APP Transgenic Mouse Model for Alzheimer Disease: Intraneuronal Amyloid Beta Paralleled by Reduced Brain Volumes

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Background and aims: Transgenic mouse models of Alzheimer disease (AD) expressing amyloid precursor protein (APP) with familial AD (FAD) mutations are useful in understanding the process of amyloidogenesis. We earlier identified a mutation in APP, APPT714I or APP-Au (Austrian), that led to an early onset of disease and pathologically patient brain was characterized by abundant amyloid deposits consisting of non-fibrillar diffuse amyloid, composed of N-truncated Aβ42 in absence of Aβ40. In vitro, this mutation leads to one of the highest Aβ42/ Aβ40 ratios among all FAD mutations.

Methods: We generated an APP-Au transgenic mouse model that expressed ten times lower transgene than endogenous murine APP. These animals were analyzed at various ages using behaviour studies, MRI and immunohistochemistry.

Results: APP-Au mice deposited intraneuronal Aβ in brain by 6 months of age. Accumulations increased with age and were paralleled by decreased brain sizes on volumetric MRI, compared to age-matched APP wild-type mice. Immunohistochemical studies in APP-Au mouse brains revealed that the majority of the intraneuronal Aβ deposits colocalized with late endosomal markers. We generated a second model expressing a double mutant form of APP (K670N/N671L + T714I) because the APP-Au mutation leads to a decrease in total Aβ. Transgene expression in these lines is half of the endogenous APP expression and analyses are ongoing.

Conclusions: Our data on the APP-Au mouse model support earlier observations of Aβ accumulation in the endosomal-lysosomal pathway and the hypothesis that intraneuronal accumulation of Aβ could be an important factor in the AD pathogenesis.

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Elevation of BACE1 Levels in Alzheimer's Disease and APP Transgenic Mouse Brains: Potential Roles of Energy Impairment and Amyloid Deposition

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BACE1 (beta-secretase) initiates cleavage of Abeta from APP and is a promising therapeutic target for Alzheimer's disease (AD). BACE1 levels are elevated in AD and may increase Abeta, but the cause of BACE1 elevation is unknown. Interestingly, glucose metabolism is reduced in AD, suggesting impaired energy metabolism may be pathogenic. Here, we investigated whether energy inhibition increases BACE1 and Abeta in APP transgenic mice. We also examined the role of amyloid deposition in BACE1 elevation. First, we used drugs (e.g., insulin, 2-deoxyglucose, 3-nitropropionic acid) to induce acute and chronic energy inhibition in APP transgenic (Tg2576) mice. We observed that BACE1 and Abeta levels increased ~2-fold in mice treated acutely (1 injection) or chronically (3 injections/week, 3 months). Chronically treated Tg2576 mice had increased plaque numbers, supporting the hypothesis that energy inhibition may contribute to AD. Next, we made a highly specific anti-BACE1 monoclonal antibody in BACE1-/- mice and with it determined that BACE1 levels were increased ~2-fold in human AD and APP transgenic brains. In our new 5XFAD APP/PS1 transgenic mouse, the BACE1 increase started at 4 months and rose in parallel with amyloid burden, demonstrating that BACE1 elevation occurred early rather than late, excluding end-stage neurodegeneration as a cause. Increased BACE1 levels were associated with amyloid plaques in 5XFAD, Tg2576, and AD brains. Immunofluorescence microscopy showed elevated BACE1 surrounding Aβ42-positive amyloid plaque cores in neuron-derived structures. We conclude that energy impairment and amyloid deposits induce BACE1 elevation, potentially causing a positive feedback loop driving Abeta production in AD.

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Inactivation of TNF Death Receptor Inhibits Amyloid Protein Generation and Prevents Learning and Memory Deficits in Transgenic Alzheimer's Mice

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Tumor necrosis factor type 1 death receptor (TNFR1) belongs to a subgroup of the TNFR superfamily. When TNFR1 binds its ligand (TNFα), the ligand-receptor complex triggers apoptotic pathways by recruiting either TNFR-associated death domain protein or Fas-associated death domain protein/mediator of receptor-induced toxicity (FADD/Mort-1); it is still unclear which of the two intracellular adapter proteins is actually involved. The receptor-induced multimerization of TRADD or FADD leads to caspase activation. We recently demonstrated that the TNFR1 cascade is required for amyloid β protein (Aβ)-induced neuronal death. Its role in Aβ plaque pathology and amyloid precursor protein (APP) processing in Alzheimer's disease (AD), however, remains unclear. In the present study, we report that deletion of the TNFR1 gene in APP23 transgenic mice (APP23/TNFR1-/-) diminished Aβ plaque formation and inhibited Aβ generation in the brain. Examining such mechanisms, we found that TNF death receptor mediated signal transduction not only contributes to neurodegeneration, but is also involved in both β-secretase and γ-secretase activity in APP23/TNFR1-/- mice, thus presenting itself as a novel therapeutic target for AD.

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Neurofibrillary Tangles and Neuronal Loss in An Inducible Transgenic Mouse Model Expressing the Repeat Domain of Tau

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We monitored the expression and aggregation of tau in transgenic mouse models in order to understand the toxicity of tau. We have generated inducible transgenic mouse lines which express different tau constructs. Here we describe two lines expressing the 4-repeat tau domain with the FTDP17-mutation Delta-K280 (K18-DeltaK280, "pro-aggregation mutant"), and the 4-repeat tau domain with the DeltaK280 deletion and two proline mutations in the hexapeptide motifs (K18DeltaK280/I277P/I308P, the "anti-aggregation" mutant). The DeltaK280 is known to accelerate the aggregation of tau, whereas the two Pro mutations inhibit tau aggregation in vitro and in cell models. The inducible transgene expression in mice was driven by the forebrain-specific CaMKII promoter. The "pro-aggregation" mutant shows the presence of soluble human tau protein as well as aggregated tau from 3 months onwards. The double proline mutant shows a similar pattern of expression, but does not form aggregated tau. By immunohistochemistry, the "pro-aggregation" mutant shows relocalization of tau from the axonal to the somatodendritic compartment in a phosphorylated form (pS262/pS256 recognized by antibody 12E8, in the KXGS motifs that are targets of the kinase MARK). Gallyas silver staining confirms the presence of aggregated tau in the limbic system starting as early as 3 months of tau expression. Consistent with the tau pathology, there is a noticeable neuronal loss in the dentate gyrus of aged mice. The "anti-aggregation" transgenic mice show similar tau expression but less phosphorylated tau at pS262/pS256 by immunohistochemistry, and no aggregated tau by Gallyas staining, and no neuronal loss. - Supported DFG.

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Memantine Lowers Beta Amyloid Levels in Neuronal Cells and Transgenic Mouse Models of Alzheimers Disease

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Background and Aims: Memantine, an uncompetitive antagonist of N-methyl-D-aspartate (NMDA) receptors, is approved for the treatment of moderate to severe Alzheimer's disease (AD). AD is characterized by the extracellular deposition of neurotoxic amyloid beta peptide, and we have previously reported that memantine can rescue brain cells from the neurotoxic effects of amyloid beta. The purpose of studies presented here was to investigate the mechanism of neuroprotection from amyloid beta toxicity, and to determine whether memantine treatment can alleviate cognitive difficulties in transgenic mouse models of AD.

Methods: A series of studies, performed in different laboratories, used human neuroblastoma cells, primary rat cortical neurons, and transgenic mice to investigate the effects of memantine treatment on amyloid beta levels and on cognitive impairment associated with an AD-like brain pathology.

Results: We demonstrated that memantine at therapeutic doses lowers the levels of peptides produced by human neuroblastoma cells, primary rat cortical neurons, and transgenic mice with high brain levels of amyloid beta. We also report that memantine reduces the cortical and hippocampal plaque burden and improves cognitive performance of transgenic mice in several behavioral tests.

Conclusions: The mechanism of action of memantine may affect pathways of amyloid production and accumulation. Taken together, these data suggest a disease-modifying potential for memantine in AD.

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Insulin-Degrading Enzyme and Alzheimer`S Disease: Insights From Newly Developed Pharmacological Inhibitors and Activators

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Background And Aims: Insulin-degrading enzyme (IDE) is an atypical zinc-metalloprotease that hydrolyzes the amyloid β-protein (Aβ), insulin and other medically relevant substrates. Despite IDE's demonstrated importance in the pathogenesis of both Alzheimer's disease (AD) and diabetes, only a limited number of very poor pharmacological tools are available with which to probe its function.

Methods: drug design, high-throughput screening (HTS), ELISAs, dynamic light scattering (DLS).

Results: We used a substrate-based rational design approach to develop IDE inhibitors ~1,000,000 times more potent than currently available inhibitors. In addition, through HTS, we identified small molecules that significantly activate IDE in a highly substrate-selective manner. Both cell-permeant and -impermeant IDE inhibitors increase steady-state levels of Aβ in cells overexpressing the β-amyloid precursor protein, confirming the existence and importance of extracellular pools of IDE to the regulation of Aβ levels. Pharmacological inhibition of IDE also increases the secretion of insulin from a pancreatic beta cell line and potentiates the action of insulin within cells, suggesting they may have utility in the treatment of diabetes. Some IDE activators interact with an uncharacterized nucleotide polyphosphate binding domain within IDE, and their degree of activation correlates with increases in hydrodynamic radius as detected by DLS, suggesting that they may promote the "open? state of IDE revealed by recently reported crystal structures.

Conclusions: These and other results we have obtained highlight the broad utility of this new class of pharmacological agents for dissecting the molecular basis of AD, diabetes, and their interrelationship.

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Significant Aß Lowering in CSF and Plasma After Systemic Administration of a Potent Small Molecule BACE1 Inhibitor in Non-Human Primates

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BACE cleavage of Amyloid Precursor Protein (APP) is one of the first steps in the generation of amyloid peptides, including the potentially neurotoxic Aß42 species. We have previously shown that potent cell-permeable BACE inhibitors can lower brain Aß levels when introduced directly into the lateral ventricles of mice (Sankaranarayanan et al, submitted) or injected systemically (Sankaranarayanan et al, ICAD, Madrid 2006). We have further optimized various amyloid-reducing BACE inhibitors and now report in vivo BACE inhibition in a unique, chronically implanted non human primate model. The compound utilized in the present study has an enzymatic IC50 of 1.5 nM and cell culture IC50 of 50 nM, and PK properties suitable for in vivo use. In CSF and plasma samples collected from N=6 conscious rhesus monkeys treated with this BACE1 inhibitor, we observed a significant baseline adjusted reduction in CSF Aß40 (42 %, $p<0.001$) and CSF Aß42 (35%, $p<0.001$). Using a novel SELDI based method, CSF Aß38 was also reduced (20 %, $p<0.05$). Importantly, CSF sAPPβ levels significantly decreased (10%, $p<0.01$), while sAPPα levels trended upwards (10 %, not significant), as anticipated from our previous mouse studies. In the periphery, plasma Aß40 was dramatically reduced (65 %, $p<0.001$). These results are the first reported demonstration of in vivo pharmacological evidence in primates that acute brain Aß lowering can be achieved after peripheral administration of a BACE inhibitor.

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BACE1 Cleavage and Release of Neuregulin-1

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The generation of amyloid peptides (Aß) from the amyloid precursor protein (APP) depends on sequential cleavage by ß-secretase and gamma-secretase enzyme activities. Cleavage by gamma-secretase (previously identified as presenilin-1) produces Aß peptides mainly of 40 or 42 amino acids in size. BACE1 (ß-site APP cleaving enzyme-1), an aspartylprotease, was recently shown to be the major ß-secretase in the brain, based on the loss of ß-cleavage in BACE1 -/- mice and reduced Aß levels in mouse brains upon treatment with a BACE1 inhibitor. Recently it has been shown that the EGF-like ligand Neuregulin-1 (NRG1), and its receptors, members of the ErbB receptor tyrosine kinase family are also substrates for regulated intramembrane proteolysis mediated by ß-secretase. Genetic studies in mice have demonstrated the multiple essential roles of this signalling system in glial cell and cardiac development. NRG1 cleavage was studied in HEK 293 cells and in BACE -/- mice, and myelination of peripheral nerves was analysed by electron microscopy. Very high levels of BACE1 were observed at time points when peripheral nerves become myelinated. Deficiency of BACE1 resulted in the accumulation of unprocessed NRG1. BACE1 -/-mice displayed hypomyelination of peripheral nerves and aberrant axonal segregation of small diameter afferent fibers very similar to mice with mutations in type III NRG1, or Schwann cell-specific ErbB2 knockouts. Thus, BACE1 is required for myelination and correct bundling of axons by Schwann cells most likely via processing of type III NRG1 (Willem et al., Science Vol.314, pp664, 2006).

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Frequency of GBA and LRRK2 Mutations in Lewy Body Disorders

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The presence of mutations in Glucocerebrosidase (GBA) or in leucine-rich repeat kinase 2 (LRRK2) gene has been described as a risk factor for the onset of Lewy body disorders (LBD). These disorders are characterized neuropathologically by the presence of -synuclein aggregates in different brain regions and clinically by varying degrees of dementia and parkinsonism. Recently, a reported study sequenced the GBA gene in 75 autopsy specimens with different LBD and identified mutations in 23% of cases with dementia with Lewy Bodies. Here, we perform a similar study and sequenced all GBA exons in 108 brain specimens from the Columbia University Brain Bank. The cohort , in a range from 44 to 89 age of death, includes 35 controls and 73 cases with neuropath confirmed LBD. Additionally, we sequenced LRRK2 exons 35 to 46 (the kinase domain) and genotyped 5 known mutations and 17 reported SNPs across this gene in all brain specimens. Our results indicated that 24.6 % (18/73) of LB cases are GBA mutation carriers (1 PD case, 12 Diffuse LBD, 2 Alzheimer LB variant, 4 DLB), while 4.1% (3/73) harbor LRRK2 gene variants (1 PD, 1 DLB, 1 ALBV). There was no association between the presence of GBA mutations and the presence of LRRK2 mutations. None of the controls carried any mutations except for the GBA variant T369M identified in one control. The results of the current work confirm that GBA mutations are relevant genetic risk factor for the development of LBD.

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Non-Rem Sleep Disturbances in Dementia With Lewy Bodies: Report on Three Video-Polysomnographic Studies

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Diagnostic criteria for “probable or “possible” Dementia with Lewy bodies (DLB) have been recently revised (Third Report of the DLB consortium, 2005), recognizing three “core features” and additional “suggestive features”, including rapid eye movement (REM) sleep behavior disorder (RBD). As far as we know, only few cases of non-REM (NREM) sleep disturbances with reported bizarre behaviour has been described in DLB patients (Zarranz JJ, 2005), but not registered at video-PSG.

We report cases of three patients admitted to our Institute to assess DLB diagnosis. All of them presented severe sleep disturbances. Video-polysomnographic (PSG) study was performed.

Patient A (male, 72 ys old): “probable” DLB. At video-PSG, RBD was shown, but also NREM sleep paroxystic events were registered, resembling rhythmical movements during sleep (RMD) similar to “legbanging”, with rest tremor at awakening. Patient B (female, 78 ys old): “possible” DLB. Reported complex and bizarre sleep behaviour disturbances, registered at video PSG during NREM sleep, possibly recalling RMD of “bodyrocking”, a parasomnia rarely described in adults. Patient C (male, 80 ys old): “possible” DLB. Clinically no parkinsonism, but SPECT showed low putamen dopamine transporter (DAT) activity. Presented from the beginning confusional arousals, RBD and periodic movements of limbs (PMLs), all registered during video-PSG.

In three patients with clinical diagnosis of DLB, we documented the presence of both REM and NREM sleep disturbances.

Sleep disorders in DLB could be more heterogeneous than known, with their physiopathogenesis possibly involving multiple mechanisms. Video-PSG could help better describing them, even in comparison to other neurodegenerative dementias.

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Effect of Behavioral and Psychological Symptoms on Patient and Caregiver Quality of Life in Alzheimer's Disease

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Background and aims: Relatively few studies have explored the association between behavioral and psychological symptoms of dementia (BPSD) and quality of life (QOL) of patients and their caregivers. This study aimed to examine the effect of BPSD on patient and caregiver QOL in Alzheimer's disease (AD)

Methods: Fifty-one AD patients and their caregivers participated. Measures about patients were Neuropsychiatric Inventory (NPI), Korean version of QOL–Alzheimer's Disease (KQOL–AD), Activities of Daily Living (ADL), Clinical Dementia Rating (CDR), and Korean version-Mini Mental State Examination (K-MMSE). Caregiver QOL was assessed with KQOL–AD and General Health Questionnaire/Quality of Life-12 (GHQ/QOL-12).

Results: Patient QOL-AD on patient ratings was negatively correlated with appetite/eating change and NPI scores. Patient QOL-AD on caregiver ratings was negatively correlated with hallucinations, depression/dysphoria, and NPI scores. Caregiver QOL assessed by the GHQ/QOL-12 was negatively correlated with agitation/aggression, depression/dysphoria, and NPI scores and was negatively correlated with distress related to agitation/aggression, depression/dysphoria, and NPI scores.

Conclusion: BPSD of AD patients was associated with low QOL of both patients and caregivers. Thus, interventions of BPSD need to improve both patient and caregiver QOL.

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Leisure Activities and Cognitive Function in Community-Dwelling Elders

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Background and aims: Leisure activity has been hypothesized to reduce the risk of Alzheimer disease in foreign countries, but in our country, data regarding an association are lacking.

Methods: To determine whether leisure activity in a community-based sample delays cognitive decline, we selected 272 community-dwelling elders (72.3 years old) in Shimane Prefecture. We divided the sample population into 2 groups: leisure activity group (186) and non-leisure activity group (86).

Results: There were no significant differences between the two groups in subjective well-being, depression score or ADL; however, the leisure activity group had a significantly ($p<0.05$) higher Hasegawa scale score than the non-leisure activity group. Also, the leisure activity group was curious and sociable compared with the non-leisure activity group.

Conclusions: These results suggest that leisure activity is associated with cognitive function.

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Prospective Validation of Neuropathologic Criteria of Third Consortium for Dementia With Lewy Bodies (CDLB)

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Objectives: To determine the validity of revised clinical and pathologic CDLB criteria for dementia with Lewy bodies (DLB).

Background: The revised clinical criteria for DLB place greater emphasis on REM sleep behavior disorder (RBD) as a supportive feature. The revised neuropathologic criteria give low-, intermediate- and high-probability of clinical DLB related to severity of Lewy body (LB) pathology and inversely related to Alzheimer pathology. There is no prospective study to determine the validity of the revised criteria.

Methods: We systematically evaluated core features (visual hallucinations, fluctuations, parkinsonism) and the suggestive feature, RBD, in 46 patients who were participants in a prospective, longitudinal study of neuropsychology of DLB. All cases had clinically possible (2) or probable (44) DLB.

Results: Of the 44 probable DLB cases, five had one core feature plus RBD, while all others had at least two core features. The two possible DLB cases had one core feature (parkinsonism). At autopsy only two cases did not have LBs (specificity 96%); one had progressive supranuclear palsy (probable DLB) and the other multiple infarcts (possible DLB). Of the 44 cases with LBs, 2 were low-, 11 were intermediate- and 31 were high-probability. All cases had diffuse (39) or transitional (5) LBs; none had brainstem-dominant LBs.

Discussion: This study confirms the specificity of CDLB clinical criteria. More than 95% of the cases met pathologic criteria of intermediate- or high-probability. Neither of the low-probability cases had brainstem-dominant LBs; rather they had advanced Alzheimer pathology. Nevertheless, both had all core clinical features.

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Is Memory Impairment Dependent on Executive Dysfunctions in PD?

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Background and aims: Neuropsychological deficits in Parkinson disease concern disturbance of speed, executive, visuo-spatial and memory functions. Cognitive profile indicates damage of subcortical structures, especially their connections with frontal areas. Aim of this work was to explore the nature of memory and executive functions in PD.

Methods: 32 participants, 16 PD patients (8 women and 8 men) aged 70-81 (mean 76) and 16 healthy controls, matched with age and level education went clinical examination. Rigidity, postural instability and bradykinesia were dominant neurological signs in PD patients. Duration of disease totaled from 2 for 7 years, the mean result of HY was 2,5 and mean MMSE score was 27,5. Neuropsychological assessment of memory, executive and visuo-spatial functions was conducted with special set of methods in dr Luczywek's adaptation.

Results: Analysis of neuropsychological results revealed significant differences in auditory verbal learning, delayed and working memory scores, verbal fluency, Similarities, TMT and Complex Rey Figure Test (CRFT) in favor of control group. PD patient were significantly slower, but there were no differences in simple visual and auditory tasks between the groups.

Conclusions: Deficits of executive functions have turned out to be the most differentiating. Simple tasks scores are similar in both the groups, but PD patients performance decreased with increasing complexity of tasks. Time factor differentiates both groups in all task.

Cognitive slowing and deficits of executive functions predominate in image of PD. Distractibility in PD patients results rather from disturbing influence of frontal signs than primary memory dysfunctions.

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Application of the Middleheim Frontality Score for the Discrimination of Frontotemporal Dementia From Alzheimer's Disease in Greek Demented Patients

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Background And Aims: Discrimination of frontotemporal dementia (FTD) from Alzheimer's disease (AD) is often a challenging task in clinical practice. This is the first study to assess discriminatory power of Middelheim Frontality Score (MFS), a behavioural assessment tool developed recently, with respect to a Greek population.

Methods: Patients with probable AD (N = 101) and FTD (N = 99) were included. Diagnosis of dementia was made according to the DSM-IV criteria. Patients with AD and FTD fulfilled the NINCDS-ADRDA and Neary criteria, respectively. The MFS consists of ten items, each one rated from 0 to 1. Maximum total score is 10. Information was obtained through an interview of the patient and her/his caregiver, following patient's neuropsychological assessment.

Results: Comparing mean total MFS scores, FTD patients had significantly higher scores (6.16±1.3) than AD patients (2.4±1.19) ($p < 0.001$). A positive and highly significant correlation was shown between the total MFS score and diagnosis FTD ($r = 0.848$; $p < 0.001$). Applying a total MFS score of 5 as discriminatory cut-off, a specificity of 97.03% and a sensitivity of 94.94% were achieved.

Conclusions: The MFS is a useful behavioural assessment tool that reliably discriminates FTD from AD patients in a sample of Greek demented patients.

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An Exploratory Study of Estrogen-Related Factors and Incidental Lewy Body Disease (ILBD)

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Background and Aims: ILBD is ten-times more common than Parkinson disease (PD). It may represent preclinical PD, aborted PD, or a non-specific finding. Reduced estrogen may predispose to PD and increased estrogen may protect against PD. There is no data regarding estrogen-related factors and ILBD. The aim of this study was to compare the frequency of gender and estrogen-related factors in persons with and without ILBD.

Methods: We employed the Tissue Registry of the Rochester Epidemiology Project to identify subjects ages 60 and older at death during the time period 1990-2004. We excluded subjects with medical records documentation of parkinsonism, tremor, dementia, or other neurodegenerative diseases, and required multiple physician evaluations within the last five years of life. Formalin-fixed, paraffin-embedded brain tissue blocks were stained for H&E and alpha-synuclein. The blocks were examined for Lewy bodies and neurites in selected brain regions. The medical records were abstracted to identify estrogen-related factors. We considered gender, oophorectomy, body mass index (BMI), and estrogen replacement therapy; and performed logistic regression analyses.

Results: Of 93 subjects included, 12 had ILBD and 81 did not. The ages at death, gender distributions, and frequencies of medical contacts were similar in both groups. ILBD was not associated with oophorectomy or estrogen status, but was associated with BMI (OR = 0.49, CI = 0.25-0.95, P = 0.035).

Conclusions: Of the estrogen-related factors considered, only BMI was associated with ILBD. It is possible that ILBD and PD may not be related. Ongoing clinicopathological study may resolve this uncertainty.

The work was supported by the Morris K. Udall Parkinson's Disease Research Center of Excellence (P50 NS40256), by the NIH grant 2R01 ES10751, by the Mayo Alzheimer's Disease Patient Registry (UO1 AG06786), and by the Mayo Alzheimer's Disease Research Center (P50 AG16574).

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Contrasting Patterns of Neuropsychiatric and Functional Impairment in Lewy Body Dementia and Frontotemporal Dementia

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Background and Aims: Lewy Body Dementia (LBD) and Frontotemporal dementia (FTD) are both characterized by neuropsychiatric disorders and impairment in function.There is little data on whether the same or different patterns of neuropsychiatric and functional deficits occur in these two disorders. By using the same assessment tools across a cohort of patients in these two groups, we wished to elucidate these clinical patterns.

Methods: A cross sectional cohort was collected using standardized diagnostic criteria across 12 Quebec centres.

Results: 17 LBD patients (mean age 69, mean education 10 years) were studied, along with 27 FTD patients (mean age 69, mean 12 years education). Both groups were similarly impaired on the Folstein MMSE, with a mean score of 23.5. The LBD group was significantly more depressed on the Geriatric Depression scale, and on the Neuropsychiatric Inventory (NPI) sub-scale for apathy. The FTD group showed a significantly greater score on the NPI subscale for euphoria, was more impaired in executive functions, and showed greater loss of insight, along with a significantly lower subjective rating of their own memory loss. Nevertheless, the LBD group was more impaired, both on IADLs (measured on the Pfeiffer FAQ), and on the Barthel's Index of functional ability.

Conclusion: apathy and depression in LBD may play a greater role in functional limitation, than loss of insight and frontal executive dysfunction in FTD.

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Anomia and Its Origins in Lewy Body Dementia and Frontotemporal Dementia

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Background and Aims: Lewy Body Dementia (LBD) and Frontotemporal dementia (FTD) are both characterized by anomia. Both disorders share frontal lobe pathology, although LBD is also characterized by dysfunction in basal ganglia and posterior cortex as well. There is little data on whether the same or different patterns of language deficits occur in these two disorders. By using the same assessment tools across a cohort of patients in these two groups, we wished to elucidate these clinical patterns.

Methods: A cross sectional cohort was collected using standardized diagnostic criteria across 12 Quebec centres.

Results: 17 LBD patients (mean age 69, mean education 10 years) were studied, along with 27 FTD patients (mean age 69, mean 12 years education). Both groups were similarly impaired on the Folstein MMSE, with a mean score of 23.5, and had a similar degree of anomia (36/60) on the Boston Naming Test. In terms of semantic knowledge, both groups were equivalently impaired on semantic probe questions. Nevertheless, the FTD group showed a significantly greater impairment in semantic fluency and phonemic fluency (reflecting frontal lobe deficits), while the LBD group showed greater impairment on visuospatial functions (posterior cortical deficits).

Conclusion: Similar degrees of anomia were seen in the LBD and FTD groups, but while the FTD group's deficits were driven by impaired semantic and phonemic search processes, the LBD group's problems were driven more by perceptual deficits.

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Apraxia in Cortical Versus Subcortical Dementia

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Introduction: Ideomotor limb apraxia is a common sign in patients with Alzheimer's disease (AD). In patients with Huntington's disease (HD) the occurrence of apraxia is still controversial. Recent studies described an occurrence of apraxia in HD (Hamilton2003), but until now there has not been a detailed comparison between apraxia in AD and HD.

Methods: To assess the occurrence of apraxia, 23 in-patients with AD and 24 in-patients with HD, treated at the Department of Psychiatry at the Graz Medical University, were examined in imitation of hands' and fingers' movements, execution of gestures and pantomimic movements. AD- and HD-patients did not differ in gender distribution and in severity of dementia (assessed by Mini Mental State Examination), but due to the earlier beginning of the illness HD-patients were significantly younger (44.2a) than AD-patients (74.0a).

Results: In AD-patients apraxia occurred from 12.9% in the assessment of hands' imitation up to 29.0% in the assessment of fingers' imitation and gestures; in HD-patients the occurrence of apraxia was found from 36.8% (gestures) to 73.7% (pantomime). In the comparison of AD- and HD-patients' assessment of fingers' imitation and gestures no difference was found, in the assessment of hands' imitation and pantomime HD-patients scored significantly lower than AD-patients.

Conclusion: Until now apraxia was an exclusive sign of cortical dysfunction but obviously apraxia also may occur in subcortical dementia. There are distinct differences in occurrence of apraxia in AD and HD, for until now we do not

know yet the underlying pathological pathway causing apraxia in each disease.

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Hippocampal Pathology in Patients With Multiple System Atrophy and Dementia

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Cognitive symptoms are not characteristic in multiple system atrophy (MSA) and the clinical criteria of MSA state that dementia is an exclusion sign. In spite of this, several published cases with pathologically proven MSA had dementia.

The hippocampal formation play a strategic role in cognitive functions and hippocampal pathology is present in MSA.

In our study we examined the pathology of the hippocampal formation in patients who had clinically diagnosed dementia and pathologically proven MSA (without other significant pathology) in order to define differences between demented and non-demented patients.

The clinical data of the patients were independently studied by two neurologists who were blind to the neuropathological results. The diagnosis of dementia was made according to DSM-IV. criteria. Patients with no dementia had a CDR score of ≤ 0.5.

Alpha-synuclein and GFAP immunohistochemistry was used to detect glial and neuronal inclusions and astrocytes in the temporal lobe, respectively.

Ten demented (D) and eight non-demented (ND) patients were studied. There was a significant difference between the D and ND groups regarding the weight of the brain. Astrocytic pathology was similar between the groups. Neuronal inclusions also were similarly distributed in the different hippocampal areas in the D and ND groups. The number of PLBs in the white matter of the subiculum was significantly different (D: 39.3±7.1/section, ND: 21.1±5.9/section).

Our preliminary results show that the pathology of the hippocampal formation is different in MSA with and without dementia.

Acknowledgements: The work was supported by the Hungarian Scientific Research Found grant OTKA T46487.

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Clinical and Neuropsychological Measures in Differential Diagnosis of Lewy Bodies Dementia and Alzheimer's Disease

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Background: Some preliminary evidence exists that the pattern of clinical symptoms and cognitive impairment in dementia with Lewy bodies (DLB) is different from Alzheimer's disease and might be useful in differentia;l diagnosis.

The aim of this study: To determine which clinical or neuropsychological feature(s) best predicted a diagnosis of DLB or AD in the early stage of dementia.

Methods: Sixty two subjects matched for age, gender, years of formal education and dementia severity scores have been examined, 20 of those were clinically diagnosed with DLB, 23 with AD and 19 were cognitively intact controls.

Results: There were significant differences in clinical variables among dementia groups despite a similar duration of disease. The NPI, HIS, UPDRS, and ADL scores were significantly worse in the group with probable DLB than in the group with probable AD. The presence/recent history of apathy and visual hallucinations were significantly more frequent in DLB. The onset of neuropsychiatric symptoms was earlier in DLB and symptoms were more severe in this group. The DLB cases also had an increased prevalence of a positive history of REM sleep behavior disorder and anosmia. The DLB group was more impaired on category fluency (animals), visuoperceptual and constructional, and attention tasks.

Conclusions: DLB patients have a different profile of clinical symptoms and neuropsychological deficits early in the course of dementia as compared to the AD group. These features might be useful in differential diagnosis.

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Neuropsychological Patterns of Parkinsonian Disorders : Comparison With PD, MSA, PSP and DLB

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Background: Although motor impairment is the hallmark in parkinsonian disorders, congnitive impairment, particularly frontostriatal pattern, is well established. Despite of growing importance of cognitive symptoms, the study of cognitive assessment of these patients remains very limited. The aim of this study is to compare the pattern of cognitive function and to assess which cognitive domain could be discriminable among various parkinsonian disorders.

Method: Five subject groups (19 DLB patients, 30 PSP, 20 MSA , 28 PD, 19 control) participated in this study. They were all matched for age, education duration and duration of the symptom. All subjects were evaluated using the Seoul Neuropsychological Screening battery. Detailed clinical history, motor examination and clinical disability were also rated .

Results: The performance of four patient groups was significantly impaired compared to control group. The impairment of DLB patients was prominent compared with other patient groups in most tests. The overall performance of patients with PSP and MSA was lower than that of patients with PD. Geriatric depression score is highest in patients with MSA. Verbal memory was the most important in discriminating DLB from PSP. While naming, visuospatial function, word fluency were helpful in discriminating between MSA and PD, visuospatial function and memory were helpful between PD and control.

Conclusion: Patients with DLB show the most prominent cognitive dysfunction, and then come PSP, MSA and PD in that order. Unlike previous study, PSP and MSA patients

showed comparable cognitive impairment and MSA patients showed poor performance compared to PD patients.

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Neuroprotective Effects of Losartan Applied Intranasally in the Transgenic APP/PS1 Mouse Model of Alzheimer's Disease (AD)

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The local angiotensin receptor system of the brain has been shown to play an important role in processes such as cognition and fear.

While the anti-hypertensive therapeutic losartan (angiotensin receptor type 1 antagonist (AT1-R)) has been shown to improve cognitive function in patients with AD, the molecular and cellular mechanisms of its cognition-improving effect in AD remains unclear.

In the present study the influence of losartan on expression of genes involved in processes of inflammation, degeneration and regeneration of neural tissue, as well as on the production of Abeta and acetylcholine was investigated in a transgenic mouse model (APP/PS1) of AD after long-term application of losartan (2-3 months). The data obtained revealed an increase in the expression of genes involved in regulation of tissue integrity (LAMA5), neuroregeneration and hippocampal synaptic plasticity (upregulation of CREB-RP, PTPRZ1) that was accompanied by inhibition of inflammation (upregulation of CISH, PPAR). In addition, losartan decreased the expression of ATF5, the negative regulator of neuronal differentiation. After 2-3 months of intranasal treatment with losartan a strong decrease in production of Amyloid beta protein and upregulation of choline acetyltransferase was detected in the brains of APP/PS1 mice in immunohistochemical studies and by quantification of amyloid plaques.

In conclusion, the local angiotensin receptor system plays an important role in the pathogenesis of AD. The blockade of AT1-R may improve the regeneration and functionality of neural tissue by its influence on production of acetylcholine, Abeta, local inflammation and other factors involved in neuroregeneration.

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A Preliminary Study on APP, Beta and Gamma-Secretase Expression in ALS Transgenic Model

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Background and Aims: The molecular bases underlying the pathogenesis of neurodegenerative diseases are gradually disclosed. Rare, inherited mutations causing familial forms of these disorders have provided important insights into the molecular networks implicated in disease pathogenesis. Evidence is accumulating to suggest that such chronic neurodegenerative disorders as Alzheimer's disease (AD) and Amyotrophic Lateral Sclerosis (ALS) are caused by a combination of events that impair normal neuronal function. An hypothetic link between AD and ALS was suggested by finding of both an higher amount of amyloid precursor protein in the spinal cord anterior horn neurons and of A beta peptides in ALS patients skin. The aim of our study is to investigate, if an alteration exists in the expression of APP, BACE1 or gamma-secretase components involved in AD pathogenesis in the ALS model G93A mice.

Methods: Three different cortical cultures were prepared: murine, expressing human SOD (hSOD) and G93A mutated hSOD cells. A real time PCR method was used to analyse genes expression. Protein expression was analyzed by Western Blot.

Results: Our preliminary study performed on mRNA and protein levels, shows a significant over-expression of both APP and BACE1, in cortical G93A cultures compared to hSOD, used as controls. An increase of PSEN1 and PSEN2 mRNA levels are also evident, but without any statistical significance.

Conclusions:The APP and BACE 1 modulation, significantly showed during embryonic day, suggests for their potential role in ALS.

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Dopaminergic Projection From the Midbrain in Dementia With Lewy Bodies

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Background: Dopaminergic projections from midbrain are believed to play a role in symptoms in dementia with Lewy bodies (DLB). Dopaminergic neurons in A9 project to putamen, while those in A10 project to the nucleus accumbens. Dopaminergic neuronal loss is variable in DLB; however, it remains to be elucidated if it correlates with clinical symptoms.

Methods: All 24 DLB patients (19 men; 5 women) were participants in a prospective, longitudinal study of neuropsychology of DLB. The dopaminergic marker, tyrosine hydroxylase (TH), was measured by immunofluorescence and image analysis in the striatum and correlated with neuronal cell loss in A9 and A10, as well as clinical features (UPDRS and Neuropsychiatric Inventory - NPI). Neuronal cell loss in A9 and A10 was assessed as none, mild, moderate or severe.

Results: A wide range of neuronal loss was observed in A9 and A10. Neuronal loss in A9 correlated with last UPDRS ($r=0.35$, $p<0.05$). The degree of neuronal loss in A9 correlated with TH in putamen ($r=-0.68$, $p<0.001$) and A10 correlated with TH in nucleus accumbens ($r=-0.044$, $p<0.05$). There were significant correlations between TH in the putamen and last UPDRS ($r=-0.41$, $p<0.05$) and the annualized rate of change in

the UPDRS (r=-0.51, p<0.05). The NPI did not correlate with A10 neuronal loss or TH in the accumbens.

Conclusion: Dopamine neuronal loss is variable in DLB. Degeneration of A10 neurons correlates with accumbens TH, but not psychiatric symptoms. Degeneration of A9 neurons correlates with putamen TH as well as severity and rate of change of parkinsonism.

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Synuclein-Mediated Microglial Activation in Vivo and in Vitro

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Parkinson's disease (PD) is an age-related slowly progressive neurodegenerative disorder with preferential loss of nigrostriatal dopamine neurons. The pathological hallmark of PD is the presence of intracytoplasmic synuclein-positive inclusions, so-called Lewy bodies, in the dopaminergic neurons. A study demonstrated that triplication of wild-type (WT) α-synuclein (SYN) leads to early-onset PD. In addition, a growing body of evidence suggests an inflammatory process in the substantia nigra (SN) and striatum (STR), characterized by activation of microglia, in patients with PD. The association of SYN overexpression, microglia-mediated inflammation and disease progression remains unclear. To dissect the roles of SYN and microglia in disease progression, we have employed a mouse model of overexpressing human SYN exclusively in tyrosine hydroxylase-containing neurons (SYNwt+/+). We applied qRT-PCR to identify upregulated transcription 1-month old SYNwt+/+ mice compared with non-transgenic animals (NTG). The results demonstrated that TNFα and the microglial cell marker Iba1 were increased in 1-month old SYNwt+/+. We also used Iba1 immunohistochemistry to determine numbers of activated microglia in the SN and STR. We found more activated microglia in the SN of 1-month old SYNwt+/+. To further examine the direct effect of SYN on microglial activation, we added exogenous WT SYN to primary microglia-enriched cultures from NTG mice. The data demonstrated that microglia responded to WT SYN in a dose-dependent manner. Furthermore, we identified increased transcription levels of TNFα, IL1β, IL6, COX2, NOX2 and iNOS in WT SYN-treated microglia. Together, these studies implied the involvement of WT SYN in activating microglia in vivo and in vitro.

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A Case of Frontotemporal Dementia With Family History

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The average age of frontotemporal dementia onset is much younger than that of the Alzheimer's disease. Behavioral disturbances occur at the early stage of frontotemporal dementia, and social dysfunction and personality deterioration may ensue. For this reason, it may be misdiagnosed as other psychiatric disorders. Therefore, more careful observation and concern is required for proper diagnosis. This report is about the case of a patient who had been misdiagnosed of disorders like schizotypal personality disorder, schizophrenia, obsessive compulsive disorder etc., and therefore have been treated for those. We reviewed this case based on the data of neuropsychiatric history, family history, neuropsychological test, brain MRI, and SPECT, and considered this case as frontotemporal dementia with family history. This is the first frontotemporal dementia case with family history in Korea, so hereafter we may discover the gene locus associated with this case. This study can be very useful for the following frontotemporal dementia studies in Korea.

Key words :

Frontotemporal dementia, Dementia, Brain SPECT, Brain MRI, Family history

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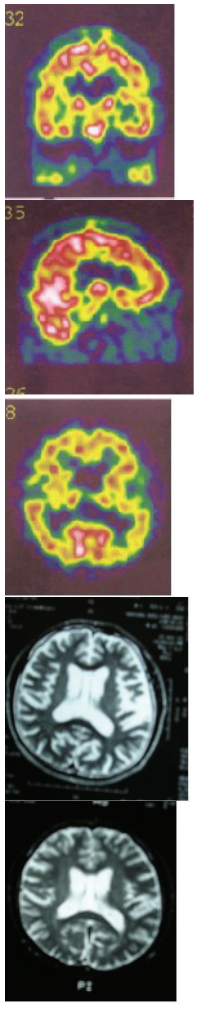

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LRRK2 Mutation Screening Reveals a Novel Mutation in a Patient With Benign Tremulous Parkinsonism

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Background and Aims: Benign tremulous parkinsonism (also known as benign tremulous Parkinson's disease or tremor dominant Parkinson's disease) is characterized by rest and postural tremor as the main feature, but mild bradykinesia, rigidity, or both also exist. Family history of tremor or parkinsonism is present in more than 60% of probands. Mutations in the Leucine-rich repeat kinase 2 gene (LRRK2) may account for up to 13% of familial and 3% of sporadic PD cases. Due to the remarkably frequency of positive family history of tremor or PD in benign tremulous parkinsonism, we attempted to test whether mutations in LRRK2 could lead to a benign form of PD.

Methods: Twenty-nine unrelated patients from Spain were included in the study. Mean age at onset was 64,2 years, and 78% had a family history of ET or PD. DAT-SCAN, neuropsychological and olfactory tests, and UPDRS scales were performed in all participants. The 51 exons of LRRK2 and their exon-intron boundaries were sequenced for all individuals.

Results: We identified a heterozygous carrier of an exon 48 mutation (c.G7168A) predicted to replace the valine at position 7168 of the Dardarin protein to methionine. This mutation was absent in 178 control chromosomes from healthy individuals and in 254 chromosomes from PD patients.

Conclusion: Our results suggest a broader clinical heterogeneity related to LRRK2 mutations. The results also points towards benign tremulous parkinsonism as a subgroup of PD, in which disabling tremor but otherwise mild parkinsonian signs are the main feature.

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Genes and Parkinson's Disease – A Report on a Portuguese Cohort

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Background: Genetic studies in families with mendelian inheritance of Parkinson's Disease (PD) have reported the cloning of several disease-associated genes. There have been described so far six of these genes, in which, mutations are considered to be pathogenic. Although the first gene reported to be involved in the onset of the disease is Alfa-synuclein, mutations are more frequent in the genes Parkin and LRRK2.

Aims: Here we report on an ongoing evaluation of coding variants in four of the six genes involved in PD performed in 124 patients selected in the movement disorders clinic of the Coimbra University Hospital.

Methods: This is a consecutive clinic case series comprised of patients who gave permission for sampling. All genes were subjected to direct sequencing, and additionally Alfa-synuclein, Parkin and PINK1 were screened for gene dosage mutations.

Results: No mutations were found in Alfa-synuclein, but the remainder genes all yielded mutations in our sample. Our findings show a relatively high percentage of cases presenting a pathogenic mutation.

Conclusions: There are two potential outcomes of the genetic analysis of PD. First, improvement of diagnostic accuracy with earlier and perhaps presymptomatic diagnosis. Second, an understanding of disease pathogenesis to facilitate modelling in cell systems and in animals so that treatments can be developed and tested. This work, despite being in process, clearly shows PD as having a genetic component, and further emphasizes the importance of genetic testing of these patients, aiming at a better and earlier diagnosis and thus of more effective clinical practice.

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Mutations in the Tdp-43 Gene Tardbp Are Not a Frequent Cause of Frontotemporal Dementia

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Background and Aims: The TAR DNA binding protein (TDP-43) is a component of brain inclusions of tau-negative frontotemporal dementia characterized by ubiquitin-immunoreactive inclusions (FTDU), the most frequent neuropathological FTD subtype. Mutations in the genes encoding progranulin (PGRN), valosin containing protein (VCP) and chromatin modifying protein 2B (CHMP2B) are identified as a cause of FTDU; however not all FTDU patients are explained by a mutation in these genes. In this study we investigated the hypothesis that the gene encoding TDP-43 (TARDBP) contributes to the genetic heterogeneity of FTDU.

Methods: We performed mutation and association analyses of TARDBP in a collection of 165 Belgian FTD patients (mean onset age 64.5 ± 10.1 years; range 29-90 years), including neuropathologically confirmed FTDU patients without mutation in the known FTD genes.

Results: We did not find a disease-related mutation in any of the six TARDBP exons or flanking intronic regions. Using the common sequence variants identified during mutation screening, we are performing genetic association studies in the 165 FTD patients and matched controls.

Conclusion: In conclusion, TARDBP mutations are not a common cause of FTD.

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Genetic Modifiers of the Phenotype of Alpha-Synuclein Associated Familial Parkinson's Disease

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Background: Familial parkinsonism is associated with mutations in the alpha-synuclein, parkin, tau, UCHL-1, DJ-1, PINK1and LRRK2 genes. While different forms of familial parkinsonism are associated with a single mutation, there is phenotypic variability within and between kindreds with the same mutation. Phenotypic differences include age of disease onset, disease progression, disease severity and neuropathology. We described a Greek-American kindred (Family H) with the alpha-synuclein A53T mutation. Its phenotype is rigidity-bradykinesia predominant.

Methods: We sequenced the parkin gene exons in Family H members and ethnically matched controls. We clinically characterized affected members. Brains were stained with routine stains and with alpha-synuclein and tau antibodies.

Results: A polymorphism in exon 4 of parkin (S167N) was identified in a nuclear family within Family H. Two A53T alpha-synuclein heterozygotes differed in their parkin genotypes. The clinical phenotype of the N167 homozygote individual consisted of early disease onset rigidity, bradykinesia, early dementia, myoclonus, sleep disorder and rapid disease course. The clinical phenotype of the S167 homozygote consisted of later disease onset rigidity, bradykinesia and late onset dementia with a longer disease course. A comparison of their neuropathological findings demonstrated differences. The N167 homozygote exhibited extensive neuronal loss in the hippocampus and alpha-synuclein-positive neurites and neurofibrillary tangles. In contrast, the S167 homozygote exhibited less neuronal loss, fewer alpha-synuclein-positive neurites and more Lewy bodies.

Conclusions: These findings suggest that parkin acts as a modifier of the A53T alpha-synuclein associated phenotype and that genotypic differences within kindreds contribute to the disease phenotype.

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Alpha-Synuclein Polymorphisms Associate With Neurofibrillary Pathology in a Finnish Elderly Population

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There is growing evidence for a clinical and pathological overlap between Alzheimer's disease (AD) and Parkinson's disease (PD). We studied whether genetic variation in alpha-synuclein associates with common forms of brain pathology in a neuropathologically verified, population based sample of very elderly Finns (Vantaa 85+, n=268). We genotyped 10 alpha-synuclein polymorphisms and tested their possible associations to histologically defined neo-cortical beta-amyloid- (Cerad score), neurofibrillary- (Braak stage), and Lewy body pathologies. Genotype distributions were compared between different groups by chi-square and Fisher's exact tests. None of the SNPs associated with beta-amyloid or Lewy body pathologies, but 5 SNPs associated with neurofibrillary pathology (Table). Polymorphisms located in the 3'-end of the intron 4 and in the intron 2 showed the most significant association. The same SNPs also associated significantly with neurofibrillary pathology in the APOE4-negative subpopulation, suggesting an effect independent from APOE. In conclusion, our results suggest that variation in the alpha-synuclein gene is a risk factor for AD-type neurofibrillary pathology in the very elderly Finnish population.

SNCA SNP	Braak Stage* (p-value)	distance** (base pairs)
intron 2		
rs2583985	0.002	-
rs2583978	0.032	5,613
intron 4		
rs2737020	0.440	15,061
rs1812923	0.042	16,400
rs3775439	0.093	46,198
rs356186	0.191	50,575
rs356164	0.593	62,463
rs356198	0.480	73,435
rs2572324	0.0004	77,141
rs356168	0.037	81,508

*130 subjects with Braak stage 0, 1 or 2 were compared with 67 subjects with Braak stage 4, 5 or 6

** Distance from the marker rs2583985

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Association of HFE Common Mutations With Parkinson's Disease, Alzheimer's Disease and Mild Cognitive Impairment in a Portuguese Cohort

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Background and Aims: Pathological brain iron deposition has been implicated as a source of neurotoxic reactive oxygen species in Alzheimer (AD) and Parkinson diseases (PD). Iron metabolism is associated with the HFE gene, and mutations in the HFE gene are often present in hemochromatosis. Here we intend to establish an association between these mutations and neurodegenerative diseases, such as AD and PD.

Methods: Genotypes were analysed for the two most common variants of the HFE gene in a series of 130 AD, 55 Mild Cognitive Impairment (MCI) and 132 PD patients. Additionally, a series of 115 healthy age-matched controls was also screened.

Results: A statistically significant association was found in the PD group when compared to the controls, showing that the presence of variant allele may confer higher risk for developing the disease.

Conclusions: Taken together these results suggest that the common variants in the HFE gene may be a risk factor for PD, but not for AD in the Portuguese population.

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Mutations in Patients With Frontotemporal Dementia That Alter Dosage Levels of Progranulin

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Mutations in progranulin (PGRN) were recently identified in ubiquitin-positive frontotemporal dementia linked to chromosome 17q21 (FTDU-17). These mutations resulted in loss of functional PGRN due to the creation of null alleles suggesting a haploinsufficiency mechanism. Therefore deletions leading to a non-functional allele and/or promoter mutations reducing transcriptional activity could be expected in FTDU patients. A Belgian series of 104 FTD patients was systematically screened for such mutations. Multiplex amplicon quantification (MAQ) and real time PCR allele quantification (qPCR) of all 13 PGRN exons suggested the presence of 4 deletions of more than 3 consecutive exons. These data are being confirmed by alternative techniques including comparative genomic hybridization (CGH). In addition, sequencing data of non-coding exon 0 and conserved regions in intron 0 revealed 2 variants in one patient each, which were absent in 444 control individuals: the previously described IVS0+5 G>C loss of allele mutation and a G-to-T transition at position 148 of exon 0. Here we measured transcriptional activity of these variants using a dual luciferase reporter assay system. The IVS0+5 G>C mutation, located in a known TAF binding site and predicted to destroy an AREB6 binding site, showed a decrease in PGRN transcriptional activity of about 25%. The variant in exon 0 had no effect on transcription. Together our data underline the importance of PGRN dosage and extended the mutation spectrum of PGRN explaining up to 14% of the genetic etiology of FTD and 27% of familial FTD in the Belgian patients which is higher than previously estimated.

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Effects of Beta Amyloid-Metal Complexes on SH-SY5Y Neuroblastoma Cells: Gene Expression Profile Analysis

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Typical neuropathological features of Alzheimer's disease include the presence of senile plaques made up of beta-amyloid (Abeta) and neurofibrillary tangles in the brain. In addition, metal ions have been shown to abnormally accumulate in the brain with aging and deeply in Alzheimer's disease. Recently, we have demonstrated that some metal ions play a relevant role in conditioning amyloid aggregation. In this connection, to investigate the effects induced by amyloid-metal complexes on gene expression on SH-SY5Y neuroblastoma cells, we performed a microarray analysis of 35130 genes with iron, zinc, copper, aluminum and their relative amyloid complexes.

This comparative study showed that the up-regulated genes after treatment with Abeta-Fe were associated with the regulation of enzyme activity (APH1A), intracellular transport (CCS), metabolism (LOX). In presence of Abeta-Zn the microarray analysis revealed selective up-regulation in level of transcripts encoded by genes associated with the intracellular signalling cascade (ESR2) and metal ion homeostasis (FTL, TF). The modulated genes of the Abeta-Cu showed the over-expression of genes implicated in cell communication (APPBP1), nuclear division (NOLC1), protein metabolism (MRPS21). These results demonstrated that different Abeta-metal complexes produced a different pattern of gene expression. However, the most important finding was obtained in the presence of Abeta-Al that produced the over-expression of genes directly related to Alzheimer's disease such as Amyloid-like protein 1 and 2 precursor and Microtubule-associated protein tau (MAPT). Based on these data, Abeta-Al complex seemed to be strongly involved in the etiopathogenesis of Alzheimer's disease.

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Genomic Analysis of Alzheimer's Disease Neuronal Cell Models

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Background and aims: Sporadic Alzheimer's disease (AD) is a trait resulting from combinations of genes and environmental factors. The apolipoprotein E ε4 allele is the main genetic risk factor for AD identified to date, although it is widely accepted that other genes should be involved in AD. Association studies in candidate genes are a valuable tool for identifying genes contributing to the development of complex diseases.

Our purpose is to find novel genes modulating AD risk and/or important in the pathogenic proccesses underlying the disease, which constitute potential therapeutic targets.

Methods: We use a two phase strategy, first analyzing cell models, and then genetic association studies in AD case control samples.

Gene expression of neuronal cell models mimicking several aspects of AD pathogenesis is analyzed in microarrays followed by a kinetic study in quantitative PCR low density arrays, to select the responder genes/functions.

Since the selected genes respond to the specific stimulus used in each model we hypothesize that, if the pathogenic process mimicked in the model is actually involved in AD pathogenesis, they could modulate susceptibility to AD, and to prove it we perform genetic association studies in case control samples.

The models include oxidative, endoplasmic reticulum and adrenergic stress.

Results/Conclusions: We have identifyed several genes which expression is modulated in the human neuroblastoma SKNMC as a response to oxidative and adrenergic stress. At leat two of them showed association with AD.

Our results are compatible with the hypothesis that oxidative stress and adrenergic stress participate in AD pathogenesis.

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Beta-Amyloid 1-42 Induced Expression Study in Rat Brain Using DNA Microarray and Qrt-Pcr Technology

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Alzheimer's disease is a common neurodegenerative disorder that is caused mainly by β amyloid (Aβ) peptide, formed by enzymatic cleavage from amyloid precursor protein.

β-amyloid monomers can polymerize forming complex aggregates eg. oligomers, protofibrils and fibrils and disturb the normal function of the neurons.

The objective of our research was to model β-amyloid toxicity. Synthetic Aβ 1-42 peptide was administered injected into the rat brain 3rd ventricle. After 72 h incubation, the cortex was dissected, total RNA was isolated and reverse transcribed to create a cDNA library. The cDNA was hybridized to the DNA microarray containing 24000 gene specific oligonucleotid sequence. It was found in the DNA microarray experiment that altogether the expression ratio of 91 genes were changed. Quantitative real-time polymerase chain reaction (QRT-PCR) method was used to measure the exact values of the expression ratio.

Our results revealed several possible mechanisms of the β amyloid neurotoxicity eg. activation of glial and astrocyte cells, disturbing the normal function of synapses and the ubiquitin proteasoma system (UPS). These findings help to understand the β amyloid induced molecular mechanisms involved in the Alzheimer's disease.

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DNA Methylation and CNS Gene Expression in AD Models

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An increasing number of evidences sustain the existence of a correlation between elevated levels of plasma Homocysteine (HCY) and Alzheimer's Disease (AD). Nevertheless, it remains still unclear whether HCY is a primary risk factor, a co-factor or a consequence in the onset of the disease.

Our aim is to investigate the relationship between the alteration of HCY, S-adenosylmethionine (SAM) levels and the biological methylation reactions with particular attention to DNA methylation and gene expression. We already demonstrated that elevated HCY levels are responsible for impaired DNA methylation with consequent PS1 and BACE over-expression and amyloid-beta over-production in a human neuroblastoma cell line and in transgenic CRND8 mice. It is well known that DNA methylation can be a tissue-specific and also sequence-specific epigenetic modification. Although we didn't observe any change in expression of other genes involved in AD (like APP, PS2, Adam10, TACE) when HCY cycle was altered, we think it is important to verify the consequences of such alteration in the nervous tissue. In order to achieve this objective, we performed a large-scale expression analysis of CNS genes using a cDNA-array with human neuroblastoma cells in different conditions, reproducing alterations of HCY metabolism that lead both to DNA demethylation and DNA hypermethylation. This approach will allow us to evidence the possible involvement of modulated genes in the onset of AD. We found that only a minor fraction of CNS genes is modulated by methylation; here we report the results regarding the Real-Time PCR analysis on the modulated genes.

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Diagnostic Usefulness of FP-CIT Spect Imaging in Dementia With Lewy Bodies

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Clinically based diagnostic criteria for Dementia with Lewy bodies (DLB) have limited accuracy. The availability of a biomarker to assist with diagnosis would be a major advance. Severe nigrostriatal degeneration with presynaptic dopamine transporter (DAT) loss occurs in DLB but not in most other dementia subtypes offering a potential system for a biological marker.

In the PDT301 study, conducted across 40 European sites, 326 patients with dementia with clinical diagnoses of probable or possible DLB, or non-DLB type dementia established clinically, had a FP-CIT (DaTSCANTM) SPECT labelling the dopamine transporter density in the striatum. The objective of the study was to assess the diagnostic efficacy of FP-CIT in the differential diagnosis between DLB and non-DLB.

Three readers, blinded to clinical diagnosis, classified the images as normal or abnormal (reduced uptake) by visual inspection. A consensus panel of three old age psychiatrists reviewed the clinical features of each case to establish a clinical diagnosis that was used as a standard of truth.

The results of this study confirm the high correlation between abnormal DAT density measured using FP-CIT SPECT and a clinical diagnosis of probable DLB. Sensitivity of 78% (range 75%-80.2%) and specificity of 90% (range 88.6%-91.4%) was reported in comparison to the clinical diagnosis of the consensus panel. No differences were seen upon sub-analysis based on age, severity of cognitive impaiment (MMSE) or parkinsonism (UPDRS) or radioactivity dose.

The diagnostic accuracy is considered sufficiently high for this to be clinically useful in distinguishing DLB from AD.

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Single-Neuron Quantification of DNA Reveals Constitutional Aneuploidy and DNA Replication in Normal Adult Brain and AD

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Cell-cycle re-entry of mature neurons, including DNA replication, might play a major role in Alzheimer´s disease (AD). A more than diploid DNA content in differentiated neurons might alternatively result from chromosome mis-segregation during development. To distinguish between these two mechanisms of aneuploidy, we quantified the single-cell DNA amount of individually identified neurons of normal adults and AD patients by three independent methods, Laser Scanning Cytometry, Chromogenic in situ Hybridisation and single cell PCR amplification of alu repeats and analyzed the link between DNA content and expression of the early mitotic marker cyclinB1. The majority of neurons contains a 2n DNA content. With all three methods, however, a constant number of about 10% of neurons containing a more than diploid DNA content is obtained in control, with about 0.5% being tetraploid. None of the 4n neurons in controls expresses cyclin B and, therefore, most likely represent constitutional aneuploidy. In AD, the number of neurons with more than 2n DNA is increased to up to 30% and 1%-2% contain 4n DNA. All the tetraploid neurons express cyclin B indicating that mature neurons have passed the S-phase. Our results indicate that two different mechanisms need to be distinguished giving rise to a more than diploid DNA content in the adult brain. Constitutional aneuploidy in differentiated neurons of the normal brain might be more frequent than previously thought. In AD, in addition, 1% to 2% of neurons have re-entered the cell cycle and entirely passed through a functional interphase with a complete DNA replication.

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Origin Recognition Complex in Alzheimer's Disease - Bridging the Gap Between Aberrant Synaptic Plasticity and Unscheduled Neuronal Cell-Cycle Re-Entry ?

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In Alzheimer´s disease (AD), a variety of cell cycle regulators are upregulated in vulnerable neurons already in early stages of AD. Moreover, a considerable number of neurons contain a tetraploid content of DNA and simultaneously express cyclin B1, indicating that they have progressed towards the S-phase and replicated their DNA. Initiation of DNA replication is a multi-step process that depends on the formation of pre-replication complexes which involve origin recognition complex (ORC) proteins. Human ORC subunits ORC1, ORC2 and ORC3 are localized in the

nucleus of proliferating cells where they regulate G(1)-S transition. In neurons, however, ORC3 might have additional functions related to synaptic plasticity. Here we show an involvement of ORC subunits in AD pathology. Levels of ORC subunits are differentially altered in the cytosolic and membrane bound fraction. ORC compounds, furthermore, are associated with neurofibrillar pathology. These results indicate in AD an abnormal expression and/or subcellular distribution and seggregation of ORC proteins which might compromize their physiological function at the DNA and membrane compartments and might, thus, help to understand the link between aberrant synaptic plasticity and neuronal cell-cycle re-entry and induction of cell death in AD.

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Role of Substance P for Induction and Course of Alzheimer's Disease

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Background: Alzheimer's Disease is most common form of dementia and one of complicated neurodegenerative diseases. Neurodegenration causes altered function and concentration of many neurotransmitter especially acetylcholine and substance P.

Objectives: Pathophysiological effects of altered substance P concentration in AD brain will be covered with context to preventive and therapeutic methods.

Methods: It is a review to study pathophysiological effects of altered substance P concentration during entire course of AD.

Results: Significant reductions of 20 to 40% in substance P-like immunoreactivity is present in AD cerebral cortex and hippocampus, most severe in inferior temporal gyrus. Substance P half-life is not significantly altered in hippocampus or caudate tissues from AD brain compared to temporal cortex. Despite modest quantitative depletion of substance P in AD cortex, there is a significant depletion of substance P-like immunoreactive perikarya. Coadministered of substance P prevent ß-amyloid-induced neuronal loss into cerebral cortex. Efferent fibers to hippocampus from mamillary body secrete an increased amount of substance P in AD.

Conclusion: It is observed that reduction in amount of substance P is not parallel with reduction in number of substance P secreting neurons in hippocampus of AD. This is in part that mamillary body efferent to hippocampus contributes for sustain substance P release. This increased production of substance P by mamillary body could be inducing step for AD by altering function of receptors and neuronal enzymes through enhanced neuronal excitation.

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Wnt Pathway Activation Occurs Early in the Course of Tau-Induced Neurodegeneration Linked to Frontotemporal Dementia

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Background and Aims: Glucogen synthase kinase-3beta (GSK-3ß) is an important tau kinase with a potential role in frontotemporal dementia (FTD) and other tauopathies. It is also a central regulator of the Wnt-pathway, which is involved in neuronal cell fate. We previously showed in a drosophila model of tau expression that increased GSK-3β leads to the enhancement of tau-induced neurodegeneration. Here, we aimed to evaluate the role of GSK-3β in the CNS of the JNPL3 transgenic mouse expressing the P301L tau linked to FTD.

Methods: We investigated cortex and spinal cord of JNP3L mice and controls using immunohistochemistry and immunoblotting to detect alterations in the expression of GSK-3β and the downstream component of the Wnt pathway, β-catenin, which is linked to cell fate. We generated an in vivo reporter model of β-catenin expression to investigate β-catenin expression changes throughout the disease course.

Results: We show that GSK-3ß becomes increasingly associated with insoluble tau in the JNPL3 mouse model of FTD during the early stages of the degenerative process, concommitent with an increase in the downstream Wnt-pathway component ß-catenin. Using the in vivo reporter system in a transgenic model, we show that this leads to activation of downstream wnt signaling.

Conclusions: Our findings indicate that Wnt pathway activation occurs during the early stages of the neurodegenerative process. This finding is important for the identification of the Wnt pathway as a potential therapeutic pathway.

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Evidence for Cortical Hypoperfusion by Arterial Spin Labeling in a Mouse Model of Alzheimer's Disease

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Introduction: Studies in AD patients showed that perfusion impairments can be detected non invasively by MR protocols based on spin labelling1. In our study we evaluated whether, MRI can detect perfusion modifications in APP/PS1 mouse models of AD. The mice were followed up in a longitudinal way, and cerebral blood flow (CBF) was assessed in different brain regions in normo and hypercapnia conditions by a quantitative spin labeling MRI technique.

Materials and Methods: APP/PS1 mice modelling cerebral amyloid deposits and PS1, amyloid free, mice were studied at 54±1weeks (nAPP/PS1=9, nPS1=10) and at 62.5±1weeks of age. A FAIR Look-Locker gradient-echo spin-labeling method 2 was used at 4.7T. (50 echos, TE/TR=1.59/150ms, α=12.5°, FOV: 20x20mm2, slice thickness: 1.5mm, input matrix 128x64). Perfusion values (CBF/λ= [(T1global/T1blood)*(1/T1selective-1/T1global)]) were calculated in the parietal cortex, the thalamus and the hippocampus.

Results: Under normocapnia and hypercapnia, at both studied ages, APP/PS1 animals showed a significantly lower CBF in the parietal cortex than PS1 mice. In other evaluated brain regions, the CBF was similar in both genotypes.

Conclusion/Discussion: Our longitudinal study showed that under normo and hypercapnia, the parietal cortex of APP/PS1 mice is less perfused than that of PS1 animals. As perfusion can be measured both in humans and animals, it might be used as a translational marker of AD.

Reference: 1. Alsop DC et al. Ann.Neurol. 47(1): 93-100; 2000; 2. Kober F et al., Magn Reson Med. 51:62-67; 2004.

Acknowledgments: Sanofi-Aventis Neurodegenerative Disease Group -ATC (INSERM)- French 'Federation for Brain Research'-Neuroscience ACI research.

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The Effect of L-Dopa and Folate Deficiency on Homocysteine and Methylation Cycle Metabolites in Mice

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Background: Studies have shown that L-dopa treatment can increase plasma concentrations of homocysteine (Hcy). In controlled studies L-dopa induced hyperhomocysteinemia in Parkinson's disease (PD) is associated with dyskinesia, depression and cognitive decline. Potential mechanisms of L-dopa induced Hcy neurotoxicity include increased oxidative stress and activation of NMDA receptors. The metabolism of L-dopa consumes S-adenosylmethionine (SAM) and produces S-adenosylhomocyteine (SAH), resulting in altered SAM/SAH ratios in tissues that affect other critical methylation reactions. Since adequate tissue stores of folate are required to maintain low levels of Hcy and maintain SAM synthesis we studied the interaction between folate deficiency and L-dopa treatment on blood Hcy and brain SAM and SAH levels.

Results: Mice maintained on control, low folate or folate deficient diets for 2 months received i.p. injections of L-dopa (100mg/kg) and benserazide (10mg/kg). Low folate and folate deficient diets increased plasma Hcy 1.5 and 10 fold respectively. L-dopa treatment significantly increased plasma Hcy 7.5 and 15.9 fold in mice on low folate and folate deficient diets respectively. In brain tissue the SAM/SAH ratio was significantly reduced in mice treated with L-dopa, an effect that was exacerbated in the presence of folate deficiency.

Conclusion: PD patients in negative folate balance or homozygote for MTHFR C677T, a common polymorphism that affects folate metabolism, may be at increased risk for Hcy induced and methylation dependent neurotoxicity.

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Structure and Function Relationship of Met1-Val60 Segment of the Tyrosine Hydroxylase Molecule Regulatory Domain

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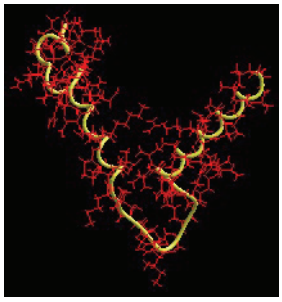
Our investigation is devoted to study the spatial organization, conformational possibilities of the tyrozine hydroxylase molecule functional active segment of the regulatory domain and its molecular dynamics in water solution. The calculations were carried by the methods of theoretical conformational analysis and molecular dynamics. The -reversible turn on the segment Met30-Ser40 resulted in parallel orientation of two -helixes in the regulatory domain structure and its stability at the molecular dynamics simulations in water solution had been shown. The energies of the various types intermolecular interactions and hydrogen bonds formations, the distances between the functional groups as well as the values of the backbone internal dihedral angles of the low-energy conformational states of the regulatory domain were calculated. The high flexibility of the Arg37, Arg38, Ser40 amino acids side chains was revealed. The effect of the amino acids chemical modifications on the spatial structure and conformational possibilities of the tyrozine hydroxylase molecule regulatory domain was studied too by the called methods. The results were compared with available experimental data and the main principles of the relationship between structure and function of the tyrozine hydroxylase molecule regulatory domain were reported.



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Aβ42 is More Rigid Than Aβ40 at the C-Terminus: Implications for Aβ Aggregation and Toxicity

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Background and Aims: Although Aβ42 differs from Aβ40 by only two residues, Aβ42 is much more prone to aggregation and more toxic to neurons than Aβ40. Protein dynamics may contribute to such dramatic difference in function.

Methods: Backbone ps – ns dynamics of native Aβ monomers were characterized by 15N spin relaxation at 273.3 K and 800 MHz.

Results: R1, R2 and NOE values are similar in Aβ40 and Aβ42, except at the C-terminus, indicating Aβ42 and Aβ40 monomers have identical global motions. Comparisons of the spectral density function J(0.87 H) and order parameters (S2) indicate that the Aβ42 C-terminus is more rigid than the Aβ40 C-terminus. At 280.4 K and 287.6 K, the Aβ42 C-terminus remains more rigid than the Aβ40 C-terminus, suggesting such a dynamical difference is likely present at the physiological temperature.

Conclusion: The Aβ42 monomer has less configurational entropy due to restricted motion in the C-terminus and likely pays a smaller entropic price to form fibrils than the Aβ40 monomer. This can partly account for the fact that Aβ42 is the major Aβ species in parenchymal senile plaques in most AD brains in spite of the predominance of Aβ40 in plasma and

CSF. The rigidity of the Aβ42 C-terminus is likely due to its preordering for β-conformation present in soluble oligomers and fibrils. The Aβ42 C-terminus may therefore serve as an internal seed for aggregation.

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Aβ40 Protects Nontoxic Aβ42 Monomers From Aggregation

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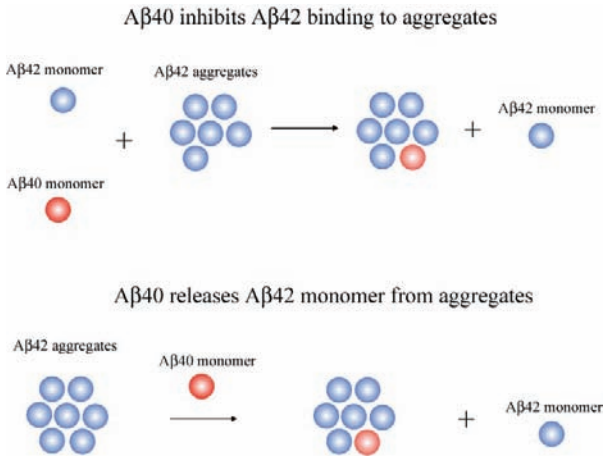
Background and aims: The leading amyloid cascade hypothesis states that the neurotoxicity of Aβ42 plays a central role in Alzheimer's. Emerging evidence from human genetics, cell biology and transgenic mice shows that Aβ40 may play a protective role in AD. However, the molecular nature of Aβ40 protection is not known.

Method: Solution NMR.

Results: we show Aβ40 monomers inhibit the aggregation of nontoxic Aβ42 monomers, in an Aβ40/Aβ42 ratio dependent manner. Aβ40 monomers bind to Aβ42 aggregates with higher affinity than Aβ42 monomers and block the binding of Aβ42 monomers. In addition, Aβ40 can release Aβ42 monomers from Aβ42 aggregates.

Conclusion: Aβ40, by protecting nontoxic Aβ42 monomer from aggregating into toxic species, plays an equally important role as Aβ42 in the pathogenesis of Alzheimer's. This leads to the novel hypothesis that Aβ40 itself can used for the prevention and therapy of AD.



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Expression of Amyloid Beta-Binding Protein 17beta-HSD10 in Hippocampi of People With Alzheimer Disease and Schizophrenia

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Lateralization of human brains probably underlies specialization of both hemispheres. Left – right asymmetry has been observed on different levels (e.g., structural, functional or neurochemical) as well as changes in its degree in people with Alzheimer disease (AD) or schizophrenia. Both disorders are accompanied by a dysfunction of mitochondria. It is suggested that the intraneuronal accumulation of amyloid beta peptides and their binding to multifunctional mitochondrial enzyme 17beta-hydroxysteroid dehydrogenase type 10 (17beta-HSD10) could be involved in AD pathogenesis. The increased expression of enzyme was found in cortical and hippocampal neurons of AD patients when compared to age-matched controls. The complex of peptides and enzyme is localized in cytosole and mediates apoptosis. Aims of the study were as follows: 1) to evaluate the degree of laterality of 17beta-HSD10 mRNA in hippocampi of controls and ii) to estimate possible asymmetric changes due to AD or schizophrenia. Totally 23 human autoptic brains were analyzed (8 controls, 9 AD and 6 psychotic patients) using real-time PCR, data were normalized to the mRNA expression levels of beta2-microglobulin. We have found the marked right/left laterality in controls and shifts to the mild left/right asymmetry in AD and schizophrenia. The shifts were associated with increased expression of enzyme in the left hippocampus, the changes were more pronounced in AD than in schizophrenia. Our results support data in literature that dominant left hemisphere is more vulnerable to neurodegeneration; however, the change in 17beta-HSD10 expression does not seem to be specific for AD.

Supported by MŠMT (1M0517) project.

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DJ-1 Suppresses Aggresome Formation in a Cellular Model of Parkinson's Disease

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Parkinson's disease is a neurologic disorder involving selective degeneration of dopaminergic neurons in the substantia nigra. The disease is characterized by oxidative stress, proteasome dysfunction, and the presence in some surviving neurons of oxidatively-modified protein inclusions named Lewy bodies. Lewy bodies are thought to evolve from 'aggresomes,' perinuclear inclusions that accumulate in cells under conditions of prolonged oxidative stress and/or proteasome dysfunction. Rare cases of early-onset Parkinson's disease are caused by mutations in the gene encoding DJ-1, a protein with antioxidant and chaperone functions. In this study, we examined the effects of DJ-1 on aggresome formation in MES23.5 dopaminergic cells. Perinuclear inclusions that stained positive for classic aggresome markers were formed in MES23.5 cells treated with rotenone, a pro-oxidant, and MG132, a proteasome inhibitor. Wild-type DJ-1 suppressed aggresome formation in neurons treated with rotenone or MG132. The familial Parkinson's mutant M26I had a decreased ability to inhibit aggresome formation compared to the wild-type protein. The antioxidant compound N-acetyl-cysteine protected MES23.5 cells against rotenone- and MG132-induced aggresome formation, albeit to a lesser extent than DJ-1. DJ-1 expression caused an upregulation of glutathione and Hsp70 in MES23.5 cells, and the relative magnitude of these effects differed depending on whether the cells were treated with rotenone or proteasome inhibitor. The results suggest that (i) dopaminergic neurons are sensitized to aggresome formation because they are subjected continuously to oxidative and 'proteolytic' stress; and (ii) a loss of DJ-1

may contribute to protein oxidation and Lewy body formation in Parkinson's disease and other neurodegenerative disorders.

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Oxidative Stress Markers in Animal Models and Neurodegenerative Diseases

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Reactive oxygen species (ROS) could act a dual role as deleterious and beneficial species.

Overproduction of ROS results in oxidative stress which is a typical hallmark of ageing and it has been implicated in various neurodegenerative diseases.

We considered an animal model (rat) and three human neurodegenerative diseases (Alzheimer's, Pick's and Binswanger's diseases) to analyze some aspects of oxidative stress in ageing and phatological conditions respectively.

Since it has been reported that metal ion dyshomeostasis might exacerbate oxidative stress damage, this study reports the concentration of some metal ions (Zn, Fe, Mn, Cu) in various areas of rat brain in relation to age. We record significant differences in these levels especially for copper and zinc.

We investigated (immunohistochemistry) the expression of metallothioneins MT (I-II), glial fibrillary acidic protein (GFAP) and, only in rats, cyclooxygenase (COX-II) in the brain as markers of oxidative stress. Results clearly demonstrate an increase of MT I-II, GFAP and COX-II expression in astrocytes in old rats respect with young. This over-expression is evident also in Alzheimer's, Pick's and Binswanger's diseases respect with the normal brain. These might represent a defence against stress factors in order to protect neurons.

Despite the heterogeneity of these models we argued that changes in the expression of MT I-II and GFAP in astrocytes might aid in basic understanding of physiological (aging) and pathological (neurodegenerative diseases) mechanisms which lead to loss brain functioning.

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Peculiar Hydrophobic Properties of the 67-78 Fragment of Alpha-Synuclein Are Responsible for Membrane Destabilization and Neurotoxicity

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α-Synuclein is a 140 residue protein linked to Parkinson's disease. Intraneural inclusions called Lewy bodies and Lewy neurites are mainly composed of α-synuclein aggregated in amyloid fibrils. Few years ago, tilted peptides have been detected in two other amyloidogenic proteins : the amyloid β peptide involved in Alzheimer's disease, and the PrP protein linked to Creuztfeldt-Jakob's disease. Tilted peptides are short protein fragments that adopt an oblique orientation when inserted into biological membranes. Tilted peptides are able to destabilize membranes. In this study, we predicted by sequence analysis and molecular modelling that the 67-78 fragment of α-synuclein is a tilted peptide. Like most of them, the α-syn 67-78 peptide is able to induce lipid mixing and leakage of unilamellar liposomes. A mutant designed by molecular modelling to decrease the destabilizing properties of the peptide was shown to be significantly less fusogenic. The neuronal toxicity was studied using human neuroblastoma cells and we demonstrated that the α-syn 67-78 peptide induces neurotoxicity. In conclusion, we have identified a tilted peptide in α-synuclein which could be involved in the toxicity induced during amyloidogenesis of α-synuclein.

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ER Stress is Induced in Alzheimer's Disease But Not in Tg2576 Mice

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The endoplasmic reticulum (ER) stress results from disrupted protein folding triggered by protein mutation or oxidation, reduced proteasome activity, and altered Ca2+ homeostasis. Evidence has accumulated showing induction in adaptive pathway of ER stress in AD. We examined the possibility that ER stress would be induced in AD. RT-PCR experiments revealed increased splicing of X-box binding protein (XBP-1), an unfolded protein response (UPR) transcription factor, in AD compared with age-matched control. Among target genes of XBP-1, expression of protein disulfide isomerase (PDI) was increased in AD. This indicates that AD is accompanied by ER stress. CHOP, caspase-4, and caspase-12 were also activated in AD. However, mediators of ER stress were not induced in Tg2576 mice. The present study suggests that ER stress in AD may be induced irrespective of beta amyloid.

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The Proteome of the Neuromelanin Granule, An Iron Storing Organelle in Dopaminergic Neurons of the Human Brain

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Background: The increase of iron in the substantia nigra (SN) pars compacta contributes to oxidative stress and to neurodegeneration in Parkinson´s disease (PD) and thus underlines the importance to investigate the iron-storing structures of the human SN. In the pigmented neurons of the human SN iron is particularly stored in cellular organelles termed neuromelanin (NM) granules. NM granules play a role in neuronal iron homeostasis and tremendously accumulate iron and bind α-synuclein in PD, but a biochemical description of these organelles has so far been missing.

Aims and Methods: Aiming at a molecular characterisation of NM granules we succeeded in their isolation and performed mass spectrometry-based proteomics of these organelles.

Results and Discussion: This study provided the first biochemical basis for the deeper understanding of NM granules, which uncovered the genetic program underlying the organellar biogenesis and allowed fundamental insight into the enzymatic environment of these organelles. On the basis of 72 proteins the NM granule may be classified as a lysosome-related organelle that contains several proteins engaged in macromolecule turnover, but also in cell signalling, cargo transport, apoptosis and protection against oxidative stress.

Further investigations will focus on candidate NM granular proteins regarding an involvement in cellular signalling and iron-binding. Organellar protein misregulation may contribute to pathophysiological processes in PD, such as deposition of iron and α-synuclein onto NM granules. Knowledge about derailed signalling mechanisms is crucial to understand the massively increased iron accumulation occurring in PD and may be gained by the investigation of NM granules in PD.

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Proteome Analysis of Amyloid Precursor Protein Intracellular Domain (AICD) Expressing Neuroblastoma Cells: Is AICD a Crucial Domain in Alzheimer's Disease?

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The Ÿ-Amyloid Precursor Protein (APP) is an ubiquitous type 1 transmembrane protein with a large extracellular domain, a single transmembrane region, and a short cytoplasmic tail. Processing of the amyloid precursor protein (APP) by the beta- and gamma-secretases leads to the production of two small peptides, the Alzheimer's disease (AD)-associated amyloid beta-peptide (AŸ) and the APP intracellular domain (AICD). Whereas the role of AŸ in the pathogenesis of AD has been studied extensively, the role of the small AICD protein is yet poorly understood. AICD is believed to participate in transcriptional processes by forming a complex with the multi-domain adaptor protein Fe65 and the histone acetyltransferase Tip60. Therefore, AICD-dependent differential gene expression could elementary change the proteome pattern of affected cells. Analysis of AICD-related mechanism is of prime importance in current AD research.

In order to elucidate the physiological/pathophysiological functions of AICD and AICD/Fe65, we perform differential 2D-DIGE proteome analysis of a neuroblastoma cell culture model that inducibly expresses 1) AICD alone, 2) AICD in combination with Fe65, and 3) Fe65 alone. Here, we present first results and the putative relevance of AICD dependent mechanism in AD.

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Analysis of Changes in Protein Expression Caused by a Failure Ubiquitin-Proteasome System in a Cell System and Transgenic Mouse Model

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Accumulation of ubiquitin conjugates and inclusion bodies associated with ubiquitin, proteasome and some disease specific proteins have been found in many chronic neurodegenerative diseases, like the neurofibrillary tangles of Alzheimer's disease, in brainstem Lewy bodies and in the bunina bodies in Amyothrophic Lateral Sclerosis. Therefore the ubiquitin proteasome system (UPS) is an interesting topic of research. In eukaryotic organisms 80-90% of all proteins are degraded by the UPS. Ubiquitin B (UBB), a highly conserved protein with 76 amino acids, mediates the proteolysis of proteins. A mutant form of ubiquitin B with a frameshifted extended C-terminus (resulting from molecular misreading) (UBB+1) has been reported to be present in the neuritic plaques and tangles in AD patients. This mutant form of ubiquitin B can not be attached to proteins, which cannot be marked for degradation for the UPS any longer.

In the presented work a cell culture system was established to analyse changes in composition of the proteasome, to detect changes of posttranscriptional modifications and to find differentially expressed proteins in presence of UBB+1. Additionally a differential analysis of brain compartments of a UBB+1 transgenic mouse model at different age stages was performed using the DIGETM technology. Differentially expressed proteins in the cell culture system and in the mouse model were identified by mass spectrometry and validated with transcriptome data and immunochemical techniques, such as western blotting and immunohistochemistry. Additionally a proteome analysis of tissue, which was collected through microdissection was carried out to get detailed insights in the different brain regions.

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Hypothesis: Finding Double-Membraned Inclusions in Duodenal Enterocytes Suggests Systemic, Extra-Neuronal Mitochondrial Involvement in Idiopathic Parkinsonism

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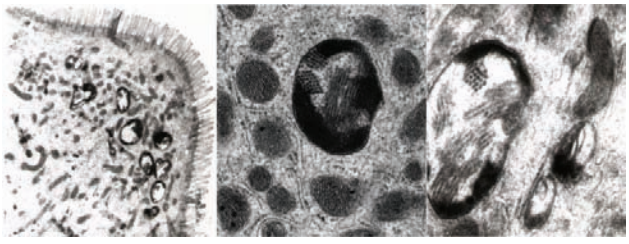
Idiopathic parkinsonism (IP) is associated with substantia nigra and platelet mitochondrial hypofunction. Dopaminergic neurone loss and Lewy bodies are found in enteric nervous system. Mitochondrial damage is compatible with chemical toxicity/infection. Rotenone and tumour necrosis factor-α impair oxido-reductase. Sub-toxic rotenone doses damage dopaminergic neurones in presence of bacterial lipopolysaccharide.

Oesophago-gastro-duodenoscopy was carried out to confirm eradication of (culture-positive) Helicobacter pylori infection in IP. Transmission-electronmicroscopy of duodenal enterocytes revealed encapsulated filamentous arrays, but no intracellular Helicobacter (DE). Findings were confirmed in same/separate biopsies (AC). Neither duodenal EM-specialist had seen similar bodies before. Electron-micrographs illustrate double-membraned bodies, at low magnification (left: multiple bodies) and higher (middle: small body amongst normal mitochondria; right: larger disintegrating body). 'DE' bodies were present in one/both biopsies from 14/28 probands with successful Helicobacter eradication, defined by histopathology, culture and molecular-biology.

Nature/relevance of DE bodies to pathogenesis of IP requires clarification. Their presence supports systemic disorder, perhaps originating in gastrointestinal tract. There is an isolated report of cerebral neurone 'mitochondrial' inclusions in Creutzfeldt-Jakob-like-disease. Prion-tubulovesicular structures are larger than DE bodies, cluster free within cytoplasm, and not found at prion-infectivity sites outside central nervous system.



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Involvement of the Calcineurin/Nfat Pathway in Neuro-Immune/Inflammatory Processes Associated With Alzheimer's Disease

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Alzheimer's disease (AD) is associated with a major neuro-immune/inflammatory (im/inf) response characterized by the release of pro-inflammatory cytokines from activated neuroglia. In immuno-competent cells, such as T-lymphocytes, cytokine induction is strongly governed by the protein phosphatase calcineurin (CN) and its downstream transcription factor, NFAT. Whether the CN/NFAT pathway regulates similar im/inf signaling cascades in resident CNS cells has not been addressed. Recently, we observed a remarkable increase in CN expression in activated astrocytes in APP/PS1 mice that show profound amyloid pathology. CN staining was particularly intense in those astrocytes surrounding amyloid deposits. In neuron/astrocyte co-cultures, overexpression of CN produced activation-like changes in astrocyte morphology and induced numerous mRNA species associated with neuroinflammation, aging, and incipient AD, suggesting a critical regulatory role of CN in these processes. In the present studies, we report a similar close association between CN, NFATc1 and activated astrocytes in human AD brain specimens collected at extremely short (~3 h) post-mortem autopsy intervals. NFATc1 also appeared prominently in nuclear fractions extracted from brain tissue homogenates, suggestive of CN-mediated nuclear translocation. In purified rat astrocyte cultures, inflammatory mediators such as interleukin 1beta robustly stimulated CN/NFAT activity. Activation of CN was associated with the release of numerous cytokines and other factors that, in turn, provided strong positive feedback regulation on CN/NFAT signaling. Together, the results indicate a potentially important role for the CN/NFAT pathway in controlling neuro-immune/inflammatory processes associated with AD and other neurodegenerative conditions where neuroinflammation is prominent.

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Coexistence of Neurotransmitters and Cause of Oxidative Stress in Parkinson's Patients

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Parkinson Disease is an old age neurological disorder faced by the neurologists in their clinical practice. The small puzzle pieces provided by the magnitude of researchers now seem to be crystallizing into a recognizable and useful model unified by a broad base of seemingly disparate ethiologic factors including infectious agents, genetic predisposition, environmental and endotoxic factors, metabolic abnormalities, traumatic events, electromagnetic radiation exposure, antioxidants, sex hormones, pharmaceutical drugs and others.

In order to study these changes along with the role of neurotransmitters, cerebrospinal fluid obtained by lumbar puncture technique is the most important and widely used diagnostic tool in evaluating the levels of neurotransmitters and their coexistence phenomena in patients with Parkinson's disease treated with L-Dopa having On and Off status. The attempt has been made to show the correlation between small peptides such as Neuropeptide Y, Substance P and Cholecystokinin both octa and tetra peptide with the classical neurotransmitters such as norephinepherine, dopaamine and serotonin.

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Neuroinflammation: What Role Does Somatostatin Play?

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Chronic inflammation is involved in the pathogenesis of neurodegenerative disorders such as Alzheimer's disease (AD). Activated glial cells are a major source of inflammatory mediators such as prostaglandins (PGs) and cytokines. The aim of the present study was to investigate the role of somatostatin (SS) in the regulation of basal and lipopolysaccharide (LPS)-induced PG production in primary neonatal rat glial cells. SS (1-100 nM) enhanced basal PG synthesis by about 2 fold in astrocytes. LPS increased PG synthesis in all cell models used. SS inhibited LPS-induced PG synthesis by 80% in microglia but, did not change LPS-induced PG levels in astrocytes. Specific agonists for SS receptors 1-4 (SSTR 1-4) (0.1-100 nM) reduced by 50-75 % the LPS-induced microglial prostaglandin E2 synthesis. However, in astrocytes SSTR 1 and SSTR 2 agonists (0.1-100 nM) increased LPS-induced PGE2 synthesis by 40-70%, whereas SSTR 3 and SSTR 4 agonists inhibited LPS-induced prostaglandin E2 synthesis by 30-60%. The opposing results obtained with the different SSTR agonists in astrocytes may explain the lack of effect observed with somatostatin, which reacts with all receptors. Our results suggest that SS may play a crucial role in the regulation of inflammatory processes in the brain. We hypothesize that differential receptor-interaction may account, at least in part, for the diversity of signaling. Further studies are needed aiming to use selective SS receptor agonists as a potential treatment strategy for neurodegenerative diseases.

"This research was supported by THE ISRAEL SCIENCE FOUNDATION (grant No. 500/04)".

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Epigenetic Regulations and Cell Cycle Genes Silencing in Mature Neurons: A Key to Reactivation of E2F in Neurodegeneration?

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It is now characterized that the molecular mechanisms of neuronal death occur through an inapropriate reactivation of the cell cycle. Several studies on simplified models, as well as examination of post-mortem tissues obtained from patients with Alzheimer's or Parkinson's diseases have evidenced the presence of E2F1 in dying neurons. This protein plays a crucial role in driving the cell cycle towards the S phase. Neurons require a permanent silencing of such genes to remain terminally differentiated. Here we describe a molecular mechanism by which several E2F-targeted genes (including E2F1) are silenced during neuronal differentiation. We provide evidence that an interplay between the gamma and alpha isoforms of the heterochromatin HP1 occurs at e2f-dependent promoters. We further demonstrate that only HP1 alpha can time a full repression of E2F-dependent transcription associated with cell cycle disruption. Transient inhibition of HP1 alpha expression within a restricted time frame drives neuronal progenitors either towards death or cell cycle progression, yet preventing the expression of the neuronal marker MAP2. Finally, our results suggest that E2F-targeted genes are packaged into higher-order chromatin structures in mature neurons compared to neuroblasts. Together, these data present new epigenetic regulations orchestrated by HP1 isoforms. This leads to heterochromatinization of E2F-targeted genes, a necessary step for permanent cell cycle exit during differentiation. Nevertheless, HP1 alpha binding is still displaceable and we are currently investigating whether these molecular events are implicated in the inappropriate reactivation of such E2F-dependent genes observed in neurodegenerative diseases.

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Amyloid-Beta Causes Apoptosis of Neuronal Cells Via Caspase Cascade, Which Can Be Prevented by Amyloid-Beta-Derived Short Peptides

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Amyloid beta 1-42 (Aβ42) and Aβ17-42 are major constituents of diffuse plaque in brains with Alzheimer's disease (AD). We demonstrate the potent cytotoxicity of Aβ42 and Aβ17-42, lesser toxicity of Aβ1-40 (Aβ40), and lack of toxicity of Aβ1-16 (Aβ16) in neuronal cells as measured by inhibition of cell proliferative response using thymidine incorporation assay, and that this cytotoxicity can be reduced with Aβ16 and eight-residue Aβ derivatives such as Aβ1-8 and Aβ9-16. FACS analysis also revealed that Aβ16 could dramatically protect against the apoptosis induced by Aβ17-42 with over 80% viable cells. We determined the caspases involved in the Aβ-mediated apoptotic pathway using caspase-specific inhibitors in MTT assays. For all Aβs, the executor was caspase 3, while the initiator was caspase 9 for Aβ42 and caspase 8 for Aβ40 and Aβ17-42. Microscopic observation of lucifer yellow-labeled neuronal cells demonstrated the occurrence of lysosomal membrane injury of the cells, corresponding to the severe cytotoxic effects of Aβ42. Our findings suggest that the apoptosis of neuronal cells due to Aβ42, Aβ40 and Aβ17-42 is mediated by the different caspase pathways and that this apoptosis can be reduced with the 8 residue Aβ-derived fragments Aβ1-8, Aβ9-16 and Aβ16.

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Identification of Genes Involved in Abeta-Induced DNA Damage in Primary Rat Neurons

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Alzheimer's disease (AD) is characterized histopathologically by the accumulation of amyloid plaques, neurofibrillary tangles, and severe neuronal loss. The amyloid-β peptide (Aβ), the major component of amyloid plaques, is believed to be a causative molecule in the pathogenesis of AD. We have previously shown that soluble Aβ induces DNA damage in primary rat neurons, as revealed by Hoechst staining. In addition, we have reported that tramiprosate, presently in phase III clinical trials for the treatment of AD, inhibits this Aβ-induced cell death. The purpose of this study was to identify molecular pathways involved in this Aβ-mediated neurotoxicity. Affymetrix oligonucleotide microarray technology was used to compare simultaneously the mRNA expression profile of 28,000 genes in primary rat neurons treated with Aβ1-42. Comparing Aβ1-42-treated and untreated rat neurons, the microarray analysis identified 168 genes that were differentially regulated by at least 40%. Functional grouping of the modified genes revealed that soluble Aβ significantly affects several different cellular pathways. In addition to extracellular matrix/cell adhesion molecules and cell cycle/DNA repair genes, the soluble Aβ1-42 preparation used in this study induced genes involved in ER stress/Unfolded Protein Response (Atf3, c-Jun, Egr1, tribbles 3 homolog, DNAJ, eif2ak3, Herpud1, Ddit3, C/EBP). These results suggest a possible link between stress-response genes and the development of DNA fragmentation and condensation induced by Aβ treatment.

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Expression of AD-Associated Genes in the Aging Rat Cortex and Hippocampus – Effect of Dietary Restriction

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Age-related disorder, Alzheimer's disease (AD), is associated with a decline in cognitive function that can, in part, be explained by changes in neural plasticity or cellular alterations that directly affect mechanisms of plasticity. Since dietary restriction (DR) is shown to be beneficial in delaying age-related changes, we examined the effect of DR on the expression of genes involved in synaptic plasticity and/or are associated with development of AD in the aging rat cortex and hippocampus. Using quantitative real-time PCR we have analyzed the effect of long-term DR (starting from 6 to 24 months of age) on the expression levels of mRNAs for the key Alzheimer's proteins, APP, ADAM 10, BACE1 and PS1 and for α-synuclein and synaptic proteins, synaptophysin and growth-associated protein (GAP-43). Real-time PCR screen revealed a) regional abundance of various mRNA transcripts with all transcripts, except α-synuclein, being more abundant in the cortex compared to hippocampus; b) region-specific, cortex vs. hippocampus, pattern of age-associated changes in mRNA levels; c) similarities in the pattern of age-related changes in the expression of synaptic plasticity-associated genes and d) region-specific influence of DR with the changes more prominent in the cortex compared to hippocampus. The obtained results are discussed in the light of their implications to aging processes and the potential of DR as a possible beneficial external manipulation of aging-related changes.

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Protein Nitrotyrosination is a Key Event in Neuronal Degeneration Associated to Alzheimer's Disease

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Oxidative stress is one of the major mechanisms involved in amyloid ß-peptide (Aß) neurotoxicity since Aß fibrils are a source of free radicals. The reaction between superoxide anion and nitric oxide produces the highly reactive peroxynitrite anion, which binds to tyrosine residues of proteins, yielding to the lost of the protein function. Triose phosphate isomerase (TPI) is largely nitrotyrosinated in both endothelial and neuronal cells treated with Aß. Moreover, TPI was nitrotyrosinated in the cortex and hippocampus from Alzheimer's disease brains. TPI nitrotyrosination in vitro poduced a significant decrease in the isomerase activity. Physiologically, TPI produces methylglioxal (MG) in a very low rate. MG is directly related with the formation of advanced glycation end-products (AGEs), which are suggested to be one of the mechanisms that involved in the ageing of the brain. We have obtained that MG production was significantly increased after TPI nitrotyrosination. Finally, TPI nitrotyrosination induced the aggregation of the protein forming ß-sheet aggregates that could be acting for the seeding of other intracellular protein aggregates such as tau protein.

This work was supported by the Spanish Ministerio de Sanidad (FIS: PI041242; Red HERACLES), Educación y Ciencia (BIO2005-01591; SAF2003-1240) and Generalitat de Catalunya (GSR2005-266).

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Tramiprosate Prevents Amyloid Beta-Induced Inhibition of Long-Term Potentiation in Rat Hippocampal Slices

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Memory impairment is among the most distinctive features of Alzheimer's disease (AD) with synapse loss seeming to be the best morphological correlate of the functional deficits observed in the mid-to-late disease stages. Amyloid-β (Aβ), the primary proteinaceous component of

amyloid plaques, one neuropathological hallmark of AD, is known to have deleterious effects on synaptic transmission, especially on long-term potentiation (LTP), an in vitro model of processes thought to underly learning and memory. It has previously been demonstrated that soluble oligomeric forms of Aβ can inhibit LTP. Tramiprosate (3-amino-1-propanesulfonic acid; homotaurine), presently in phase III clinical trials for AD, is believed to act in part by binding to toxic soluble Aβ, thereby interfering with its oligomerization and accumulation in the brain. In the present study, we have used rat hippocampal slices to determine the impact of tramiprosate in CA1 region on both basal synaptic transmission and Aβ-induced inhibition of late LTP. While tramiprosate was demonstrated to have an inhibitory effect on basal synaptic transmission at excessively high concentrations (1-10 mM), there was no such inhibitory effect at more relevant concentrations (10-200 μM). Treatment of slices with Aβ1-42 (5 μM) for 30 min induced a marked decrease in LTP (-34% of fEPSP amplitude). Preincubation of slices with tramiprosate (100 μM) for 60 min followed by co-incubation with Aβ1-42 for 30 min significantly prevented Aβ-induced inhibition of LTP, which is no longer different from the LTP obtained in control slices. Further characterization of tramiprosate mechanism of action is in progress.

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Low Concentration of Beta Amyloid Peptide Alters Protein Tertiary Structure

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A growing body of evidences suggest that intraneuronal beta-amyloid accumulation, at levels that do not compromise cell survival, can affect neuronal functions, including signal transduction pathways, enzymatic activity and protein oxidation. We recently found that exposure of human fibroblasts to nanomolar, non cytotoxic concentrations, of beta-amyloid 1-40 induced the tumour suppressor p53 to change its conformational state to acquire a novel tridimentional structure defined as "mutant-like" p53 isoform. A significant amount of mutant-like p53 isoform was also detectable in fibroblasts from Alzheimer's disease patients. Functionally, the expression of unfolded p53 isoform is associated with a lower sensitivity to apoptotic signals.

Similarly to fibroblast treated beta-amyloid as well as fibroblasts derived from AD patients, HEK cells overproducing APP751 (HEK-APP) expressed significant amount of mutant-like p53 isoform, in comparison with untransfected cells. Moreover, HEK-APP cells were more resistant to doxorubicin than control cells, suggesting an impairment of p53 mediated apoptotic process. Interestingly, HEK cells exposed to HEK-APP conditioned media expressed a mutant-like p53, suggesting that APP products released in the medium were responsible of p53 conformational change. The effect was prevented when 1) the HEK-APP conditioned media was exposed to anti-beta-amyloid neutralizing antibodyand 2) HEK-APP cells were treated with beta or gamma secretase inhibitors. These data suggest a direct involvement of APP metabolic products on modulation of p53 conformational status and figure out a new function of soluble beta amyloid peptides.

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Memantine Reverses Cognitive Deficits in Transgenic Mice With Both Plaques and Tangles

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Background and Aims: Alzheimer's disease (AD) is generally believed to result from the progressive accumulation of beta amyloid plaques and neurofibrillary tangles, which may cause significant neuronal loss. Memantine, an uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, is widely-prescribed for the treatment of moderate to severe AD, although its effect on progressive AD neuropathology remains unknown.

Methods: In this study, a battery of cognitive tasks was used to test the effect of memantine in mice carrying mutations in three genes relevant to AD pathology (PS1M146V, APPSwe and tauP301L). These triple-transgenic (3xTg-AD) mice develop both plaques and tangles in a region-specific and age-progressive manner and also exhibit learning impairment and synaptic dysfunction. Memantine (30 mg/kg/day) was administered orally via drinking water in one cohort of adult mice (aged 9 months) for 3 months ("therapeutic" trials), and in a younger cohort of mice (aged 2 months) for 10 months ("preventive" trials).

Results: In the "therapeutic" trials, memantine significantly improved spatial memory (both acquisition and retention) and inhibitory avoidance in 3xTg-AD mice, but did not significantly affect recognition of novel objects. In the "preventive" trials, memantine significantly improved inhibitory avoidance and retention of spatial memory in the transgenic mice, but it did not affect spatial learning or novel object recognition.

Conclusions: These findings carry two important implications. First, memantine is able to slow cognitive decline in younger 3xTg-AD mice. Second, memantine is able to slow or reverse established cognitive deficits in older transgenic mice, supporting use of memantine in individuals with AD.

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Assessing the Effects of Memantine in APP/PS1 Transgenic Mice Using Behavioral Studies and Ex Vivo Imaging of Amyloid Plaques

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Background and Aims: Memantine, a moderate-affinity, uncompetitive NMDA receptor antagonist, has been shown to improve learning and memory in several preclinical models of Alzheimer's disease (AD) and is widely used to treat AD clinically. Memantine has also been shown to reduce the amount of amyloid- beta (Aβ) peptides secreted by human neuroblastoma cells and rat cortical neurons, and to provide

neuroprotection against Aβ neurotoxicity. In this study, we assessed the correlation between memory-enhancing and amyloid-lowering effects of memantine in transgenic APP/PS1 mice.

Methods: Transgenic APP/PS1 mice develop amyloid deposits from the age of about 2 months. Fourteen APP/PS1 mice (7/group) were treated with either memantine (10 mg/kg; ip) or vehicle (water) for a period of 4 months starting at 3 months of age. After treatment, the mice were subjected to an object discrimination test and analyzed using ex vivo μMRI and histological examination of amyloid burden. μMRI was performed following intracarotid injection with gadolinium-labeled Aβ1-40 peptide. Coronal brain sections were then stained with thioflavin-S or processed for Aβ-immunostaining.

Results: Memantine-treated APP/PS1 mice performed the same as wild-type control animals, while the performance of vehicle-treated APP/PS1 mice was significantly impaired (p=.0081, one-way ANOVA). Compared to vehicle-treated animals, memantine-treated APP/PS1 mice had fewer Aβ plaque lesions, reduced plaque burden, and reduced Aβ immunostaining in the hippocampus and cortex.

Conclusions: Memantine treatment in this AD model reduces amyloid burden, as assessed by both histological and μMRI studies. This reduction in amyloid burden correlates with an improvement in cognitive performance.

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Intralysosomal Accumulation of Endogenous Amyloid Beta Protein Sensitize Cells to Oxidant-Induced Apoptosis

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Alzheimer disease (AD) is a neurodegenerative disorder characterized by extracellular senile plaques, mainly composed of amyloid beta-protein (A-beta). Lysosomes have been shown to associate with A-beta deposition. We have previously found that in wild type neuroblastoma cells A-beta is normally localized to small non-lysosomal cytoplasmic granules, but after exposure to oxidative stress it is autophagocytosed and accumulates intralysosomally. To find out whether intralysosomal A-beta accumulation can enhance oxidant-induced cellular damage, we compared the effects of normobaric hyperoxia (40% ambient oxygen) and 100 μM hydrogen peroxide on cultured Hek293 cells cells with different content of A-beta. Cells were either non-transfected, or transfected with empty vector, or with wild type APP (APPwt), or with Swedish mutant APP (APPswe). We have found that APPwt and APPswe cells that contain more APP and A-beta than non-transfected cells and vector controls showed increased apoptosis after exposure to either hyperoxia or hydrogen peroxide. Hydrogen peroxide-induced apoptosis was prevented by the lysosomal enzyme inhibitor leupeptin, suggesting that the increased sensitivity of cells to oxidative stress is due to enhanced lysosomal rupture following autophagocytosis of A-beta.. Furthermore, the intracellular content of APP and A-beta increased after hyperoxia exposure, apparently contributing to cell death. These findings indicate the role of intralysosomal A-beta accumulation in oxidant-induced cell death and may help explain neuronal loss in Alzheimer's disease.

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Generation of N-Terminally Truncated AŸ Species in HEK293 Cells

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N-terminally modified AŸ(N3pGlu-42) is over-represented in brains of demented patients suffering from Alzheimer€™s disease (AD). In contrast, in healthy and pathologically aged brains displaying profound plaque pathology without signs of dementia AŸ(N3pGlu-42) is only detectable in low amounts. Moreover, recent studies suggest that pyroglutamated AŸ is highly prone to oligomerization and can act as a seed for the aggregation of AŸ(1-42). Therefore, the generation of AŸ(N3pGlu-42) is possibly a key event in the development of AD pathology and the prevention of AŸ(N3pGlu-42) formation might be a unique causative approach for the treatment of AD.

In order to prove that AŸ(N3pGlu-40/42) is generated from APP during normal cell metabolism, we have investigated the N-terminal heterogeneity of AŸ(40/42) after expression of APP(WT), APP(Swedish), APP(London) and APP(Swedish/London) in HEK293. Cells transfected with APP(Swedish) and APP(Swedish/London) generate high levels of AŸ(1-40/42). In contrast, in cells transfected with either APP(WT) or APP(London), AŸ(1-40/42) is only a minor species. Further investigations on the generation of AŸ(N3pGlu-40/42) suggest that in particular APP(WT) and APP(London), which display the wild type sequence at the Ÿ-secretase cleavage site, are highly prone to produce N-truncated AŸ(N3pGlu-40/42) variants. The results point to differences in AŸ generation in sporadic and familial AD, which might have implications for future treatment strategies.

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Longitudinal Behavioral Testing and Pathological Correlates of Happ Transgenic Mice Overexpressing Human APP With London and Swedish Mutations

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Background: Progressive age related cognitive impairments were determined in a transgenic mutated human APP overexpressing mouse model (hAPP751SL). The specialty of the investigation is a longitudinal setup for behavioral and pathological characterization with exact age matching and short inter-turn time windows.

Methods: Behavioral characterization consisted of motor ability tests (RotaRod, Open Field), a spatial orientation task (Morris-water-maze) and a curiosity based task (Pokehole) longitudinally evaluated at three, six and nine months of age. Amyloid pathology was determined by brain immunohistochemistry and ELISA.

Results: Transgenics feature learning deficits in the Morris-water-maze, affecting consecutively acquisition abilities, retention and short-term memory up to the age of nine months. These impairments are accompanied by an increasing amyloid plaque pathology, determined by ELISA and immunohistochemistry, providing a new view on the timeline and the relational context between plaque burden, afflicted brain region and a time dependent selective memory loss. Curiosity measurements, turned out to be sensitive to describe disturbances in curiosity based behavior of transgenics. Beside good physical state, unhindered motor abilities and uninfluenced anxiety levels, factors that are crucial for successful testing of mice in learning paradigms, outcomes of memory tasks are strongly related to neuritic plaque pathology at nine months of age.

Conclusion: The time course of plaque development and the associated reduction of memory abilities in hAPP751SL determined in a well defined longitudinal setup opens a new view on the relationship between cognition and pathology and allows studying effects of therapeutic intervention during progression of human AD like symptoms in mice

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Structural and Functional Alterations in Brain Mitochondria From An Alzheimer Disease Mouse Model Occur Before Plaque Deposition

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Synaptic dysfunction is an early event in Alzheimer's disease patients and has also been detected in transgenic mouse models. In the present study, we analyzed proteomic changes in synaptosomal fractions from Tg2576 mice that overexpress mutant human amyloid precursor protein and from their non-transgenic littermates. Cortical and hippocampal tissue was microdissected at a stage before amyloid plaque deposition and crude synaptosomal fractions were prepared by differential centrifugation. Proteins were separated by two-dimensional difference gel electrophoresis (2D DIGE) and identified by tandem mass spectrometry. Significant alterations were detected in mitochondrial heat shock protein 70, also termed glucose-regulated protein 75, pointing to mitochondrial stress response. Subsequently, synaptosomal versus non-synaptic mitochondria were purified from Tg2576 mice brains by density gradient centrifugation. Mitochondrial proteins were separated by isoelectric focussing or Blue-native gel electrophoresis in the first dimension and SDS polyacrylamide gel electrophoresis in the second dimension. Significant changes in the protein subunit composition of the respiratory chain complexes I und III were identified. Levels of corresponding mRNAs remain unchanged as revealed impaired state 3 respiration of brain mitochondria from young Tg2576 mice. By immunoblotting, amyloid-beta oligomers were detected in synaptosomal fractions from Tg2576 mice and reduced glucose metabolism was observed in Tg2576 mice brains by [14C]2-deoxyglucose infusion. Taken together, we demonstrate alterations in mitochondrial structure and function that occur in Tg2576 mice brains before amyloid plaque deposition suggesting that mitochondria are early targets for amyloid-beta toxicity.

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Abeta Fails to Disturb LTP

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Background:Deposits of beta amyloid (Ab) and neurofibrillary tangles are the two pathological hallmarks in Alzheimer`s disease. There is recent evidence, that an impairment of synaptic activity and plasticity by soluble Ab monomers and/or oligomers may be a very early feature in this dementia. However, there is also evidence that the alterations in synaptic plasticity may depend on the type of stimulation, the Aß species and/or the physiological state of the neurons.

Methods:Using interface slices from rat hippocampus, we investigated the influence of different Ab oligomer species on synaptic long-term potentiation (LTP). LTP was induced in area CA1 of the hippocampus by different induction protocols under varying experimental conditions.

Results: We did not observe any effect of any Ab species on LTP induced by different protocols in a variety of experimental conditions.

Conclusions:The data indicate that Ab might act only if there are additional changes in the physiological state of the neuron that may vary in different experimental backgrounds, leading to different impact of Ab on synaptic activity. Further studies addressing these concomitantly required changes are necessary to clarify the mechanism of Ab action.

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Specificity of Prevention of Beta-Amyloid-Induced Neurotoxicity by Tramiprosate in Organotypic Hippocampal Slice Cultures

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The most distinctive neuropathological features of Alzheimer's disease (AD) include senile plaques composed primarily of amyloid-β (Aβ) and degenerating neurites containing neurofibrillary tangles. Many recent studies have implicated a key potential role of soluble oligomeric Aβ forms in AD pathogenesis, suggesting that some form(s) of the soluble peptide are responsible for neurotoxicity by mechanisms that remain to be defined. Tramiprosate (3-amino-1-propanesulfonic acid; homotaurine), presently in phase III clinical trials for AD, is believed to act in part by binding to toxic soluble Aβ, known to oligomerize, deposit and accumulate in the brain. In the present study, we have used a murine organotypic hippocampal slice culture (OHC) model to probe the effects of tramiprosate on Aβ-induced cell death. Here, we demonstrate that Aβ1-42 induces a time- and dose-dependent increase in cellular mortality as measured with propidium iodide (PI) staining and lactate dehydrogenase (LDH) levels in the culture media. Co-treatment with tramiprosate completely prevents both Aβ1-42-induced cell death in the dentate gyrus, the hippocampus and the entorhinal cortex and Aβ1-42-induced LDH activity in the culture media. The effect of tramiprosate was compared to the effect of taurine and two structurally related compounds, 1,2-ethanedisulfonic acid and eprodisate disodium (Kiacta). Interestingly these drugs do not affect either Aβ1-42-induced

cell death or Aβ1-42-induced LDH activity. Together, these data suggest that tramiprosate exerts neuroprotective effects against Aβ-mediated neurotoxicity.

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Tramiprosate Decreases Amyloid-Beta Induced ERK1/2 Activity in Primary Rat Neurons

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Mitogen-activated protein kinase (MAPK) family members, including extracellular signal regulated kinase (ERK1/2), c-jun N-terminal kinase (JNK), and p38, have been proposed to play important roles in neuronal cell death mediated by amyloid-β (Aβ). Specifically, Aβ has been shown to induce sustained activation of ERK1/2 in primary neurons. Further, treatment of murine hippocampal slice cultures (OHCs) with soluble Aβ results in the activation of ERK1/2, which contributes to cell death in this model. Tramiprosate (3-amino-1-propanesulfonic acid) presently in phase III clinical trials for Alzheimer's disease, exhibits neuroprotective activity against Aβ-mediated toxicity in primary rat neurons. The aim of this study was to assess the role of MAPKs in this neuroprotective activity. Treatment of primary rat neurons with soluble Aβ42 for 24 hours induces a marked increase in the phosphorylation of ERK1/2 but not JNK. Co-administration of Aβ42 with tramiprosate significantly decreases Aβ-induced ERK1/2 phosphorylation. Tramiprosate has been shown to bind the GABAa receptor with an IC50 of ~ 80 nM. Interestingly, muscimol a potent GABAa receptor agonist did not affect Aβ-induced ERK1/2 phosphorylation, suggesting that the activity of tramiprosate on ERK1/2 is independent of GABAa receptor activation. In murine OHCs cultures, Aβ42 induces an increase in ERK1/2 activity in the dentate gyrus and the hippocampal regions CA1 and CA3. The effect of tramiprosate on Aβ-mediated ERK1/2 activation in OHCs is currently under investigation. These results suggest that tramiprosate is partially neuroprotective in primary neurons via inhibition of Aβ-induced activation of ERK1/2 and this activity is most likely GABAa-independent.

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Pathological Consequences of Alzheimer's Disease Relevant Modifications of Tau and APP in An Ex Vivo Model of the Hippocampus

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Amyloid plaques and neurofibrillary tangles (NFTs) are hallmarks of Alzheimer's disease. Amyloid plaques consist of aggregated Aß, a fragment of the Amyloid Precursor Protein (APP). NFTs contain hyperphosphorylated tau protein as major component.

To determine a potential role of hyperphosphorylated tau in AD pathology, we constructed a pseudohyperphosphorylated(PHP) tau, which mimics key structural and functional aspects of AD-like hyperphosphorylated tau protein (Eidenmüller et al., Biochemistry 39:13166-13175 (2000)). Enhanced green fluorescent protein (EGFP) tagged tau constructs were cloned into Sindbis virus to allow efficient expression in neurons.

The hippocampus is one of the brain regions that are altered during AD. To analyze the role of modified tau during AD, organotypic hippocampal slices were prepared (Stoppini et al., JNeurosciMethods 37:173-182(1991)) and infected with the Sindbis Virus constructs.

To analyze APP or Aß effects on neurons expressing different tau constructs, hippocampal slices were prepared from APPSDL transgenic C57BL6 mice, which express equimolar amounts of Aß1-40 and Aß1-42 in embryonic age (Leschik et al., JNeurochem in revision).

A potential neurodegenerative effect of different tau constructs in the presence or absence of APPSDL is analyzed by low-resolution live-imaging using cLSM.

The influence of APPSDL on synaptic integrity of hippocampal neurons is determined using high resolution imaging of dendritic spines with algorithm-based analysis of spine-density and morphology (Shahani et al., JNeurosci 26:6103-6114(2006)).

Hippocampal ex vivo models from APP-transgenic mice in combination with virus-mediated targeted expression of disease relevant tau constructs provide an efficient tool to gain insights into the molecular pathology of Alzheimer's disease.

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Different Effects of Abeta1-42 Oligomers and Fibrills on AMPA Receptor Function in Vivo

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The key pathological step in the pathogenesis of Alzheimer's disease (AD) is thought to be the accumulation and aggregation of Abeta species. Aggregated amyloid has a direct cytotoxic effect, although the neuron loss is disproportionate to the extent of cognitive demise, therefore dysfunction of morphological intact synapses seem to play a pivotal role in the etiology of AD. The impairment of normal synaptic function and plasticity by Abeta is well documented; however, the exact molecular mechanisms remain enigmatic.

Here, we demonstrated different effects of Abeta1-42 oligomers and fibrils on postsynaptic function, especially on the function of AMPA receptors. The aggregation states of the solutions used were verified by several methods. Transmission electromicroscopy (TEM), quasi-elastic light scattering (QLS) studies and gel electrophoretic experiments showed that the applied protocols resulted in mostly oligomer or fibrillar amyloid assemblies respectively, which remained stable for the interval of the in vivo electrophysiological experiments.

In vivo single-unit recordings from the CA1 region showed that both aggregated amyloid forms enhance NMDA receptor evoked neuronal firing. Strikingly, the effects on AMPA elicited responses were divergent: Abeta oligomers augmented, while fibrils ablated neuronal AMPA elicited responses.

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Roles of the Glutamatergic Receptors in the Changing of the Intracellular Zinc Concentration in the Hippocampal Neurons

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In the hippocampus the ionic zinc is a neuromodulator. The co-release of the glutamate (Glu) and the Zn2+ from the presynaptic vezicules of the granule mossy fiber terminals is a central event in the CA3 region of the hippocampus. The ratio between the vesicular Zn2+ and Glu is very sensitive to any cellular stress effect. The high Zn2+ and Glu levels in the extra- or intracellular spaces are very toxic to the neurons. Membrane depolarization (e.g. addition of 100 mM KCl) induces vesicular Zn2+ release from the mossy fiber terminals, but the Glu in dose dependent manner (5, 0.5 and 0.05 mM) increases intracellular free Zn2+ level, instead of the release of the vesicular Zn2+ in extracellular space.

In our study, we investigated how glutamatergic receptors increased the concentracion of free [Zn2+]i in acute hippocampal slices, and we looked for the source of free Zn2+. We measured the changes of the free [Zn2+]i with a fluoro-plate-reader equipped with matrix scanner, and the well known zinc-sensitive fluorescence dye 4-[([6-Methoxy-2-methyl-8-quinolinyl]amino)sulfonyl]benzoic acid (TFLZn) has been used in the experiments.

According to our results the regulation pathways of the intracellular Zn2+ concentration and the ratio between the Zn2+ and Glu in the presynaptic vesicules of granule mossy fiber terminals may serve as possible targets to prevent Alzheimer's disease.

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Insulin-Degrading Enzyme Expression in the Brain and Peripheral Organs of Hypoxic and Diabetic Rats

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Background and Aims: Alzheimer's Disease (AD) is characterised by accumulation of amyloid-beta peptide (Abeta) in the cortex (Cx) and hippocampus (Hip) and insulin-degrading enzyme (IDE) is one of the Abeta-degrading enzymes that control levels of brain Abeta. Ischaemia and diabetes are believed to be associated with the risk of development of AD. The aims were to analyse the effects of hypoxia and diabetes on IDE expression in tissues.

Methods: We have analysed by immunoblotting the effect of acute hypoxia (Hyp) (7% O2, 3h) on IDE expression in brain structures as well as in the brain and other organs of rats with experimental type I diabetes.

Results: In Cx and Hip, IDE expression was initially increased after acute Hyp but then statistically decreased 24 hours after the exposure. In the striatum (Str), Hyp led to a decrease in its expression 3, 24 and 72 h after exposure with the most pronounced decrease (by 80%) after 24 h. In diabetic rats, levels of IDE expression were decreased significantly in heart (by 70-75%) and brain hemispheres (by 40%). There were no significant changes in renal expression of IDE. In the Cx and Hip of diabetic rats, IDE was also lower than in the controls (by 30 and 20%).

Conclusions: The data suggest that both hypoxia and diabetes lead to a decrease of IDE levels in the Cx and Hip – the structures where accumulation of Abeta is the most pronounced in AD. Supported by RAS: "Fundamental Sciences to Medicine", RBRF (06-04-48414), INTAS 04-832877.

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5-Lipoxygenase Activation Increases Alzheimer's Beta-Amyloid Production

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Alzheimer's disease (AD) is the leading cause of dementia in the elderly and no efficient disease-modifying treatment modality is currently available for it. Inflammatory pathways seem to have an important role in AD. 5-lipoxygenase (5LO) is widely expressed in the CNS and is the key enzyme in the production of leukotrienes, which are potent pro-inflammatory compounds; however the role of 5LO in AD has not been fully investigated yet. In this study, we sought to evaluate the effects of 5LO activation on Abeta production and amyloid precursor protein (APP) metabolism in vitro. In mouse neuroblastoma (N2a) cells stably transfected with "Swedish" mutant human APP (SweAPP N2a cells), 5LO metabolites significantly increased Abeta production. Activation of 5LO by A23187 in mouse embryonic fibroblasts (MEFs) and CHO cells similarly induced an increase in Abeta formation. This effect was blocked by 5LO inhibitors and was absent in MEFs genetically lacking the 5LO enzyme. 5LO metabolites or enzyme activation did not have any effect on APP or C-terminal fragments levels. 5LO effect on the production of Abeta42 seemed to be more prominent than Abeta40 and Abeta38, suggesting that the modulation of gamma-secretase complex may be the main mechanism. These data show a pro-amyloidogenic effect for 5LO and suggest that suppression of this enzyme can be a new strategy to modulate Abeta production in AD.

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Influence of Beta-Amyloid (1-40) on the Erythrocyte Osmotic Fragility

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Beta-amyloid (1-40) (betaA) regarded as a central neurotoxic agent found in the brains of Alzheimer's disease patients, can be also found in blood and blood vessels. In contrast to its shorter fragments (25-35 and 31-35), betaA (1-40) does not induce direct rapid toxic action towards erythrocytes (RBCs). However, multiple instrumental and biochemical data demonstrate differences between RBCs from AD patients and controls.

In this work we present studies over the betaA influence on the human RBCs osmotic fragility. This method allows us to study the impacts of lower (submicromoloar, approaching physiological) betaA concentrations. Increased levels of copper ions (CuII) and cholesterol in serum were described in blood of Alzheimer's patients. Effects of CuII ions and cholesterol as factors modyfying betaA-RBCs interactions are described. Quantitative data are accompanied by microscopic images.

From the data we can conclude that copper and cholesterol have quite opposite effects on betaA influence on the RBCs osmotic fragility. Complex formation between the peptide and copper is not sufficient to remove metal effects on RBC membranes. On the other hand, co-incubation of cholesterol with betaA potentiates stiffening effects of cholesterol on RBC membranes. We can conclude, that interaction of betaA with RBC membrane is very susceptible to the presence of all substances, that interacts with the peptide.

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The Functional Significance of Ngr1, Ngr2 and NgR3 Null Backgrounds on APP Processing and Amyloid Beta-Mediated Toxicity in Primary Neurons

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Aberrant neuritic sprouting appears to be activated in the vicinity of Abeta deposits in Alzheimer's brain. In this respect, the Nogo receptor (NgR), a strong modulator of neuronal protrusive activity, shows altered expression in Alzheimer's brain and direct binding to APP and Abeta1-40. In addition, APPswe/PSEN-1 (DeltaE9) transgenic mice bred onto an NgR1-/- background show increased accumulation of Abeta and treatment of DeltaE9 transgenic mice with NgR(310)ecto-Fc reduces Abeta plaque deposition (Park et al., 2006. J. Neurosci. 26, 386-1395). In order to further investigate the role of the NgR signaling complex in Alzheimer's disease pathology, we are comparing responses of WT and NgR1 KO hippocampal neurons treated with oligomeric Abeta to determine whether the absence of NgR1 causes increased susceptibility to Abeta toxicity. There are 3 known isoforms of NgR (1,2,3). We are therefore conducting similar experiments using NgR2 KO and NgR3 KO hippocampal neurons to determine whether NgR2 or NgR3 are potential molecules to exploit to alter Alzheimer's disease progression.

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Fe65 and APP Are Involved in the Cellular Response to DNA Damage

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APP, the precursor of the beta-amyloid peptides, is a membrane protein that plays a crucial role in the pathogenesis of Alzheimer's disease. The functions of APP and of its amyloidogenic processing are still unknown. The cytosolic domain of APP interacts with the adaptor protein Fe65. To explore the functional role of the APP-Fe65 complex, we generated Fe65 knock out mice. These mice do not show any obvious phenotype, however, when embryo fibroblasts (MEFs), isolated form Fe65 KO, were exposed to low doses of DNA damaging agents, such as etoposide or H2O2, an increased sensitivity to genotoxic stress, compared to wild type animals, clearly emerged. Accordingly, brain extracts from Fe65 KO mice, exposed to non-lethal doses of ionizing radiations, showed high levels of γ-H2AX and p53, thus demonstrating a higher sensitivity to X-rays than wild type mice.

Nuclear Fe65 is necessary to rescue the observed phenotype and, few minutes after the exposure of MEFs to DNA damaging agents, Fe65 undergoes phosphorylation in the nucleus. With a similar timing, the proteolytic processing of APP is rapidly affected by the genotoxic stress: in fact, the cleavage of the APP C-terminal fragments by γ-secretase is induced soon after the exposure of cells to etoposide. In the cells lacking Fe65 the APP C-terminal fragments are not affected by the genotoxic stress. These results demonstrate that Fe65 plays an essential role in the response of the cells to DNA damage and suggest a significant involvement of the proteolytic processing of APP in this phenomenon.

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Measuring the Intra- and the Extracellular Zinc Ion Concentration With Fluoro-Plate-Reader on Acute Hippocampal Slices

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The mossy fiber terminals of hippocampal granule cells contain high amount of vesicular free zinc ions. The main function of this bivalent cation (co-localized with glutamate) is neuromodulation. Following the dopolarization of the presynaptic membarane (e.g. electrical stimulation or KCl treatment) the vesicular zinc quickly releases into the synapse, together with glutamate. We developed a simple and rapid method to measure the vesicular zinc release and the effects of zinc binding chelators in rat acute hippocampal slices. We have performed the experiments with the help of a 96-well-type fluoro-plate-reader with matrix scanning properties and a widely used zinc-sensitive fluorescence dye 4-[([6-Methoxy-2-methyl-8-quinolinyl]amino)sulfonyl]benzoic acid (TFLZn). The dye can bind Zn2+ ions specifically with high affinity (Kd = 20 μM); the level of free zinc ion can be measured by fluorometry (λ ex. 380 nm, em. 520 nm). TFLZn is water soluble and does not harm the neurons in 0.3 mM concentration, besides it does not grab zinc ions from the zinc-binding methallothionein proteins.

This novel method can be used successfully for ex vivo modelling of the stress induced neuronal presynaptic zinc release, representative to neurodegenerative processes (e.g. Alzheimer's disease).

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Increased Kv4.2 Subunit Expression in Acute Hippocampal Slices Following Incubation With Amyloid Beta Protein 1-40

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We have previously shown that amyloid β protein can modulate the amplitude of the voltage-gated K+ channel current in hippocampal slices. Here we have studied Kv4.2 potassium channel subunit expression in rat hippocampus using immunohistochemistry, and investigated how the immunofluorescence is altered by exposure to Aβ1-40.

The hippocampii were removed from 13 day old rats (n=4) and 500μm slices were prepared and maintained in tissue chambers perfused with ACSF or ACSF containing 10nM Aβ1-40 for 2 or 12 hours. Slices were resectioned at 50μm and incubated with anti-Kv4.2 antibody and a Cy3 conjugated secondary antibody. Fluorescence micrographs were taken of 4 regions of the hippocampus and analyzed for pixel intensity. Data presented is mean increase in pixel intensity ±S.E.M and statistical differences were assessed using Student's t-test.

Incubation of hippocampal slices with Aβ1-40 resulted in a marked and statistically significant increase ($p<0.01$) in Kv4.2 immunofluorescence after only 2 hours, which appeared to decrease in magnitude after 12 hours incubation. The largest increase in immunofluorescence was seen in the CA1 region (609.44% ± 200.12 after 2 hours, 70.50% ± 11.51 after 12 hours). Similar increases were also found in the CA2, CA3 and dentate gyrus regions.

We conclude that the increased K+ current seen in hippocampal slices after 2 hours incubation with Aβ1-40 is likely to be a consequence of increased expression of Kv4.2 subunit. Thus Aβ1-40 has a regulatory effect on Kv4.2 expression in rat hippocampal slices.

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Amyloid Beta Toxicity is Enhanced by Alpha-Synuclein in Experimental Model of Alzheimer's Disease

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Alpha-synuclein (ASN) and its neurotoxic fragment NAC (non -amyloid beta component of Alzheimer's disease amyloid) are suggested to play a crucial role in amyloid beta aggregation and neurodegeneration. However, the precise mechanism of ASN action remains unclear. In the present study, the role of ASN and NAC peptides on cells viability and molecular processes leading to apoptosis were investigated. The study was performed on PC12 control, PC12 cells transfected with human amyloid precursor protein (APPwt) or bearing the Swedish double mutation (APPsw) using immunochemical, spectrophotometrical and spectrofluorometrical methods. Our data presented that non-aggregated ASN and NAC enhanced free radicals level and induced PC12 cells death in concentration dependent manner by 50% and 70%, respectively. Analysis with Hoechst 33342 indicated that apoptotic signaling was activated. ASN induced caspase-3 activation by about 40% and poly(ADP-ribose) polymerase (PARP-1) degradation influenced PARP-1 and poly(ADP-ribose)PAR regulator transcription and DNA repair machinary. Inhibitor of caspase-3 (Z-DEVD-FMK, 100 microM) and mitochondrial permeability transition pore blocker (cyclosporine A, 2 microM) partially prevented ASN-evoked cell death. These findings indicate that ASN and its degradation product NAC induce apoptotic pathway in PC 12 cell lines and the significance of their effects depends on the AB peptide concentration and on molecular events activated by intracellular AB.

Supported by the Grants: PBZ-MIN-001/PO5/16 and 2PO5A4129

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Amyloid ß(25-35) Increase No, Heat Shock Proteins and Decrese Spatial Memory

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The extracellular plaques mainly contain ß-amyloid (Aß) peptide that increases the reactive oxygen species (ROS) in patient with Alzheimer Disease (AD). The Aß peptides cause neurotoxicity and increase oxidative stress. This events cause neuronal and synapse lost that induce memory impairments in in-vivo models. The aim of this work was to evaluate the effect of amyloid ß(25-35) into hippocampus and temporal cortex on NO levels, heat shock protein (HSP) and spatial memory. Male Wistar rats (250-300gr) were injected with 1 µl (100 µM) of amyloid ß(25-35) into hippocampus (Hp) or temporal cortex (TC), after one month the spatial memory of animals were evaluated. Finally, the HSP90, GFAP and DAPI inmunohistochemistry were assessment in Hp, FC and TC. The nitric oxide (NO) was measured in Hp, FC and TC by Griess method. The Aß(25-35) increased significantly the NO into Hip, CF and CT when it was deposited into Hp and TC. The memory test shows that time of latency to escape in Aß(25-35) group was major in water maze, so its evident the impairment of spatial memory by Aß(25-35). Finally we found inmunoreactivity to HSP90, GFAP and DAPI into hippocampus and FC and TC increased Aß(25-35) group. We suggest that stress oxidative plays an important role in neurodegenerative process by Aß(25-35). Our results strongly favor the hypothesis of neurotoxiticty by Aß(25-35) in which stress oxidative are increased following to impairment in memory of rats.

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Effects of Oligomeric and Fibrillary A**β**(1-42) on Rat Hippocampal Neurons

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It is hypothesized that Aβ1-42 plays a major role in the development of Alzheimer's Disease. Aβ1-42 exists as oligomeric or fibrillary aggregates and may ultimately form plaques. Some studies show that Aβ1-42's toxicity is mediated through generation of reactive oxygen species (ROS).

In the present study we examined the effects of oligomeric (protofibrils) and fibrillary Aβ1-42 on hippocampal neuronal cells prepared from P0 rats and grown in defined medium. Their effects was also compared to the effects of freshly dissolved Aβ25-35.

Oligomeric Aβ1-42 and fibrillary Aβ1-42 was prepared from hexafluoroisopropanol solubilized peptides according to Dahlgren et al. (2002) JBC 277:32046.

The cells were characterized by the mitochondrial activity marker MTT and the ROS level indicator carboxy-H2DCFDA.

The results are expressed as percentage of untreated cells.

	3 hours			24 hours			
72 hours							
	Oli	Fib	25-35	Control	Oli		
Fib	25-35	Oli	Fib	25-35			
MTT	52	86	110	100			
30	48	54	27	57	71		
ROS	88	83	119	100	81	73	
	53	71	54	-			

When treated for three or 24 hours with 20 μM of the three types of A? we found that the cells showed a large decrease in median mitochondrial activity and also in a time- and dose-dependant manner. Oligomeric Aβ1-42 lowered the activity both ealier and significantly more than fibrillary Aβ1-42 and Aβ25-35. The results further indicate that the Aβ1-42 effect was not mediated by ROS.

The interaction between Aβ effects and Apolipoprotein E isoforms will also be presented.

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Aggregated Amyloid Beta Peptide Increases cPLA2 Expression in Rat Cortical Neurons

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Recent data have revealed that the aggregated form of amyloid beta peptide (Aβ1-42) may be the proximate effector of the neuronal injury and death occurring in Alzheimer's disease (AD). However, the molecular mechanisms associated with the neuronal cell death induced by the aggregated Aβ1-42 remain to be elucidated. Cytosolic Phospholipase A2 (cPLA2) is a requisite component in the cascade of events leading to the production of eicosanoids during acute and chronic inflammation. In the central nervous system (CNS) cPLA2 likely functions in maintaining basal cellular physiological processes, as arachidonic acid and its metabolites have been implicated in normal CNS physiological processes, including synaptic receptor signal transduction, synaptogenesis, and neurotransmitter release. In this study, we investigated whether aggregated Aβ1-42 affects cPLA2 expression in neuronal cells. Primary rat cortical neurons were prepared from E16-18 rat. 6-7 days after culture preparation the cells were incubated for 24 hours in the presence of 1μM aggregated Aβ1-42. This treatment caused a significant increased in cPLA2 expression. The ability of aggregated Aβ1-42 to affect cPLA2 expression suggests a role for cPLA2 in the pathophysiology of AD.

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Inhibition of Microglial Cytosolic Phospholipase A2 Decreases A-Beta-Induced Oxidative Burst

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Previous studies have demonstrated that amyloid-β peptide (Aβ) which accumulates in Alzheimer's disease (AD) brain tissue contributes to the characteristic neurodegeneration. In addition, the finding that cytosolic Phospholipase A2 (cPLA2) is associated with amyloid deposits in AD brain suggests that cPLA2 may be actively involved in AD pathogenesis. The goal of this study is to elucidate the role of microglial cPLA2 in the inflammatory processes present in AD brain. We show that Aβ activates microglia causing them to undergo morphological changes from resting ramified cells to the ameboid activated state. Microglia cells treated with Aβ show increased CD68 expression, pro-inflammatory cytokines mRNA expression, superoxide production and cPLA2 expression at the mRNA and protein levels. Prevention of the elevation of cPLA2 expression caused inhibition of superoxide production stimulated by Aβ. This result indicates that similar to other phagocytic cells, cPLA2 regulates NADPH oxidase activity and establishes the involvement of cPLA2 in regulation of the oxidative stress present in neurodegenerative disorders.

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Role of Beta-Amyloid Precursor Protein in Neurite Outgrowth and Synaptogenesis in Primary Neuronal Cultures

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Background: The amyloid precursor protein (APP) is a central player in the development of Alzheimer's disease (AD). Proteolytic processing of APP results in production of the toxic amyloid-β (Aβ) fragment that is the primary constituent of the senile plaques found in the AD-affected brain. However, to date the role played by APP in neuronal physiology remains unclear.

Methods: To examine the role of APP in neurite outgrowth and synaptogenesis, primary rat cortical neuron (PRCN) cultures were allowed to differentiate in culture and neuronal and synaptic markers as well as secreted APP and Aβ were monitored over the course of two weeks.

Results: Synaptic markers SNAP-25 and synaptophysin, which is known to be reduced in AD-affected brains, increased dramatically over this period as measured by Western blotting. Simultaneously, APP secretion increased in direct proportion with Aβ1-40 as measured by ELISA.

Conclusions: Coincident increases in synaptic markers, APP and Aβ1-40 secretion suggest that APP and APP processing are involved in neurite outgrowth and synaptogenesis. Western blot results of secreted and cell-associated APP also suggest splice variant specific induction of APP in this process. While the exact function of APP remains to be discovered, these data suggest that APP may not be a single homogenous entity, but rather that several forms of APP have distinct contributions to neuronal physiology. Ongoing experiments will further clarify this function of APP.

Supported by NIH and Alzheimer Association grants

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Altered Expression Pattern of Different Synaptic Markers in APP Transgenic Mice

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Alzheimer's disease (AD) is characterized by deposits of amyloid beta peptide (A?) and a progressive impairment of memory formation. Recent studies indicate that depletion of synaptic proteins may play a crucial role in the Aß-induced cognitive deterioration occurring already before plaque deposition. We investigated whether defects in the expression of different synaptic proteins in brain regions important for memory function could be observed in Tg2576 mice which overexpress the human Swedish APP mutation.

Fourteen month old female Tg2576 mice (n=13) and wild type littermate controls (n=8) were perfusion-fixated, and their brains processed immunohistologically and analyzed for optical densities of synaptic marker staining in hippocampal and neocortical brain regions. Only regions without plaques were studied in order to quantify effects of soluble rather than deposited A?. We found a significant decrease in the expression of Drebrin by 40-55% in several hippocampal regions and the neocortex of Tg2576 compared to wild type controls (t-test; $P<0.001$). Expression of phosphoPAK in the dentate gyrus and of PSD-95 in the mossy fiber terminal region in CA3 was also reduced ($P<0.05$). In contrast, Homer-1 and Dynamin-1 remained unchanged while Synaptophysin immunostaining was increased in some synaptic regions of the hippocampus, suggesting that only distinct synapses and/or pathways are impaired by APP overexpression.

Our data confirm and extend the observation that synaptic integrity is disturbed in Tg2576 and conclude that a histological, region-specific determination of synaptic markers could be a useful tool for testing efficacy of AD-modifying therapies.

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Recapitulation of Sporadic Pick's Disease in a Novel Tau Transgenic Mouse

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Filamentous deposits made of microtubule-associated protein tau are a hallmark neuropathological characteristic of several neurodegenerative diseases including Alzheimer's Disease, Pick's Disease (PiD) and progressive supranuclear palsy. Recapitulation of tauopathic disease in mouse models indeed provides a valuable tool for investigating neurodegenerative pathways and pathogenic mechanisms in these highly enigmatic disorders.

Following identification of a pathogenic K369I missense mutation in exon 12 of Tau (chromosome 17q21) in a human sporadic PiD case (Neumann, et al. 2001), our laboratory generated a transgenic mouse, expressing K369I-mutant (K369I Tg) tau in neurons. Histological and immunoreactivity studies of K369I Tg brain pathology have revealed a close resemblance to human PiD, including comparable species of insoluble tau, brain volume loss and identical argyrophilic staining profiles. Moreover, this is an early-onset phenotype with cortical tau hyperphosphorylation from 4 months and Bielschowsky Silver-positive neurons at 12 months. These promising characterization results suggest the K369I Tg mouse to be a useful tool not only for studies into PiD itself, but also for studies into pathogenic mechanisms in the whole tauopathic spectrum.

Neumann, M., W. Schulz-Schaeffer, R. A. Crowther, M. J. Smith, M. G. Spillantini, M. Goedert and H. A. Kretzschmar (2001). "Pick's disease associated with the novel Tau gene mutation K369I." Annals of Neurology 50(4): 503-513.

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Eeg Comparison in Early AD, LBD, PDD Patients With a 2 Years Follow-Up

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Background and Aim: criteria for diagnosis of LBD diagnosis include EEG abnormalities as supportive features. Our aim was to evaluate whether EEG can differentiate early Lewy Body Dementia (LBD), Alzheimer's disease (AD) and Parkinson's disease with dementia(PDD) patients, and which EEG representation method is best showing differences.

Methods: In 40AD, 36LBD, 35PDD patients EEGs were recorded at admission to the study, when MMSE scores were 20-25/30 and ADAS-cog scores were>16/70. EEGs were analysed with classic visual inspection and with several quantification methods.

All patients were assessed for the presence of cognitive fluctuations, neuropsychiatric symptoms, REM sleep behaviour disorder, and assessments were repeated after 2 years.

Results: EEG from parietal and occipital derivations differentiated LBD and AD patients($p=0.001$). 5.6-7.9 Hz activities, dominant or intermingled with alpha or lower frequencies were observed in all LBD and 45.7% of PDD patients. In AD alpha was well represented. Differences were best evidenced when Dominant Frequency(DF), and DF variability were represented in Compressed Spectral Arrays showing EEG changes across time, with salient patterns interpretable by visual inspection.

At follow-up abnormal EEG on posterior leads was prevalent in LBD(100%), less represented in AD(27.5%). In PDD the prevalence of EEG abnormalities increased to 74.3%.

Conclusion: absent alpha and DF in the 5.6-7.9 range with variability of DF differentiated early LBD from AD. Early in the course of PDD these findings were observed in a minority of patients, but became prevalent at follow-up.

These findings are helpful for early diagnosis, and support and clarify consensus guidelines on LBD-PDD.

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Oxidative Stress-Induced Alpha-Synuclein Phosphorylation and Oligomerization Are Linked to Increase of CK2 and Lysosomal Enzymes

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Intracellular accumulation of alpha-Synuclein(a-Syn) as filamentous aggregates is a prominent pathological feature of synucleinopathies. Ser129 phosphorylation or C-terminal truncation has been demonstrated to facilitate a-Syn aggregates formation in vitro. To elucidate the mechanisms involved in a-Syn aggregation, we used human neuroblastoma cells with tetracycline-off (TetOff) induction of human wild-type a-Syn and FeCl2 exposure as an experimental model. Lysates from cells regarded as Syn(+)/Fe(+) were compared with those from Syn(+)/Fe(-), Syn(-)/Fe(+) or Syn(-)/Fe(-) cells by immunofluoresence, immunoblotting and enzymatic assay. The results showed that Syn(+)/Fe(+) cells contained the largest amount of a-Syn inclusion, oligomer and phosphorylated a-Syn monomer. FeCl2 exposure also led to increase in the level/activity of CK2a and cathepsin D. Olgomers were demonstrated to contain a-Syn truncated at C-terminus. C-terminally truncated a-Syn of size(~10 &12kD) smaller than monomer were detected in both Syn(+)/Fe(-) and Syn(+)/Fe(+) cells. Treatment of Syn(+)/Fe(+) cells with CK2 inhibitors, DRB/TBB, blocked phosphorylation and oligomerization of a-Syn and inclusion formation. The production of oligomer in Syn(+)/Fe(+) cells was also inhibited by lysosomotropic agent, NH4Cl or cathepsin D inhibitor, pepstatin A. These treatments also led to reduction of truncated a-Syn. Together, the results indicate that CK2 is a major phosphokinase for a-Syn, and oligomer formation and a-Syn phosphorylation are closely associated, and that lysosomal enzymes play a role in generating a-Syn fragments for oligomer assembly.

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Toxicity and Localization of Alpha-Synuclein is Dependent on Vesicular Transport in the Yeast-Based Model of Alpha-Synucleinopathy

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The function of alpha-synuclein, protein involved in the pathology of Parkinson, is still unclear but was recently suggested to be important for vesicular transport.

In order to define a role of vesicular transport and vesicular pathways in alpha-synuclein-dependent toxicity we used the Saccharomyces cerevisiae model of alpha-synucleinopathy, since yeast expresses many functional orthologues of mammalian vesicular proteins. We expressed alpha-synuclein as a native or as a EGFP-tagged fusion protein in several deletion mutants of vesicular proteins.

Similar to its localization in mammalian cells, both native alpha-synuclein and alpha-synuclein-EGFP were bound to the plasma membrane in wild type yeast cells. When we screened mutant yeast strains lacking proteins that are proven or suspected to be involved in vesicular transport, we observed distinct patterns of alpha-synuclein mislocalization. This often coincided with enhanced toxicity of alpha-synuclein.

Our data provide clear evidence for connections of alpha-synuclein with the ESCRT complex, SNAREs, the HOPS complex and typical proteins of the Golgi complex. These proteins are all related to endosomal and ubiquitin-dependent transport, which links alpha-synuclein to different vesicular transport systems.

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Effect of the Peptidyl-Prolyl Cis/Trans-Isomerase Pin1/Ess1 on Protein Tau Expressed in Yeast

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The interaction of the MAP protein tau with microtubules is dynamically controlled by reversible phosphorylation, as a result of the activities of different kinases and phosphatases. Abnormal phosphorylation of tau results in aggregation of tau and formation of paired helical filaments (PHF) and neurofibrillary tangles (NFT), which are neuropathological hallmarks of Alzheimer's disease (AD).

We recently developed humanized yeast-based models that allow us to investigate in more detail the biochemistry of protein tau (Vandebroek et al., 2006; Vandebroek et al., 2005). We adapted these models to study the influence of human Pin1 on tau. Pin1 is a peptidyl-prolyl cis/trans-isomerase that binds to phosphorylated Thr231-Pro in tau, thereby facilitating dephosphorylation of tau by the phosphatase PP2A (Buée et al., 2006). As such, Pin1 was shown to restore the MAP-function of hyperphosphorylated tau and was suggested to prevent tangle formation. In yeast, the ortholog of Pin1 is encoded by ESS1, an essential gene. We demonstrate that in strains harbouring a temperature sensitive mutant of Ess1 the phosphorylation of tau is dramatically enhanced at the non-permissive temperature, indicating that similar to its human ortholog, Ess1 facilitates dephosphorylation of tau. In addition, the lack of functional Ess1 coincided with changes in the conformation of tau as detected by the MC1 antibody, which specifically recognizes pathogenic tau isoforms. Consistently, we observed tau-dependent toxicity in the Ess1ts strains when grown at the non-permissive temperature. Interestingly, all of the above described effects were completely restored upon co-expression of human Pin1 in the Ess1ts strains.

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Screening for Phosphorylation of Tau 4r/2n in Saccharomyces Cerevisiae Knockout Mutants

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Aggregation of tau protein is a hallmark in neurodegenerative diseases summarized as tauopaties, including Alzheimer's disease. The physiological role of protein tau, its binding to MT is regulated by phosphorylation and alternative mRNA splycing. Two major types of kinases are involved in phosphorylation of protein tau, i.e. proline-directed kinases like GSK3 beta, cdk5, cdc2 and JNK, and non-proline directed kinases, like PKA, PKC, CaMKII and MARK. We recently developed yeast-based models to study the human tau in detail. These humanized yeast models recapitulated robustly the important aspects of a tauopathy, i.e. hyper-phosphorylation, conformational change and self-aggregation. In addition the rapidity of genetic modification of yeast was capitalized upon the finding that major phospho-epitopes on human tau are produced by Mds1 and Pho85, the yeast orthologues of the two major mammalian tau-kinases, i.e. GSK-3 beta and cdk5, respectively.

In this work, we performed an epitope scanning to assess changes in tau phosphorylation upon deletion of three additional yeast GSK-3β homologues. We demonstrate that one of those, i.e. Mck1, is specifically involved in the formation of the AT-100 phospho-epitope. We also monitored the phosphorylation of the KXGS motifs in the microtubuli-binding repeats of tau upon deletion of the yeast kinases sharing homology to mammalian MARK. In addition, we present data on the contribution of PKA signalling pathway in the regulation of tau phosphorylation. Finally, we tested the involvement of dual-specificity kinases and demonstrate that Yak1, a Dyrk-family kinase, is essential for the generation of the phospho-tyr18 epitope on tau expressed in yeast.

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Neuropathological and Biochemical Characterization of Founder FTLDU-17 Patients Carrying Pgrn IVS1+5G>C

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Background & Aims: Mutations in the PGRN have recently been identified as a causative factor in FTLDU families linked to chromosome 17q21 (FTLDU-17). The aim of this study was to perform neuropathological and biochemical characterization of PGRN mutation carriers.

Methods: Brains and lymphoblasts from PGRN-IVS1+5G>C, PGRN-G125X, and VCP-R159H mutation carriers as well as sporadic FTLDU and Pick's disease patients were studied neuropathologically and by immunoblotting methods for a variety of proteins including the recently identified TDP43.

Results: A considerable heterogeneity in the degree of frontotemporal atrophy and in the relative prevalence of neuronal intranuclear (NII) or cytoplasmic (NCI) inclusions in cortical-layer-II was observed within PGRN-IVS1+5G>C carriers ('PGRN'), as well as between PGRN-carriers and patients with VCP-R159H ('VCP') and sporadic FTLDU patients ('Sp'). Roughly, for NII: VCP>PGRN=Sp; Cat-eye shaped NII: VCP>>PGRN=Sp; NCI: VCP=PGRN=Sp DR7; and, neuritic pathology: VCP=PGRN=Sp. In 50% of the PGRN-carriers, dentate gyrus was also involved. PGRN immunoreactivity had punctuate cytoplasmic staining and did not specifically stain NCI (or NII). In contrast, TDP43 staining was observed not only in the neuronal and glial cell-inclusions, but also diffuse staining was present in neurites and neuronal cytoplasm. Moreover, not all inclusions seemed to be stained by TDP43. Occasional staining of TDP43 in other types of inclusions, i.e., Pick bodies, was also observed.

Conclusions: These data support earlier observations that TDP43 is an important component of FTLDU neuronal-inclusions. A lesser staining with TDP43, however, suggests that this protein might be in a different conformation, or more likely, sequestered by other proteins in UBQ inclusions.

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Caspase-3 Causes the Formation of Human-Tau-Derived Toxic Fragments During Neuronal Apoptosis

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Truncated tau proteins are a hallmark of human Alzheimer's disease (AD).The cleaved state of tau influences its physiological ability to bind microtubules, to assume AD-related pathological conformations, to aggregate and assemble into filaments and to induce neuronal death .A transgenic rat model overexpressing truncated human tau has been shown to cause in vivo neurofibrillary tangles, demonstrating that cleaved forms of tau are sufficient to produce AD-like neurofibrillary degeneration by inducing oxidative stress. Previously, we have shown that adenovirus- mediated overexpression of 25-230 human tau fragment evokes a potent neurotoxic effect in primary neuronal cultures by sustained stimulation of NMDA receptor (Amadoro et al.,2006). In order to assess whether the 25-230 fragment is actually produced during apoptosis, we attempted to ascertain its presence using a site-directed, caspase 3- cleaved antibody (Rohn et al., 2002) against amino-terminal consensus cleavage site D25 of tau protein (QGGYTMHQDQ). We provide biochemical evidence that N-tau 25-230 fragments, consistent with the sizes produced by caspase -3 and calpain cleavage of different tau isoforms, are generated in staurosporine-treated differentiated human SY5Y. These findings support the notion that activation of apoptotic mechanism(s) may be directly involved in AD pathogenesis, possibly also via generation of tau fragments, indicating that inhibition of caspase-mediated cleavage of this protein(s) may be protective in vivo.

Amadoro et al.,P.N.A.S.103(8),2892-7 (2006); Rohn et al., Neurobiology of Disease 11,341-354 (2002).

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3-Dimensional Analysis of Brainstem Lewy Bodies and Lewy Neurites

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Background & Aims: Lewy body pathology is the hallmark of Parkinson's disease, but their shapes are various. Lewy bodies are spherical and Lewy neurites are slender like a thread. We reconstructed three-dimensional Lewy body pathology to clarify the morphological varieties of Lewy body pathology and speculated about the formation process of Lewy body pathology.

Methods: Four postmortem brainstem tissue samples were obtained. They were cryosectioned at 60-micron meter and sections were immunofluorolabeled with anti-neurofilament, anti-ubiquitin, and anti-alpha-synuclein antibodies. These triple-labeled images were captured by a confocal laser microscope and we observed the relationship between three epitopes of Lewy body pathology. Serial optical sections with an interval of 0.1-micron meter were obtained and reconstructed for three-dimensional analysis.

Results: We discovered that the axial sections of Lewy bodies and those of Lewy neurites have a common feature. Ubiquitin- and alpha-synuclein-immunoreactive structures formed concentric layers and neurofilament-immunoreactive structures rimmed the inner layers. Many Lewy bodies had several branches, which have the same layered structure as Lewy bodies. Some Lewy bodies connected with Lewy neurites.

Discussion & Conclusion: We consider the boundary between Lewy bodies and Lewy neurites is ambiguous because 1) Lewy bodies and Lewy neurites have common layered structures of same antigens and 2) there is successive change between Lewy bodies and Lewy neurites morphologicaly. We suppose the formation process of Lewy bodies have close relations with that of Lewy neurites.

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Alpha-Synuclein Dynamics in Neural Cells: Role of Lysosomal Glucocerebrosidase

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Background: Genetic, neuropathological and biochemical evidence has implicated an aberrant metabolism of alpha synuclein (alpha−S) in the development of several neurodegenerative disorders including Parkinson Disease (PD). This group of synucleinopathy diseases arises from a neurotoxic gain-of-function effect associated with increased steady-state levels of alpha−S intracellularly. One possible mechanism for such increases in intracellular alpha−S load might be a decrease in the degradation of αS, either through proteasomal or lysosomal dysfunction. Among the known lysosomal storage disorders, a loss-of-function mechanism caused by markedly reduced activity of acid beta glucocerebrosidase (GCase) underlies three distinct variants of Gaucher disease (GD). A subset of GD patients develop atypical parkinsonism, and heterozygous mutations in the GCase-encoding gene, GBA, act as susceptibility factor in the development of late-onset, sporadic PD. AIMS: We aimed to examine the inter-relationship between alpha−S dynamics and GCase activity in neural cells.

Methods: We transiently overexpressed GCase (wild type or GD-linked mutants), either alone or with its co-factor prosaposin, in MES cells and examined intracellular alpha−S load, as detected by both Western blot and a sensitive enzyme-linked immuno-adsorbant assay (ELISA).

Results: Overexpression of wild-type GCase led to a reduction in intracellular alpha−S load, and this effect was enhanced by co-expression of prosaposin.

Conclusions: Increasing GCase activity in the central nervous system, either by enzyme replacement therapy or pharmacological chaperoning, may represent a novel neuroprotective treatment strategy for Parkinson disease and related synucleinopathies.

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LRRK2 (Dardarin) is a Component of Alpha-Synuclein, Tau and Motoneuron Disease Inclusions

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Backgroung And Aims: Genes mutated in Mendelian neurodegenerative disease, such as alpha-synuclein or microtubule associated protein tau, encode proteins found in inclusions of both familial and sporadic disease. Mutations in leucine-rich repeat kinase 2 (LRRK2, or dardarin) are the cause of familial autosomal dominant Parkinson's disease linked to the PARK8 locus with a diverse neuropathology including alpha-synuclein and tau inclusions and motoneuron disease features. We therefore hypothesised that LRRK2 could be a component of inclusions found in sporadic neurodegenerative disease.

Methods: We studied LRRK2 expression in normal and pathologic brain tissue by immunohistochemistry.

Results: In controls we found widespread neuronal cytoplasmic expression. In synucleinopathies we found LRRK2 deposits in ~90% of nigral Lewy bodies (LBs) and in a high proportion of cortical LBs, in axonal spheroids in cases of Parkinson's disease and dementia with Lewy bodies, and in glial cytoplasmic and neuronal intranuclear inclusions of multiple system atrophy. We frequently found LRRK2 in neuronal tau tangles, tufted astrocytes and coiled bodies of progressive supranuclear palsy, in tau tangles of Alzheimer's disease and in Pick bodies of Pick's disease. Analysis of motoneuron disease cases showed LRRK2 to be deposited in round hyaline and Lewy body-like inclusions in the spinal cord and in axonal spheroids. Marinesco and Hirano bodies

were also strongly positive for LRRK2. We show that LRRK2 lies predominantly in the central core of most inclusions.

Conclusions: These new findings suggest a key role for LRRK2 in neurological diseases involving protein aggregation and the existence of convergent mechanisms of neurodegeneration.

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Detection of C-Terminally Truncated Alpha-Synuclein in Its Aggregated Form by a Modified Protein Aggregate Filtration Assay

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Parkinson's disease and dementia with Lewy bodies are caused by the pathological deposition of aggregated alpha-synuclein as Lewy bodies and Lewy neurites. Truncated isoforms of alpha-synuclein have been postulated to trigger aggregation of the full-length alpha-synuclein since C-terminal truncation of alpha-synuclein significantly increased its aggregation propensity in vitro [1,2]. In vivo soluble truncated isoforms were detected resulting either from alternative splicing (SYN112) of alpha-synuclein mRNA [3,4] or from posttranslational proteolytic cleavage enhanced in Parkinsons disease [1,2]. Interestingly, SYN112 was three-fold elevated in DLB compared to controls [4]. However, so far C-terminally truncated isoforms were detected only indirectly after partial solubilization of alpha-synuclein aggregates and not directly in its aggregated form.

Due to the inherent insolubility of alpha-synuclein aggregates we developed a protein aggregate filtration assay for the sensitive and selective detection of alpha-synuclein aggregates which is based on microfiltration of liquid samples as brain homogenates [5]. Using a modification of this assay we are able for the first time to detect C-terminally truncated alpha-synuclein in its aggregated form. We provide direct evidence that aggregated truncated isoforms may trigger aggregation of full-length alpha-synuclein in vivo. This might be similar to Alzheimer where the more aggregation prone Abeta42 precedes the aggregation of Abeta40.

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Haplotype Specific Expression of Alternatively Spliced N Terminal Exons at the Mapt Locus

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Background: The human MAPT locus is defined by two haplotypes, H1 and H2, with H1 being over-represented in the sporadic neurodegenerative dementias, Progressive Supranuclear Palsy and Corticobasal Degeneration. Variants of the H1 haplotype have also been found to be associated with Alzheimer's disease and Parkinson's disease. Recently, it has been shown that the H2 haplotype is under positive selection in European populations and may be protective. We propose that polymorphisms in the MAPT haplotype sequence are responsible for subtle differences in MAPT expression leading to a haplotype-specific susceptibility to neurodegenerative disease.

Methods: Previously, we have shown that the H1 chromosome significantly overexpresses transcripts containing exon 10, compared to the H2 chromosome. To further investigate haplotype effects on expression at this locus, we have now expanded the study to examine allele-specific expression of N-terminal alternative transcripts. The expression assays use haplotype-defining coding SNPs to perform allele-specific primer extensions, followed by quantitation of the extension products on the Sequenom MALDI-TOF platform.

Results: We have assayed MAPT expression in fourteen heterozygous, pathology-free post-mortem brain samples. Analysis shows approximately 70% greater expression of transcripts containing both N-terminal alternatively spliced exons (exons 2 and 3) from the protective H2 haplotype, compared to H1. This haplotype expression difference is present both in the frontal cortex and globus pallidus.

Conclusions: This evidence suggests the inclusion of both N terminal inserts, exons 2 and 3, in transcripts might play a functional role in the protective nature of the MAPT H2 haplotype.

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The Inhibitor of Apoptosis Protein (Iap) Survivin : A Putative Pin1 Partner for Regulating Neuronal Fate in Neurodegenerative Diseases ?

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Alzheimer's and Parkinson's diseases are characterized by the intraneuronal aggregation of Tau and alpha-synuclein into tangles and Lewy bodies respectively. Regulation of these proteins by the peptidyl-prolyl cis/trans isomerase Pin1 has been described recently. Pin1 binds to phosphorylated Tau, facilitates its dephosphorylation by PP2A and restores its ability to stabilize microtubules. Pin1 interacts with synphilin-1, an alpha-synuclein partner and facilitates the formation of alpha-synuclein inclusions. Furthermore, alteration of Pin1 has been reported in Alzheimer's disease and Pin1 colocalizes with Lewy bodies in Parkinson's disease. Thus, Pin1 seems to be one of the rare proteins involved in both Alzheimer's and Parkinson's diseases.

We have identified Survivin as a new neuronal partner of Pin1 in neuroblastoma SY5Y cells. Survivin belongs to the IAP family of proteins. It inhibits apoptosis and plays a key role during early brain development. Moreover Survivin is a regulator of microtubule dynamics like Tau. As a major G2/M regulator, Survivin is expressed in proliferative cells and weakly detected in adult neuronal cells. After brain trauma, Survivin is upregulated in neurons and would favour their survival. In our studies, Pin1 downregulates Survivin in neuroblastoma cells. This result leads to the hypothesis that, in degenerating neurons, alteration of Pin1 could favour abnormal reexpression of the cell cycle marker Survivin. This could contribute to apoptosis inhibition and promote neurofibrillary degeneration. Further studies on Pin1 and

Survivin relationship should allow to better understand the regulation of neuronal fate in neurodegenerative diseases.

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Visuospatial Apraxia in a Professional Graphic Artist With Corticobasal Ganglionic Degeneration: Clinical and Neuropathological Correlation With Artistic Works

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Background: Corticobasal ganglionic degeneration (CBGD) is a tauopathy with a clinical complex of asymmetrical extrapyramidal signs with progressive behavioral, cognitive and language impairment. The objective is to present the clinical and neuropathological features in a patient with CBGD with his many works of art over the several year course of his illness.

Methods: Neurological examination, neuropsychological and neuropathological examination correlated with graphic designs and paintings by the patient.

Results: The patient's artwork showed the first subtle signs of his illness, before any cognitive or behavioral changes. Concurrent with the gradual loss of spatial proportion, use of color and creative design in his paintings, the patient began to loose language ability and developed behavioral changes. In his last year, his drawings showed complete inattention to the left side of the subject matter while his neurological examination showed only moderate left sided asymmetrical rigidity and inattention. Loss of color in his painting was dramatic in this artist who had been rich in the use of color. Neuropathological findings showed tau-positive ballooned neurons, tangle-like inclusions, neuropil threads and Pick bodies in the cortex. The right parietooccipital cortex had significantly greater atrophy and degeneration than the left.

Conclusions: Visuospatial apraxia and loss of color recognition may be clinically underappreciated in patients with CBGD. Recognition of these features may allow earlier diagnosis in some patients.

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Lymphocyte Subset Markers and Cytokine Production in Peripheral Blood of Patients With AD

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Objective: To explore the differences of a) lymphocyte subset markers, b) cytokine release in supernatants of peripheral blood activated with LPS and c) proliferation.

Methods: 88 patients (46 male and 42 female; age range, 64 to 78 years) with AD and 24 age- and sex-matched healthy subjects (HS) were included in the study. Flow cytometry was carried out by using monoclonal antibodies. Lymphocyte proliferation was calculated as a stimulation index: (SI)=(OD experimental/OD background unstimulated) and IL-1β, IL-6 and TNF-α were measured in supernatants of peripheral blood activated with LPS and cultured for 72 hours at 37ºC.

Results: The mean percentage of CD3+ and CD4+ T cells in the lymphocyte population was significantly higher in AD patients than in the healthy group. However, the percentage of CD45RO was significantly reduced ($p<0.05$) in the AD group (33.8±3.6) with respect to the HS group (45.6±2.9). Blastogenic responses to Con A, PHA and PWM and NK cytotoxicity revealed a diminished proliferation response to Con A (SI: 19.8), PWM (SI: 20.8) and PHA (SI:23.0) mitogen stimulation as compared to controls (Con A: 22.8; PWM: 25.5; PHA: 26.3). The LPS-induced increase in all cytokines was greater in the AD group (IL-1β, 128 pg/mL, $p<0.05$; IL-6, 88 pg/mL, $p<0.01$; TNF-α, 95 pg/mL, $p<0.01$) with respect to the HS group (IL-1β, 77 pg/mL; IL-6, 55 pg/mL; TNF-α, 62 pg/mL).

Conclusions: The results presented in this study suggest that peripheral immune cells may participate in cytokine up-regulation, and possibly contribute to both brain tissue inflammation and neuronal damage.

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A Transgenic Rat Model of Early Alzheimer's Disease

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Animal models Alzheimer's disease (AD) of are extremely valuable for the discovery and development of new treatments. Most of these models have been generated in mice. However, for decades the rat has been the preferred model for pharmacological and behavioural studies. We report the generation of transgenic rats expressing human APP695 with the Swedish double mutation (tgAPPswe). The highest expression of the transgene was observed in cortex, hippocampus and cerebellum. Immunohistochemical examination of brain tissue revealed extracellular A 42 staining, either as cerebrovascular deposits or very rare diffuse plaques in the deep layers of the cortex, but the amyloid pathology was limited and occurred at a high age, above 15 months. Western blot analysis of hippocampal and cortical brain homogenates showed hyperphosphorylated tau in older animals, but no neuronal and synaptic loss. We further analysed the tgAPPswe rats for behavioural deficities. Our findings showed that these rats are hyperactive and displayed impaired acquisition of learning at 14 months of age. We also investigated the brain of transgenic rats by in vivo MRI at the age of 12 and 16 months. Examination of the MR images shows alterations in the hippocampus and lateral ventricles in transgenic rats compared to age-matched controls. These changes visualized by MRI are unique as they show an age related change that has not been seen in other models. We

believe that these tgAPPswe rats represent a unique model of early AD.

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A Study of LRK-1 Function in the Viability of Dopaminergic Neurons of C. Elegans Following 6-OH-DOPA Lesioning

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A rapidly expanding body of literature reports that mutations in Leucine Rich Repeat Kinase 2 (LRRK2) are strongly associated with autosomal-dominant Parkinson's disease (PD). Since loss of dopaminergic neurons is a well-documented pathological feature of PD, these highly compelling genetic data prompted us to investigate the role of lrk-1, the C. elegans ortholog of LRRK2, in the viability of dopaminergic neurons of C. elegans. To do so, we generated transgenic lrk-1 lines and determined the extent of dopaminergic neuronal degeneration in response to the neurotoxin, 6-OH-DOPA. Four transgenic lines expressing wild-type or mutant lrk-1 were created on a background null for lrk-1. Mutant lrk-1 lines were G1937S (equivalent mutation to PD-linked G2019S in LRRK2), K1787R (predicted to be kinase-dead) and G1937S, K1787R double mutant. Neurodegeneration for all transgenic lines was assessed by comparison to a negative control line (null for lrk-1). In the absence of neurotoxin, no spontaneous degeneration of dopaminergic neurons was observed for any line. On treatment with 6-OH-DOPA, no marked differences in neurotoxin sensitivity were observed either for wild-type or PD-linked mutant G1937S lines by comparison to the negative control. The predicted kinase-dead and double mutant lines did however demonstrate increased 6-OH-DOPA sensitivity. The data indicate that PD-linked mutant lrk-1 does not exacerbate neurodegeneration of dopaminergic neurons in C. elegans. Furthermore, the data suggest that lrk-1 kinase activity may play a role in promoting dopaminergic neuronal survival, indicative of a possible divergent role to that of mammalian LRRK2.

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Release of Glutamate in Rat Striatum by Mpp+ is Not Essential for Dopaminergic Neurotoxicity and is Not Derived From Glutathione

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Background and Aims: Perfusion of the parkinsonian neurotoxin 1–methyl-4-phenypyridinium (MPP+,10 mM) into the rat striatum evokes a delayed release of glutamate (Glu) leading to the proposition that the interactions of this amino acid with its receptors may cause excitotoxic damage to dopaminergic neurons. MPP+ also mediates a similar delayed release of glutathione (GSH). These observations suggested that the rise of extracellular Glu mediated by MPP+ might be the result of hydrolysis of released GSH by γ-glutamyl transpeptidase (γ-GT). The present study explored these propositions.

Methods: MPP+ dissolved in artificial CSF was perfused into the striatum of awake rats and microdialysates analzed for dopamine (DA), GSH, and Glu by HPLC. Dopaminergic neurotoxity was assessed by a two-day test/challenge procedure and tyrosine hydoxylase immunohistochemistry.

Results: Perfusions (30 min) of 0.7 or 1.3 mM MPP+ evoked neurotoxic damage to dopaminergic terminals but without the release of Glu or GSH. Higher concentrations of MPP+ caused more extensive dopaminergic neurotoxicity and release of Glu and GSH. However, neither this Glu release nor MPP+ induced dopaminergic neurotoxicity were affected by the γ-GT inhibitor acivicin. These observations indicate that the rise of extracellular levels of Glu is not essential for the neurotoxicity of MPP+ . Furthermore, the rise of extracellular Glu caused by perfusion of higher concentrations of MPP+ is not the result of the γ-GT-mediated hydrolysis of released GSH. It is possible that the rise of Glu evoked by perfusion of high concentrations of MPP+ may reflect, at least in part, its release from astrocytes.

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Two-Photon Imaging of Neuron-Microglia-Plaque Interactions in the Living Mouse

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Background: In Alzheimer's disease (AD), microglia, the resident immune cells of the brain are found in large numbers clustered around fibrillar amyloid plaques. However, it is unclear how significant a role they play in the process of amyloid deposition or removal as well as in injury to adjacent neurons. Here we investigate at high spatio-temporal resolution the interactions between neurons, fibrillar amyloid deposits and surrounding microglia in the living mouse brain.

Methods: In vivo transcranial two photon microscopy (TPM) was performed in mutant mice modeling cerebral amyloidosis (TgCRND8) crossbred with a mouse that expresses green fluorescent protein (GFP) in microglia (CX3CR1GFP/+). A subgroup of double mutant mice were also injected with viral vectors to drive the expression of Red Fluorescent Protein (RFP) in neuronal subtypes. Time-lapse images of microglia, amyloid plaques and neurons labeled with GFP, Methoxy-XO4 and RFP respectively, were obtained over intervals of minutes to months to establish a temporal correlation between plaque formation or loss, microglia accumulation and formation of dystrophic neurites.

Results: We demonstrate the feasibility of using in vivo two-photon time lapse imaging over long intervals to study the interactions between microglia, plaques and neurons. We also show evidence that fibrillar amyloid plaques are very stable over months while microglia have a very limited capability for removal of fibrillar amyloid deposits.

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Pink1 and Parkin Function in the Same Pathway to Regulate Mitochondrial Function

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Mutations in PTEN-induced kinase 1 (pink1, PARK6) and parkin (PARK2) cause familial and sporadic forms of Parkinsons disease (PD). pink1 encodes a putative serine/threonine kinase with a mitochondrial targeting sequence. We show that removal of Drosophila pink1 results in male sterility, apoptotic muscle degeneration, defects in mitochondrial morphology and increased sensitivity to oxidative stress. Expression of human pink1 in Drosophila restores mitochondrial function in pink1 mutants, demonstrating functional conservation. Loss of Drosophila parkin shows phenotypes similar to loss of pink1. Genetic tests show that pink1 and parkin function in the same genetic pathway, with pink1 functioning upstream of parkin to regulate mitochondrial function. Biochemical studies suggest that Pink1 is localized to mitochondria, and that Pink1 binds to Parkin.

Though pink1-mediated PD is thought to be largely recessive, several mutations have been found in heterozygous patients, raising the possibility that these mutations may have gain-of-function, haplo-insufficient (loss-of-function) or dominant negative effects. Expression of human disease version of pink1 (G309D and Q456Stop) at physiologic (endogenous) levels in wildtype flies has no phenotype, whereas expression in the pink1 null background results in partial function. These results suggest that the human mutations G309D and Q456Stop exert their disease-causing effects as a result of partial loss of pink1 function.

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Type-Specific Evolution of Amyloid Plaque and Angiopathy in APPsw Mice

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To clarify how Aβ deposits start in the brain, we examined the early to late stages of senile plaques and amyloid angiopathy in APPsw mice. All types of human senile plaques were observed in the mouse brains. The premature forms of cored plaques appeared first in the cerebral cortex of mice at 7-8 months old. Then,amyloid angiopathy emerged, followed by diffuse plaques consisting of Aβ1-42. Modifications of the N-terminus of Aβ were late phase phenomena. The premature forms of cored plaques were composed of central Aβ1-40 amyloid cores, surrounding amorphous Aβ deposits, and accumulation of Aβ in some peripheral cells. These cells were incorporated in amyloid cores, and these plaques developed to large cored plaques composed of Aβ1-40 and Aβ1-42. The size and number of cored plaques were increased with age. These findings indicate different evolution paths for cored plaques and diffuse plaques, and suggest the presence of a pathway that initiates with the intracellular accumulation of Aβ1-42 and leads to the development of classic plaques in human brain tissues.

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Transgenic Mice Carrying APPswe/PS1(dE9) Mutations Exhibit Epileptic Seizures

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Alzheimer's disease (AD) increases the risk of epilepsy 9-fold and is one of the most common underlying factors for epilepsy in the elderly. The occurrence of seizures is especially high in rare familial cases of AD with mutation in presenilins. However, until now there is no animal model for AD-related epilepsy.

We recorded video-EEG in 20 transgenic male C57BL/6J mice carrying mutated human APPswe and PS1dE9 genes (APdE9 mice) and their wild-type littermates (n= 10) at two age points, 11-13 and 17-18 weeks of age. APdE9 mice progressively developed seizures as they aged, so that by the end of the second recording period 65 % of APdE9 mice but none of their wild-type littermates displayed electrographic seizures and 38% of APdE9 mice exhibited secondary generalized seizures. Compared with seizure-free mice, APdE9 mice with seizures had a modest increase in mossy fiber sprouting, as revealed by Timm's staining. A separate group of 4-month-old APdE9 and control mice were tested for their behavioral reactivity by using the acoustic startle reflex. The response was more robust in APdE9 mice than in wild type littermates. Finally, brain slices were prepared from an additional group of 3.5-month-old APdE9 and control mice for patch-clam recordings. Pyramidal cells in layer 2/3 of the neocortex were hyperexcitable at this age, as a result of the temporally increased capacity of glutamate release from their excitatory afferents.

The APdE9 mouse provides a promising model to study molecular mechanisms of AD-related epilepsy.

Support: Northern-Savonia Cultural Foundation, Alzheimer's Association

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Mutant Presenilin 1 Alters Synapse Formation

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Mutations in presenilins are the major cause of familial Alzheimer's disease, but the precise pathogenic mechanism by which presenilin (PS) mutations cause synaptic dysfunction leading to memory loss and neurodegeneration, remains unclear.

Methods:Here we studied synaptic transmission in autaptic hippocampal cultures from transgenic mice expressing human PS1 with the A246E mutation.

Results: We could demonstrate that mutant PS1 significantly depressed the amplitude of evoked AMPA and NMDA receptor-mediated synaptic currents. Analysis of the spontaneous miniature synaptic activity revealed a lower frequency of miniature currents, but normal miniature amplitude. Both electrophysiological alterations could be rescued by the application of a gamma-secretase blocker indicating that a gain of function mechanism is involved.

In conclusion these findings strongly suggest that the expression of mutant PS1 in cultured neurons depresses synaptic transmission by causing a physical reduction in the number of synapses. This hypothesis is consistent with morphometic and semiquantitative immunohistochemical analysis revealing a decrease in synaptophysin-positive puncta in PS1 mutant hippocampal neurons.

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Effects of Minocycline on Neuroinflammatory Gene RNA Levels in 5 Month Old Tastpm Mice

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Neuroinflammation is a key feature of chronic neurodegenerative diseases such as Alzheimer's Disease. This central inflammatory response may be a consequence of specific neuronal lesions or may contribute to the formation of such lesions or indeed may act in combined role. As such neuroinflammation represents a clear target for drug intervention that could ameliorate neuronal cell loss in chronic neurodegenerative diseases.

We used TASTPM mice, a model of amyloid deposition in which pro-inflammatory genes are known to be significantly up-regulated, to investigate the effect of the tetracycline antibiotic, minocycline. TASTPM mice aged 5 months were dosed twice daily for 7 days with minocycline at 50 mg/kg or vehicle. After treatment the mice were euthanased and blood and brains harvested. RNA was purified by extraction in Trizol and used for Taqman analysis. GAPDH expression was used as a housekeeper gene and the expression of MIP-1alpha, MIP-1beta and GFAP were assessed as markers of neuroinflammation.

MIP-1alpha, MIP-1beta and GFAP expression were significantly up-regulated in the cortex of vehicle treated TASTPM mice compared to WT mice (confirming previous gene expression findings). Treatment of TASTPM mice with minocycline significantly inhibited MIP-1alpha and MIP-1beta expression ($p=0.045$ & $p=0.032$ respectively). No significant effect on GFAP expression was observed. Our results indicate that the TASTPM mouse model has utility as a pharmacodynamic marker model for anti-inflammatory drug treatment.

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Role of Cyclooxygenase Inhibitors on Intracerebroventricular Colchicine-Induced Cognitive Dysfunction and Oxidative Stress in Rats

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Background and Aim: Alzheimer's disease is a progressive neurological and psychiatric disorder. Oxidative stress and neuroinflammation have been implicated in pathophysiology of Alzheimer's disease. The present study was aimed to evaluate the effects of cyclooxygenase inhibitors against colchicine-induced cognitive dysfunction and oxidative stress in rats.

Materials and Methods: Following intracerebroventricular (i.c.v.) administration of colchicine (15 mg/5µl), rats exhibited poor retention of memory in Morris water maze and elevated plus-maze task paradigms and oxidative stress in rats. Chronic treatment with naproxen (per se; 20 and 40 mg/kg, p.o.) or valdecoxib (per se; 5 and 10 mg/kg, p.o.) daily respectively for a period of 25 days beginning 4 days prior to colchicine injection significantly improved colchicine-induced cognitive impairment.

Results: Intracerebroventricular colchicine injection resulted in free radical generation characterized by alterations in oxidative stress markers with a significant increase in malondialdehyde and nitrite levels and depletion of reduced glutathione levels in the brains of rats. It also caused a decrease in acetylcholinesterase activity. Besides, improving cognitive dysfunction, chronic administration of cyclooxygenase inhibitors (naproxen and valdecoxib) significantly reduced elevated malondialdehyde, nitrite levels and restored reduced glutathione levels and acetylcholinesterase activity. The results of the present study indicated that naproxen (per se; 20 and 40 mg/kg, p.o.) or valdecoxib (per se; 5 and 10 mg/kg, p.o.) treatment has a neuroprotective role against colchicine-induced cognitive impairment and associated oxidative stress.

Conclusion: The present findings further support the potential use of cyclooxygenase inhibitors in treatment of neurodegenerative diseases such as Alzheimer's disease.

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Memantine Improves Cognitive Performance in Mouse Model of Lewy Body Disease

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Lewy body dementia is characterized post-mortem by alpha-synuclein positive aggregates in cortical and limbic areas of the brain. Clinical presentation is defects especially in frontal cortical executive functions. We have established a transgenic mouse model where human alpha-synuclein is expressed under the mouse alpha-synuclein promoter. This leads to expression in several brain areas, with highest expression in cortex, hippocampus and thalamus. Human alpha-synuclein accumulates in cell bodies in these areas. Proteinase K digestion was used to show that alpha-synuclein forms proteinase resistant aggregates the same areas. These mice were tested for cognitive deficits in several tasks at 12 months of age. We found that the transgenic mice were impaired in learning new odor-reward association compared to their wild type littermates. The few data on memantine in patients with Lewy body dementia are inconclusive. We tested memantine in the same mouse group at 16 months of age by giving 5 mg/kg memantine ip or vehicle once daily for one week prior to testing and continuing the dosing daily during the test which took place in the following three weeks. In the new test the transgenic mice showed similar retention of already learned association than wild type mice but they were again impaired in learning new odor association compared to the wild type littermates. Transgenic mice that had received Memantine, however, showed improved performance in the task.

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Neurorescue-Neurogenesis of Dopamine Neurons by Rasagiline in Post-MPTP Treated Mice

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Background and Aims: Rasagiline (Azilect) is a novel anti-Parkinson's disease (PD) drug with neuroprotective properties. We determined the neurorescue activity of rasagiline in post-MPTP treated mouse model of PD.

Methods: MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, (24mg/kg/day 24 mg/kg, i.p, per day) was administrated for 4 days to C57/Bl mice, followed by a further 4 days resting period At day 8 the mice received rasagiline (0.05mg/kg/day, orally) or water (control) for 10 days. Transcriptomics and proteomic analysis of about 800 proteins in midbrain of rasagiline and MPTP-treated mice were examined. All the findings were corroborated immunohistochemically, neurochemically and by Western analysis.

Results: Rasagiline given chronically after exposure to MPTP, prevented the significant decline in striatal dopamine levels and tyrosine hydroxylase (TH) activity, and almost completely restored TH positive neuron viability. The most prominently enriched protein cluster altered by rasagiline corresponded to the signal transduction functional class, which related to the neurotrophic factor responsive-tyrosine kinase receptor (TRK) pathway. Immunohistochemical analysis corroborated the involvement of the Ras-Phosphoinositol-3-kinase (PI-3K)-Akt signaling pathway that was specifically restricted to TH containing neurons in the substantia nigra pars compacta. These results were supported by the increase in the phosphorylative inactivation of the Akt substrates, glycogen synthase kinase (GSK)-3β and Raf.

Conclusions: The activation of the TRK pathway by rasagiline and its documented induction of BDNF and GDNF transcript and protein levels may contribute to rasagiline neurorescue/regenerative property. The present findings may add to the existing preclinical data in support of a possible disease modifying activity of rasagiline.

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Evaluation of the Effects of Various Dopaminergic Neurotoxins in Rats and Mice

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Parkinson's disease (PD) is a progressive neurodegenerative disease. While symptomatic treatments work effectively in the early stages, it is of great importance to find agents that may halt or repair the nigrostriatal damage. As part of our efforts to look for such agents we have characterised a number of rodent models.

We evaluated the effects of unilateral infusion of 6-OHDA into the substantia nigra (SN) or the striatum, acute (4 x 20 mg/kg every 2 hr) and sub-chronic (8 x 30 mg/kg once a day) MPTP, acute and sub-chronic methamphetamine, subcutaneous and MFB infusion of rotenone and subcutaneous administration of the proteasome inhibitor PSI. In all studies we used tyrosine hydroxylase, behavioural and neurochemical end-points.

Data indicated that SN infusion of 6-OHDA produced a robust nigrostriatal lesion and functional deficits (amphetamine and apomorphine induced rotations) while striatal infusion produced a slow partial lesion. Acute and chronic MPTP produced robust depletions, but the sub-chronic model had much less side-effects in the mice. Acute methamphetamine also produced robust dopamine depletions, but we also observed changes in noradrenaline and serotonin in certain brain areas. We failed to see any nigrostriatal injury after systemic rotenone or PSI, but saw a progressive nigrostriatal lesion after MFB infusion of rotenone.

In conclusion, it is clear that all of the toxins studied can produce dopamine depletion. However, the dose, dosing protocol, route of administration and end-points need to be carefully optimised before assessing neuroprotective agents. We are currently evaluating neuroprotective agents in the 6-OHDA and MPTP models.

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Cognitive Deficits and Cortical Pathology in Alpha-Synuclein Transgenic Mice

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Alpha-synuclein is a presynaptic protein which is a major component of pathological inclusions in neurons in Lewy body dementia, and Parkinson's disease. We have expressed the human alpha-synuclein in mice under the mouse alpha-synuclein promoter. This leads to expression of the human protein in a very similar pattern to the mouse protein, with high expression in frontal cortical areas and hippocampus. These mice were tested in several cognitive tests at 12 months of age. They show a deficit in odour recognition test in learning of new odour pairs. Pathological examination of the brains shows widely distributed proteinase K resistant alpha-synuclein fibrils in cortical areas and hippocampus. This mouse may be a useful model for Lewy body dementia and dementia in Parkinson's disease.

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Characterization of a New Neuron-Specific Promoter for Gene Expression (mTUB)

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Background: In the pharmaceutical research it is an advantage to have a disease-specific animal model with predictive value for optimal testing of future therapeutics. To produce such an animal model for some neuronal disorders like Alzheimer's disease, a strong neuron-specific promoter is a requirement. The promoter decides if, how and to what extent the transcription of a gene into mRNA takes place.

Methods: The mTUB promoter is composed of the short strong mThy-1 promoter region comprising both nonameric promoter sequences, parts of regulatory sequences of mThy-1 intron A and the SV40 late poly A resulting in a short potent neuron-specific promoter.

Results: Different transgenic cells and mouse models expressing the reporter protein eGFP or therapeutic genes like hAPP under the control of the mTUB promoter have been established. The reporter protein eGFP was a helpful tool for characterization of promoter properties. It could be shown that mTUB drives an increased tissue-specific transgene expression in neuronal cells and neuronal tissue.

Conclusion: The characterization of different mTUB-hAPP transgenic mice is on the way. The new hAPP-swedish/london and the hAPP-austrian transgenic models are estimated to show early and high plaque load, cognitive impairments and behavioral disturbances. First results will be presented.

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Characterization of a p75NTR Transgenic Mouse Model

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Background: p75 neurotrophin receptor (p75NTR) is a type I transmembrane receptor structurally related to the tumor necrosis factor receptor superfamily of death receptors. p75NTR binds non-neurotrophin ligands, including amyloid beta peptides (Abeta), known to abnormally accumulate in brains of Alzheimer Disease (AD) patients, and pro-NGF (Nerve Growth Factor) which is shown to be upregulated in AD brains. The abnormal accumulation of Abeta in the form of senile plaques is one of the main characteristics of AD. It has also been shown that two members of the neurotrophin receptor superfamily, TrkA and p75NTR, differentially regulate the processing of APP. TrkA reduces, whereas p75NTR activates the β-cleavage of APP.

Methods: In our study we have generated several mouse lines transgenic for human p75NTR gene under the control of the murine Thy-1 promoter in order to induce neuronal overexpression of human p75NTR.

Results: p75NTR mRNA and protein expression were detected in different brain regions as well as in spinal cord of tg mice. Histological data comprising detection of apoptosis are also demonstrated.

The Morris-Water-Maze test showed a significant cognition and memory decline in 8 months old transgenic mice, whereas the same animals were inconspicuously in Open-Field test and showed no motor deficits in RotaRod tests.

Conclusion: In future experiments vulnerability of these mice will be tested using neurotoxic compounds like MPTP.

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mGluR5 Antagonism Reverses Motor Deficits in a Transgenic Mouse Model of Parkinsonism

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Background and Aims: Glutamatergic system components are well positioned within the CNS to play roles in the pathogenesis of neurodegenerative disorders and have received interest as a Parkinsonian (PD) motor dysfunction therapeutic via restoration of dopamine-glutamatergic homeostasis. Antagonism of metabotropic glutamate type 5 receptors (mGluR5) reverses motor deficits in several rodent models of Parkinsonism. The "D-line" transgenic mouse has been genetically engineered to overexpress wildtype human alpha-synuclein under the PDGF promoter, and have significant deficits in motor and spatial learning behavior. We previously reported increases in mGluR5 expression in motor and spatial learning-associated brain regions of these mice.

Our goal was to determine whether mGluR5 antagonism could ameliorate motor deficits in D-line transgenic mice.

Methods: Motor performance of non-transgenic and transgenic mice was evaluated using the pole apparatus test – a measure of motor coordination and strength.

Results: Pretreatment of transgenic mice with mGluR5 antagonist MPEP (2-Methyl-6-(phenylethynyl)-pyridine) reversed deficits in the pole apparatus test – a test of motor performance. Studies are underway to extend these findings to additional behavioral paradigms (motor and spatial learning), mGluR5 antagonists, and transgenic mouse lines overexpressing alpha-synuclein under other promoters.

Conclusions: This result indicates that altered mGluR5 expression may modulate motor performance deficits in the alpha-synuclein transgenic mouse model of Parkinsonism. Taken together, these results may provide insight to glutamatergic alterations in Parkinsonian disorders.

Supported by The Branfman Family and MJ Fox Foundations, NCRR RR04050 RR043050 (Mouse BIRN), and NIH LM 07292.

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Neuroprotective Effects of Cerrebrolysin on Neurofibrilary Pathology in APP-Transgenic Mice That Received Neuronal Gene Transfer With AAV2-Tau

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Alzheimer's (AD) continue to be the most common cause of cognitive and motor alterations in the aging population. Accumulation of Abeta-protein oligomers and phosphorylated tau might be responsible for the neurological damage. We have previously shown that Cerebrolysin reduces the synaptic and behavioral deficits in APP tg mice by decreasing amyloid production by regulating APP phosphorylation via modulation of GSK3 and CDK5 activity. These kinases also regulate Tau-phosphorylation and are involved in promoting neurofibrillary pathology. In order to investigate the neuroprotective effects of Cerebrolysin on Tau pathology, a new model for neurofibrillary pathology was developed using somatic gene transfer with AAV2-Tau (P301L). Two sets of experiments were performed, in the first thy1-mutant APP tg mice (3 m/o) received bilateral injections of AAV2-Tau into the hippocampus, in the second APP tg neonates received intracerebral injections of AAV2-Tau. Control experiments were performed with non-tg mice. Animals were analyzed 3 months after the injection. Preliminary neuropathological analysis showed that compared to injections in non-tg mice, in APP tg mice intrahippocampal injections with AAV2-Tau resulted in localized increased accumulation of phosphorylated tau. Neonate mice injected with AAV2-Tau displayed a more widespread distribution phosphorylated Tau that included the neocortex and limbic system. Mice from both groups are being treated with Cerebrolysin for 3 months at 5ml/kg. This study supports the concept that Amyloid beta protein and Tau interact to induce neurofibrilary pathology and that somatic gene transfer of AAV2-Tau is a valuable alternative tool to develop models of Tau pathology in tg mice.

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Microgliosis in the Olfactory Bulb of Parkinson Patients and MPTP-Treated Mice: A Role for Interleukin-1?

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Parkinson's disease (PD) is one of the most common neurodegenerative disorders, in which a progressive degeneration of midbrain dopaminergic neurons is associated with increasing impairments in motor function. Besides, and even before overt degeneration of nigrostriatal dopaminergic neurons, neuronal pathology can be observed in the olfactory bulb (OB).

Neuroinflammation plays an important role in the pathogenesis of Parkinson's disease (PD). The present study questioned whether this neuroinflammatory response differs between the olfactory bulb, as an early affected region, and the late affected nigrostriatal system. Indeed, increased microgliosis was shown by immunohistochemistry (CD68) in post-mortem olfactory bulb of PD patients compared to control subjects. Also in olfactory bulb of MPTP-treated mice, microgliosis and increased expression of IL-1alpha, IL-1beta and IL-1ra mRNA was observed early after treatment as measured by q-PCR and confirmed by immunohistochemistry. These observations implicate that neuroinflammation is not restricted to the nigrostriatal system. MPTP-induced microgliosis in striatum and olfactory bulb was reduced in IL-1alpha/beta knockout mice, indicating that IL-1 region-specifically affects microglia reactivity. Importantly, MPTP induced differential regulation of IL-1 receptors as detected by q-PCR. mRNA levels of IL-1RI and, to a lesser extent, IL-1RII were increased in striatum. Interestingly, in the olfactory bulb only IL-1RII mRNA was enhanced. This may indicate that differential regulation of IL-1 signaling serves as an important mechanism to modulate neuroinflammatory activity after MPTP treatment and possibly during PD.

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Age-Dependent Axonal Degeneration and Behavioural Impairment in the APP/PS1ki Mouse Model of AD

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Deficits in axonal transport have been recently implicated to play a significant role in the progress of neurodegenerative diseases like Alzheimer's disease (AD). These defects can manifest as axonal swellings or spheroids, which correspond to axonal enlargements and aberrant accumulation of axonal

cargoes, cytoskeletal proteins and lipids. The APP/PS1ki mouse model for Alzheimer's disease exhibits abundant early intraneuronal Aß42 accumulation with concomitant hippocampal CA1 neuron loss starting at 6 months of age. We analyzed the brain and spinal cord of this mouse model and provide compelling evidence for axonal degeneration. The APP/PS1ki mice showed characteristic axonal swellings, spheroids, axonal demyelination and myelin ovoids in an age-dependent manner. Abundant accumulation of intraneuronal N-modified Aß, Thioflavin S-positive material and ubiquitin was found within the somatodendritic compartment of neurons. Furthermore, at the age of 6 months, dramatic age-dependent changes in body weight and other physical parameters were observed, accompanied by a significant reduced ability to perform working memory and motor tasks. While 2 month-old APP/PS1ki mice were inconspicuous in all of these tasks and properties, a massive age-related impairment in a variety of motor and cognitive behavioural paradigms was noted. Onset of these behavioural alterations correlates with robust axonal degeneration in brain and spinal cord, as well as with abundant hippocampal CA1 neuron loss. We conclude that the accumulation of intraneuronal Aß-amyloid peptides might induce axonal transport deficits, which eventually lead to axonal degradation in AD.

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Brain Insulin and Insulin Receptor Dysfunction Triggers Alzheimer-Like Pathology in An Experimental Rat Model

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Background & Aims: Decreased brain glucose/energy metabolism and cognitive deficits similar to those found in sporadic Alzheimer's disease (sAD) were reported in streptozotocin (STZ)-intracerebroventricularly (icv) treated rats, suggesting them as an experimental sAD model. Brain insulin receptor (IR) signaling cascade dysfunction found post mortem in sAD, has been reported recently also in STZ-icv rat model, in which brain insulin and IR activity, and tau protein as both a terminal substrate in IR signaling cascade, and a pathological marker of sAD, were here investigated.

Methods: Gene expression of insulin and IR, and alterations of IR-beta subunit, protein tyrosine kinase (TK) activity and tau protein were measured by means of quantitative RT-PCR, ELISA, PTK assay and Western blot, respectively, in rat frontoparietal cortex (CTX) and hippocampus (HPC), three months following the STZ-icv (1 mg/kg) treatment.

Results: The expression of insulin-1 and -2 mRNA was found significantly decreased in HPC and CTX, respectively. Both regions demonstrated significantly decreased IR mRNA expression. Significantly increased concentration of phosphorylated IR-beta subunit has been found in CTX, with no change in HPC, while significantly increased TK activity was found in HPC only. Total and phosphorylated (GSK-3 related) tau protein was significantly increased in HPC of STZ-icv rats.

Conclusion: STZicv-induced discrepancy between reduced IR gene expression and increased phosphorylated IR tyrosine residues/TK activity may indicate imbalance between its phosphorylation/dephosphorylation causing IR dysfunction, and subsequently triggering tau hyperphosphorylation, which points to the brain insulin/IR dysfunction as a possible pathological core in the generation of sAD.

Supported by Croatian MZOS and DAAD.

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Absence of Ret Signaling in Mice Causes Progressive and Late Degeneration of the Nigrostriatal System

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Background And Aims: Support of ageing neurons by endogenous neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) may determine whether the neurons resist or succumb to neurodegeneration. GDNF has been tested in clinical trials for the treatment of Parkinson's disease (PD) a common neurodegenerative disorder characterized by the loss of midbrain dopaminergic (DA) neurons. BDNF modulates nigrostriatal functions and rescues dopaminergic neurons in PD animal models. The physiological roles of GDNF and BDNF signaling in the adult nigrostriatal DA system are unknown.

Method: We generated mice with regionally selective ablations of the genes encoding the receptors for GDNF (Ret) and BDNF (TrkB).

Results: We find that Ret, but not TrkB, ablation causes progressive and adult onset loss of DA neurons specifically in substantia nigra pars compacta (SNpc) and degeneration of DA nerve terminals in striatum and pronounced glial activation.

Conclusion: These findings establish Ret as a critical regulator of long-term maintenance of the nigrostriatal DA system and suggest conditional Ret mutants as useful tools for gaining insights into the molecular mechanisms involved in the development of PD.

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Modulation of Presenilin 1 Activity in Zebrafish Embryos

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Analysis of developmental effects of changes in Presenilin protein structure may increase the understanding of the role of presenilin mutations in Alzheimers Disease (AD). Changes in Presenilin protein structure alter the cleavage of the Amyloid prescursor protein resulting in accumulation of Amyloid-beta, a common feature of AD. Some inherited AD cases present with altered presenilin transcript splicing. Zebrafish possess orthologues of human presenilin1 and presenilin2 genes and have been demonstrated as a model for study of presenilin1 activity. In zebrafish embryos we can block Presenilin1 translation and splice acceptor sites by using morpholino antisense oligonucleotides (MOs). Loss of presenilin1 activity in zebrafish embryos results in disturbed neurogenesis and somite formation (resembling Notch pathway mutants) and quantitative PCR analysis on 2 day old embryos demonstrates an increase in preseniln1 transcription. Furthermore this increase in transcription is also evident in embryos with modulated presenilin splicing. These results, together with additional protein expression analyses, further substantiate the use of zebrafish as a model to investigate Presenilin protein structure and function.

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Multi-Functional Assessment of Diet-Induced Hypercholesterolemia on Brain Cholesterol Metabolism in Aged Brown Norway Rats - A Model for Alzheimer's Disease?

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Disrupted cholesterol homeostasis in the brain and the peripheral circulation is associated with the etiology of Alzheimer's disease (AD). Since the brain's cholesterol metabolism is segregated, attenuated cholesterol metabolism in the AD brain is unlikely to be a direct consequence of elevated plasma cholesterol. Accordingly, we hypothesized that there should be signals derived from the peripheral circulation secondary to the chronic hypercholesterolemia (HC) that will compromise normal metabolism in the brain. We created a diet-induced HC condition in young, middle-aged, and older male Brown Norway rats (8-, 17- and 26-mo) by feeding cholesterol+cholic acid supplemented purified diet (CCA) for 3-4 months. The CCA diet effectively created a "humanized" HC in the peripheral compartment by significantly increasing total cholesterol in plasma and liver by 1.4-fold and 4-fold, respectively and shifting lipoprotein peaks from HDL to VLDL. Total peripheral metabolic change was also revealed by a change in the Urinary Metabolomic profile. Unlike the peripheral lipid changes, the brain's lipid profile was solely age-dependent with TC and free cholesterol peaking at midlife. Hippocampus microarray analysis revealed that the prolonged HC in old rats was associated with >2-fold change in expression of >2000 genes. Immunohistochemistry showed Aβ deposition in the hippocampus and cerebral cortex of the old animals. A Morris water maze test revealed a decline in memory induced by HC in the older rats (significant time x diet interaction). These data suggest that the HC Brown Norway rat can be used to effectively assess communication between the brain and peripheral circulation.

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The Arctic Alzheimer Mutation Alters Amyloid-Beta Deposition in Transgenic Mice

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Background and Aims: APP transgenic models are valuable Alzheimer's disease models to investigate Abeta amyloidosis. Senile plaques in most APP-transgenic mice are effortlessly cleared by various Abeta antibodies and their plaques are highly soluble at mild conditions of chemical extraction. In contrast, in human AD brain, Abeta plaques are resistant to even harsh solvents treatment. We wanted to investigate if the highly protofibrillogenic Arctic APP-mutation alter the senile amyloid plaque pathology and created transgenic mice with both Arctic (E693G) and Swedish (K670N, M671L) APP mutations (Tg-ArcSwe). The character of Abeta deposits and secondary amyloid-associated neuropathology in the plaque vicinity were compared to the Tg-Swe transgenic mouse model at advanced ages.

Methods: Human Abeta deposits in transgenic mouse brain were analysed with histochemical staining at light microscopic level and with electron microscopy. In addition, Abeta peptide composition was biochemically analyzed with ELISA assays and MALDI-TOF.

Results: Histological and biochemical analyses of structure and composition of Abeta deposits are indicative of differences between senile plaques made out of wt Abeta and Arctic Abeta.

Conclusions: A greater pool of highly insoluble Abeta and altered secondary tissue response in the vicinity of senile plaques indicates that plaques in Tg-ArcSwe mice are more similar to the chemically resistant senile plaques of AD human brain than plaques in Tg-Swe mice.

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Endogenous Drosophila Appl and Fly A-Betą Induces Amyloid Deposit Formation; Neurodegeneration and Behavioral Deficits

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Invertebrates have proven to be valuable models for deciphering the fundamental molecular mechanisms of a variety of human neurological diseases. Recently, Drosophila has also been used to study the pathogenic function of Amyloid Precursor Proteins and their involvement in Alzheimer's Disease. Transgenic expression of human A-beta

peptides in flies leads to the production of thioflavinS-positive amyloid plaques, neurodegeneration, and behavioral deficits. Recently, we also showed that expression of human APP695 itself resulted in the deposition of amyloid deposits and age-related neurodegeneration in flies, and that these effects were exacerbated by co-expression of additional copies of the secretases involved in APP processing. We now show that APPL, the Drosophila orthologue of the Amyloid Precursor Protein (APP), is also capable of producing intracellular fibrils, amyloid deposits, progressive neurodegeneration, and age-dependent behavioral deficits. Overexpression of APPL in the brain was sufficient to induce these phenotypes, however, expressing only the predicted A-beta fragment of APPL or co-expressing APPL with the Drosophila BACE-like enzyme we have recently identified exacerbated both the morphological and behavioral deficits seen in aging flies. Since the primary sequences of human and fly A-beta peptides are only partially conserved, secondary structural aspects of these amyloidogenic peptides must determine their pathogenesis. These studies indicate that Drosophila provides the first non-transgenic model for investigating the mechanisms by which amyloid peptides may lead to neurotoxiciy and behavioral deficits.

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Generation and Characterization of a Conditional LRRK2 G2019S Transgenic Mouse Model

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Mutations in the LRRK2 gene have been linked to an autosomal dominant Parkinson's disease (PD). Dardarin, the gene product of LRRK2, belongs to a family of leucine-rich repeat kinase proteins, containing a leucine-rich repeat (LRR) domain, a RAS-like GTPase domain, a kinase domain, and a WD40 domain. The G2019S substitution within the kinase domain of Dardarin represents the most prevalent genetic mutation in PD, resulting in an increase of Dardarin kinase activity, and suggesting a gain-of-function mechanism and a central role for Daradrin kinase activity in the progression of PD. To better understand how G2019S mutation in Dardarin contributes to the pathogenesis of PD, we have generated a conditional LRRK2 G2109S transgenic mouse model to achieve high expression of mutant Dardarin protein in a spatial and temporal-dependent fashion. We inserted LRRK2 G2019S cDNA into an expression vector under the control of tetracycline operator. We have obtained multiple lines of LRRK2 G2019S mice, which express various levels of mutant Dardarin protein in the forebrain after having been crossed with CamKII-tTA transgenic mice. The higher expressers of Dardarin G2019S mutant mice began to display decreased spontaneous locomotor activity at 2 months of age. Moreover, increased protein phosphorylation was observed in the Dardarin G2019S mouse brain lysate. In summary, we have successfully developed a mouse model over-expressing Dardarin G2019S mutation. Our preliminary data suggest that increased Dardarin protein kinase activities may underlie the decreased locomotor activities of Daradrin G2019S mutant mice.

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Cortical Atrophy in Mouse Lemur Primates: Association With Visual Impairments and Neuropathological Alterations

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Cerebral atrophy is a common pattern in Alzheimer's disease and in many other neurodegenerative processes (1). It is also reported in aged mouse lemur primates (2). These small animals (12 cm, maximal life span of 12 years) can also display diffuse amyloid depositions and tau pathology while aging (3).

We assessed the location of cerebral atrophy in 30 mouse lemurs aged from 1.9 to 11.3 years. 3D MR images (isotropic resolution=234µm) were recorded on a 4.7T Bruker system. An automatic segmentation technique was used to detect cerebro-spinal fluid (CSF) voxels (2). Then, CSF voxels from various functional regions (frontal, temporal, parietal areas…) were manually labeled and counted. Cortical atrophy was detected in only one young animal. The prevalence of cortical atrophy increased with age (25% and 75% in middle aged and old animals). Some animals displayed a focal atrophy while in some others the atrophy process involved the whole brain (4 aged animals). The latter animals had major visual impairments. Only one of these mouse lemurs had amyloid deposits. This animal also had microhemorrhages throughout the brain. Histological evaluations also revealed glial accumulation in the animals presenting with brain atrophy. Our data suggests that neuropathological alterations other than amyloidosis should be investigated to explain the brain atrophy in mouse lemurs.

1. Valk et al., Magnetic Resonance in Dementia, 2002. 2. Dhenain et al., MRM, 2003. 3. Mestre-Frances et al., Neurob Dis, 2000.

Acknowledgements: Small animal imaging program (CNRS/INSERM/CEA), longevity GIS, 'Neuroscience ACI research' programs (French Minister for Research).

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Molecular Modeling of Amyloid Aggregation in Posodopora Anserina

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Aggregation of amyloid proteins is involved in many neurodegenerative disorders such as Alzheimer's disease (AD), type 2 diabetes, Parkinson's disease, and prion diseases. Prions are increasingly turning up in different organisms, particularly yeast and other fungi. The formation and transmission of several prions in yeast and fungi are known to be based on the formation of amyloid fibrils by particular proteins, for example Ure2p in S. cerevisiae and HET-s in Podospora anserina.

Prions are believed to be infectious, self-propagating polymers of otherwise soluble, host-encoded amyloid proteins.

This concept is now strongly supported by the recent findings that amyloid fibrils of recombinant prion proteins from yeast, fungi and mammals can induce prion phenotypes in the corresponding hosts. However, this concept of infectious protein may be extended to others amyloid proteins or peptides. Indeed, it has been recently shown that Abeta amyloid aggregates from human patients are able to induce AD like phenotype in a mouse model.

Our previous work on the HET-s prion protein of Podospora had allow to demonstrate the infectivity of the Het-s prion protein and to correlate in vitro structural elements with in vivo amyloid aggregation.

In AD, it become obvious that a crucial point in designing therapeutics and molecular markers is to define structural features of in vivo Abeta amyloid aggregates. For this reason, we decide to realize in vivo molecular studies of Abeta amyloid aggregation using the model of Podospora anserina.

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The Nmda Antagonists Memantine, Sdz-220,581 and MK801 Produce Dissociable Patterns of Performance on a Cognitive Test Battery in the Rat

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There is a large unmet medical need for agents that offer cognitive enhancement to treat the dementia associated with Alzheimer's disease. Current agents, such as memantine and the acetylcholinesterase inhibitors, offer provide modest therapeutic benefits. Robust in vivo assays that assess different cognitive domains are required to test the effects of novel agents. In this study, the NMDA antagonists memantine (un-competitive), SDZ-220,581 (competitive) and MK801 (non-competitive) were profiled through a within-subject cognitive test battery in the rat. The battery comprised five stages: (1) discrimination acquisition (reference memory) in which naive animals learned a simple visuo-auditory discrimination in a discrete-trial procedure; (2) delayed discrimination (working memory) in which a delay was imposed between presentation of the stimulus and the levers; (3) reversal learning (executive function) in which the lever-signal contingency was reversed (4) extinction in which only one lever was now rewarded and (5) extinction consolidation (drug-free) in which the extinction test was repeated the following day.

SDZ-220,581, MK801, but not memantine produced a dose-dependent disruption of acquisition. Only SDZ-220,581 disrupted performance of the delay task; all three compounds disrupted the reversal test. Only MK801 disrupted the first extinction test. In contrast, during the consolidation test, all three compounds disrupted performance. All three compounds produced a dose-dependent increase in head entries. These data show that different NMDA antagonists can produce distinct and dissociable effects on cognitive processes. Furthermore they suggest that the battery of cognitive tests presented do indeed tap into different cognitive domains.

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Protein Expression Profile in AD: A 2 De Study in the 3xTg-AD Mice

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The triple-transgenic Alzheimer mouse (3xTg-AD ,Oddo et al, 2003) expresses mutated PS1 and APP (PS1M146v; APPswe) as well as the tauP301L transgenes, and undergoes plaques deposition and appearance of neurofibrillary tangles with a temporal- and region-specific profile that closely mimics the pathological changes found in the AD brain. Thus, the model represents a valuable tool for investigating the molecular mechanisms underlying the different stages, from preclinical to symptomatic, of the AD brain pathology.

In this study, we employed a proteomic approach (two-dimensional gel electrophoresis (2D-E) and mass spectrometry (MS)) to investigate changes in protein expression in the brain of 3xTg-AD mice (Silvestri et al, 2006).

We analyzed total proteins expression in brains from either 18 month old 3xTg-AD or non Tg (control) mice combining 2D-E and MS. Differentially expressed spots (P at least < 0.05) between the two experimental groups were identified and divided in several categories.

In this set of preliminary experiments, we found significant differences in the expression of several presynaptic proteins involved in neurotransmitter synthesis, storage and release (esocytosis and endocytosis), in the expression of proteins involved in energy metabolism and cytoskeleton maintenance. Our results seem to provide further evidence for the hypothesis of AD as a condition strongly associated with synaptic derangement and dysfunction.

Oddo et al , Neuron. (2003) 39(3):409-21

Silvestri E et al J Proteome Res. (2006) 5(9): 2317-27

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Learning and Hippocampal Synaptic Plasticity is Enhanced at First and Impaired Later in a Mouse Model of Alzheimer's Disease

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We have characterised a new Alzheimer mouse model (APPPS1-21) which expresses the Swedish mutation of APP and a mutated form of human PS-1. In the Open Field analysis, the APPPS1-21 mice showed hyperactivity at the age of 3 month ($p<0.01$), but not at 5 and 8 months. Exploratory behaviour was changed in older animals from 8 months onwards ($p<0.001$). In a spatial 4-arm maze reversal task, APPPS1-21 mice performed worse after 8 months when reversing the baited arm ($p<0.001$), but not before. At 15 months of age, animals were also impaired in the acquisition

(p<0.001). In a 6-arm maze, APPPS1-21 animals were better at the acquisition of the task at 5 months of age (ANOVA p<0.01), but no difference was seen at 6.5 months. At 15 months, performance of APP animals was much reduced (p<0.001). We also tested LTP in vivo in area CA1 of the hippocampus. Surprisingly, LTP was much enhanced at 5 months of age in transgenic mice (ANOVA p<0.001), but was absent in 8 months old mice (ANOVA p<0.0001). APP expression seems to have transient facilitating effects on LTP and memory formation. However, as beta-amyloid levels rise and plaques build up, impairment sets in.

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Comparison of Effect of Bilateral Carotid Occlusion, a Rat Model of Alzheimer's Disease, in Young and Middle Aged Rats

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Cerebral hypo-perfusion occurs during aging, and is considered a risk factor for Alzheimer's disease (AD). Permanent bilateral carotid artery occlusion (2VO) in rats reduces cortical blood flow and glucose utilisation, impairs spatial memory and has been proposed as a model of pathological changes in AD. Aim: To compare the effects of 2VO on spatial memory and hippocampal gene expression in rats aged 3, and 10 months old. Methods: Bilateral 2VO was performed under equithesin anaesthesia. Spatial memory was assessed in the Morris water maze and gene changes were determined using the "whole rat" DNA AffyChip. Results: Rats of both age groups showed a similar increase in locomotor activity in an unfamiliar open field 18 days after 2VO and deficits in reference and working memory after 30 days. While 2VO induced up-regulation in about 400 genes and down-regulation of about 350 at both ages, their identities differed considerably in the two groups. In young rats, gene changes were associated with a reduction in dendrite morphogenesis and in the regulation of microtubule assembly with a compensatory increase in neurogenesis and mitochondrial activity. Older rats showed a decrease in 9 genes associated with myelination, and a compensatory increase in 12 genes regulating synaptic activity. These data show for the first time that rats respond quite differently at the genetic level to the same insult of 2VO according to their age. This should be taken into account when using this model to assess the effect of potential anti-AD drugs.

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The Phenotype of the LRRK2 Knock-Out Mouse Model

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Mutations in the Leucine Rich Repeat Kinase LRRK2 are responsible for autosomal-dominant late-onset Parkinson´s Disease linked to chromosome 12q12. At present this is the most common cause of late-onset PD. Mutations within LRRK2 are found in up to 13% of familial PD cases compatible with dominant inheritance and in 1-2% of sporadic PD patients. To date, seven missense mutations have been shown to segregate in families, but several other new mutations have also been described. All mutations result in a spectrum of disease similar to typical late-onset idiopathic PD.

We generated a knock-out mouse model for LRRK2 in order to study the normal function of the protein. First we validated our model with Western, Northern and Southern analysis. We are able to show that the LRRK2 protein is completely absent in our model. At the age of seven month male and female mice are viable and fertile. Using cortical and hippocampal primary cultures where LRRK2 protein is usually highly expressed we provide evidence that LRRK2 is involved in vesicular trafficking and membrane recycling. This is of particular interest because it elucidates one possible pathway of LRRK2 function in vivo. Even though the disease is transmitted in an autosomal-dominant manner it is still not clear if a gain of function of the known mutations is also linked to a loss of normal function of LRRK2 in neurons of the adult brain.

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Region Dependent Nmda Receptor Loss in Apoe Knockout Mice: Autoradiographic Studies

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Background and Aims: Alzheimer's disease (AD) is a devastating neurodegenerative disorder which preferentially strikes women. The hall mark of AD is cognitive decline, and the glutamatergic system, specifically the NMDA receptors, plays a major role in cognitive processes. We tested the hypothesis that NMDA receptor availability will be lower in apoE knockout (APOKO) mice than in wildtype mice.

Methods: Brains of C57bl mice (wildtype and APOKO, 8-10 months old) were fresh-frozen and cryo-sectioned in multiple consecutive series. Sections at the level of striatum and hippocampus were incubated with the selective non competitive NMDA antagonist [3H]MK801. Non specific binding was assessed on consecutive sections in the presence of excess unlabeled MK801. Sections were scanned by beta imaging and quantitative regional analyses were performed with beta vision software.

Results: APOKO females (N=11) had significantly decreased NMDA binding compared to control females (N=7), which was also region dependent [significant 2 way ANOVA by genotype (P=0.004) and region (P=0.001)]. The decrease was especially pronounced in hippocampal regions (e.g. CA1, 39.1%; DG, 38.4%; CA3, 37.5%) which are linked to cognitive performance, while there was no apparent difference in the striatum and cingulate cortex. We also tested for differences between APOKO females (N=11) and APOKO males (N=10). While there was a trend for lower NMDA binding in the females, it did not reach statistical significance.

Conclusion: APOKO animals, which have been shown to exhibit cognitive deficits compared to wildtype, also have

decreased NMDA receptor availability in regions that are important in cognition.

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Assessment of Microglial Activation in a Mutant APP.PS1 Transgenic Mouse Model of Alzheimer's Disease Using [3h] Pk11195 and Real-Time Autoradiography

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The aim of this study was to examine the temporal and spatial changes in microglial activation in the APPswe x PS1.M146V (TASTPM) mouse, a transgenic model of Alzheimer's disease, where amyloid beta (Aβ) deposition can be detected from 3 months of age and cognitive deficits observed by 6 - 8 months of age (Howlett et al, 2004). We have used [3H]PK11195, a specific and high affinity radioligand for peripheral benzodiazepine receptors (present in activated and proliferating microglia) and CD68 immunoreactivity as markers of microgliosis. Coronal brain cryosections were collected from TASTPM or wild type mice (n=4/group) over an age range of 2-12 months. Autoradiography was carried out with 1nM [3H]PK11195 and non-specific binding was defined by the addition of 10 μM PK11195. Sections were analysed by real-time autoradiography using a Beta Imager 2000. Adjacent slides were immunolabelled with antibodies to CD68 and appropriate biotinylated or fluorescent secondary antibodies.

A significant increase in specific [3H]PK11195 binding was detected in cerebral cortex and hippocampus of TASTPM mice aged 8-12 months compared to either wildtype animals or TASTPM mice younger than 6 months of age. These findings compared favourably with immunohistochemical analyses which revealed increased CD68 immunoreactivity by 8 months in the cerebral cortex and hippocampus of TASTPM mice which was co-localised with Aβ deposits.

We have shown that [3H]PK11195 is a sensitive tool for in vitro evaluation of temporal and spatial inflammatory changes that occur in a transgenic mouse model of Alzheimer's Disease.

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Presynaptic Glutamatergic Changes in the Appswe/Ps1de9 Mouse Model of Alzheimers Disease

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Recent research indicates that the cognitive symptoms of Alzheimer's disease could be due to synaptic dysfunction caused by soluble forms of abeta and that these effects of abeta may be linked to alterations in GluR function. Specifically, it has been shown that soluble abeta causes reductions in synaptic levels of GluR1, internalization of NMDARs at the synapse, depression of NMDAR currents, and reduction of LTP. Additionally, presynaptic markers such as SNAP-25 and synaptophysin are also down regulated by abeta. However, most studies examining the effects of abeta on AMPAR/NMDAR expression and transmission have utilized cultured neurons, and it is therefore not clear whether abeta also causes alterations in the glutamatergic system in vivo, for instance in transgenic models with excess abeta in the brain.

We studied whole cell AMPA and NMDA currents and LTP in hippocampal slice preparations and assayed cortical PSD-like fractions for their content of the GluR subunits GluR1, GluR2/3, NR1, NR2A, and NR2B, as well as the postsynaptic marker PSD-95 in 18 MO APPswe/PS1dE9 mice. We found no difference in any of these makers suggesting that postsynaptic function is unaltered. However, using microdialysis we sampled hippocampal glutamate in 3 and 9 MO mice and found that potassium evoked release of glutamate was severely compromised in the oldest mice while basal levels of glutamate were unaltered at either age.

These data support the hypothesis that excess brain abeta contribute to alterations in synaptic function in vivo but suggest that the earliest changes may be related to presynaptic impairments.

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Long-Term Evaluation of Acute and Sub-Chronic MPTP Models in the Mouse

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Despite the development of several transgenic models of Parkinson's disease (PD), the MPTP model in the mouse remains viable and is still the benchmark for evaluating disease-modifying therapies. The systemic administration of the neurotoxin MPTP produces reliable and selective degeneration of the nigrostriatal pathway, a hallmark feature of PD. We have characterized two models of MPTP, an acute model based on a single high dose (40mg/kg) and a sub-chronic model (30mg/kg/day x 5 days), in terms of neurochemical, immunohistochemical and behavioral endpoints. Multiple groups of 10 weeks old male C57/bl6 mice were treated with MPTP and subsequently evaluated at different time intervals of 1, 2, 3, 4, 8 and 12 weeks for behavioral effects on distance traveled, striatal dopamine (DA) concentration. Furthermore, immunohistochemical analyses of the nigrostriatal pathway were used to quantify injury to dopaminergic terminals with tyrosine hydroxylase (TH) & dopamine transporter (DAT) immunoreactivity. The objective of these studies is to develop better understanding of the temporal relationship between different measures of the nigrostriatal pathway function enduring neurotoxin-induced injury. The discrepancies between DA conc. and TH & DAT IR in the striatum, particularly at the later time points following the MPTP insult, suggest that multiple measures are necessary to gain a more complete understanding of the functional integrity of the nigrostriatal pathway in the MPTP models

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Retromer-Deficient Mice Overproduce Endogenous A-Beta and Model Key Features of Late-onset Alzheimers Disease

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Backgroud: The primary molecular defects that contribute to late-onset Alzheimer's disease (LOAD) remain undetermined. Recently, the proteins VPS35 and VPS26 were found to be selectively reduced in the brains of LOAD patients. VPS35 and VPS26 are key elements of the retromer, a coat complex that transports type-I transmembrane proteins from the endosome back to the trans-golgi network. Because the retromer is an integral complex, reducing VP26 reduces all retromer elements, leading to retromer dysfunction and the mis-sorting of retromer cargo.

Aims: To phenotype and characterize VPS26 heterozygote knock-out mice.

Methods And Results: Western blot analysis showed that both VPS26 and VPS35 are reduced in the brains of the knock-out mice, establishing that they are retromer-deficient. ELISA showed that wildtype endogenous Aβ40 and Aβ42 are overproduced in the brains of retromer-deficient mice. Increased brain Aβ was associated with selective defects in hippocampal function, as demonstrated by impairments in hippocampal-dependent memory such as the modified radial arm maze, and by defects in hippocampal long-term potentiation. Mechanistically, co-immunoprecipitation studies showed that the amyloid-precursor protein (APP) is a cargo of the neuronal retromer, suggesting that retromer dysfunction mis-sorts APP. This proposed mechanism was confirmed by immunocytochemistry, showing that retromer dysfunction leads to increased cellular co-localization of APP and its cleaving enzymes.

Conclusions: These findings A) confirm the relevance of retromer dysfunction to LOAD pathogenesis; B) suggest a cellular mechanism for why Aβ levels are increased in LOAD; and, C) introduce a mouse-model of LOAD.

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Characterisation of Ask1 Knock-in Mice: Cortical Neurons From Ask1 Ki Mice Are Not Resistant to Amyloid Induced Neurotoxicity

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Ask1 kinase lies upstream of p38 and JNK kinases and is activated via a number of stimuli, including oxidative stress and proinflammatory stimuli such as TNFalpha. This kinase may therefore be involved in a number of disease states. Published data on Ask1 knock-out (KO) animals demonstrated a reduction in in vitro neuronal cell death following incubation with Abeta 25-35 peptide, suggesting a potential role in Alzheimer's disease. Through the introduction of a point mutation we have generated an Ask1 kinase dead knock-in (KI) mouse to more accurately mimic the effects of a small molecule inhibitor, i.e. the presence Ask1 protein but inactivated kinase. Recombinant kinase assays were used confirm that protein purified from the kinase dead construct was inactive in vitro. Moreover, signalling via Ask1 was reduced in cells from Ask1 KI animals. Neurotoxicity experiments performed with primary cortical neurons incubated with Abeta 1-42 peptide showed no increase in viability in the KI neurons compared to wild-type neurons. Additional experiments were carried out utilising co-cultures of rat cortical neurons and cortical mouse microglia stimulated with LPS/IFNgamma. No difference in cortical neuron viability was seen when neurons were co-cultured with Ask1 KI microglia compared to wild type co-cultures. Our results demonstrate a clear difference in cellular responses between cells taken from Ask1 KO compared to those from the Ask1 KI mice.

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App23 Transgenic Mice Show Altered Response to the Glutamate Nmda Receptor Antagonist MK801 and Cognitive Deficits in Spatial Memory

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Background: Synaptic dysfunction is an early manifestation in Alzheimer's disease (AD). An association between Ab, over-expressed in AD, and the neuronal alteration has been suggested, but the mechanism by which Ab affects synapse remains unclear. Changes in the expression of glutamate receptors have been shown in cortical neurones of Tg2576 APP mutant mice.

Aim: We studied the effect of a non-competitive NMDA receptor antagonist, MK801, on locomotor activity and spatial memory in APP23 transgenic (APP23tg) mice over-expressing human amyloid-precursor protein.

Methods: Mice were injected intraperitoneally with saline or 0.2, 0.5 mg/kg MK801 or 0.8 and 3.2 mg/kg amphetamine, and their locomotor activity recorded for 2 h. Learning and memory were examined using an 8-arms radial maze. After training to high levels of performance mice were injected with saline or 0.2 and 0.35 mg/kg MK801. The spatial working memory performance of APP23tg and wild type (WT) mice in a delayed-non-matching to sample (DNMTS) task was evaluated using a 60 sec delay.

Results: Our study shows that in APP23tg the effect of MK801, on locomotor activity was significantly lower that in WT mice. In contrast amphetamine increased locomotion to a similar degree in both APP23tg and WT mice. In addition, APP23tg mice showed learning and memory impairments in the spatial working memory tasks.

Conclusion: APP23tg mice seem to present alteration in the NMDA glutamatergic transmission. Consistent with the involvement of glutamatergic system in synaptic plasticity and cognitive functions, such alteration appears to correlate with deficits in spatial learning and memory.

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A Stress-Enhanced Model of Parkinson's Disease in Drosophila

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Background and Aims: Combination of both environmental stress and susceptible genetic background has been known to account for the occurrence of both familial and sporadic forms of PD. To understand better the pathological mechanisms of PD, a stress-enhanced model of PD in Drosophila model was created and characterized.

Methods: Transgenic flies overexpressing human alpha-Synuclein (a-Syn), which is a gene tightly associated with familial PD disease and is the primary structural component of Lewy body fibrils, were exposed to oxidative stress by feeding them paraquat or 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP), which can induce indistinguishable Parkinson's symptoms in humans and other models.

Results: The effects of these oxidants on PD progression in the fly model were quantified using a climbing assay. The previously observed decreases in climbing ability and dopaminergic neurons and increases in the appearance of a-Syn positive inclusion bodies all were accelerated in our stress-enhanced PD model.

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Requirement for Activity at Both D1 and D2-Type Receptors in a Mouse Model of Late Stage PD

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L-DOPA and apomorphine are more efficacious than other dopamine agonists (i.e pramipexole) in late stage Parkinson's disease (PD). One obvious distinction between these compounds is that both L-DOPA and apomorphine can stimulate D1 and D2-type dopamine receptors while agents such as pramipexole only activate the D2-type receptors. MPTP is a neurotoxin that selectively destroys dopamine-containing cells and has been used extensively in mice as a model for PD. In our hands administration of MPTP (2x15mg/kg, given 3h apart) produces approximately 60% depletion of striatal dopamine. AMPT (α-methyl-para-tyrosine) is an inhibitor of tyrosine hydroxylase and when administered to MPTP mice produces progressive decrease in motility that peaks around 3h and is maintained for 4h before motility gradually increases again. The decrease in motility reflects a state of severe dopamine depletion. The MPTP + AMPT mouse model was developed in order to recapitulate the clinical situation of superiority of D1/D2 compounds. Various dopaminergic compounds were tested for their ability to reverse the motility deficits produced in this model. Both L-DOPA/Benserazide and apomorphine restored locomotion in the mice in a dose-dependent manner. In contrast, the D2 agonist, pramipexole, did not. Other compounds are currently being tested. This model can be used to evaluate the efficacy/'superiority'of novel compounds for treatment of late-stage PD.

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Characterisation of Dopamine Neuronal Damage and Neuroprotective Effects of Salicylate in MPTP and 6-OHDA Models of Parkinson's Disease

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It is well established that MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and 6-OHDA (6-hydroxydopamine) can produce dopaminergic damage in mice and rats and thereby provide model systems of dopamine neurodegeneration seen in Parkinson's disease (PD). Although both compounds are dopamine neurotoxins, there are many differences in the mode of their effects, as well as in the mechanisms by which neurotoxicity is produced.

In order to identify and test novel disease modifying targets, we compared and characterised two sub-chronic mouse MPTP dosing regimens (8 days x 30 mg/kg, i.p. or 7 days x 10, 20 or 30 mg/kg, i.p. every other day) and two acute rat 6-OHDA (4 microgram infused into the substantia nigra (SN), or 10 microgram infused into the caudate putamen) paradigms using immunohistochemical, neurochemical and rotational behaviours as end-points. Additionally, we tested the efficacy of the anti-inflammatory agent, salicylate, (50 or 75 mg/kg, i.p.) in each model.

The data provide a better understanding of the time course of dopamine neuronal damage by the two neurotoxins using multiple end-points. Additionally, salicylate provided robust protective effects against both striatal and nigral damage in the two MPTP models but was inactive in either the SN or retrograde 6-OHDA lesion model. These results are consistent with previous reports of distinct mechanisms contributing to dopamine neuronal damage by the two neurotoxins. These results will facilitate the selection of a target-appropriate model system and help design intervention trials at an appropriate time point in these models.

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Alpha-Synuclein Expression in 6-Ohda Rat Model of PD

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Alpha-synuclein is a member of a relatively new family of proteins considered to be involved in pathogenesis of PD and other neurodegenerative diseases. Due to the localization of alpha-synuclein in presynaptic terminals, its role in synaptic function and plasticity was postulated as well. 6-OHDA rat model of PD is characterized by acute loss of dopaminergic neurons, but specific alpha-synuclein aggregates were not reported. Therefore, this model could point to the possible role of alpha-synuclein in plasticity after chemical injury given that

some compensatory sprouting of dopaminergic neurons exists after period of extensive neuronal death.

Changes in alpha-synucelin expression were followed in the striatum, mesencephalon, and structures anatomically or functionally related to the nigrostiatal system, 40 days after unilateral intrastriatal 6-OHDA injection. Using Western blot analysis, we detected the increase in alpha-synuclein expression in ipsilateral striatum, mesencephalon and cerebellum, compared to sham operated controls. Observed increase was the most prominent in the cerebellum. Given that the changes in non-striatal brain regions are of particular interest as they could be involved in the motor and cognitive impairment following dopaminergic denervation and considering the proposed role of alpha-synuclein in plasticity, colocalization of alpha-synuclein with presynaptic markers, GAP-43 and synaptophysin, was examined by confocal microscopy as well. The obtained results support depict point out to the role of alpha-synuclein in synaptic plasticity.

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MPTP Increases Gstp1 Expression and Jnk Phosphorylation in C57bl/6 Mouse Midbrain and Striatum

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Idiopathic Parkinson's disease (PD) is a neurodegenerative disease of multifactorial aetiology known to involve aging, genetic predisposition and environmental aggressions. Several studies have demonstrated that the Bcl-2 family of apoptotic proteins and the c-Jun N-terminal kinase (JNK) are key mediators of MPTP-induced neuronal cell death in animal and cellular models of PD. Epidemiological studies revealed that Glutathione S-transferase P1 wild-type allele is an individual protective genetic trait in sporadic PD. Furthermore, Gstp1 can act as a ligand-binding protein and an endogenous switch for the control of JNK catalytic activity. However, the role of Gstp1 on MPTP-induced neuronal toxicity has never been evaluated.

In this study we investigate the expression of Gstp1 and selected apoptotic markers in the C57BL/6 mouse midbrain and striatum, upon single dose MPTP administration. Gstp1 and tyrosine hydroxilase levels were evaluated by immunohistochemistry. The relative concentrations of the Gstp1, p-JNK/JNK, Bcl-2 and Bax proteins were estimated by Western blot. JNK activity was measured by non-radioactive kit. Direct interaction of Gstp1 and JNK was determined by JNK immunoprecipitation followed by immunoblotting with anti-Gstp1 antibody.

The results to be presented show that: Gstp1 is actively expressed in both mouse midbrain and striatum, and MPTP induces a transient significative increase in Gstp1 expression. Furthermore, the results indicate that the apoptotic cascade is activated upon MPTP treatment with concomitant transient increase of JNK enzymatic activity. The putative role of Gstp1 on protection/aggression against MPTP induced dopaminergic –cell death will be discussed.

Supported by FCT and FEDER (POCI 2010): project POCI/SAU-FCF/58171/2004.

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Molecular Profiling of a 6-Hydroxydopamine Model of Parkinsons Disease

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Convection enhanced delivery of 6-hydroxydopamine (6-OHDA) to the rat striatum results in a model of Parkinson's disease. An important feature of this unilateral model is the progressive loss of dopaminergic (DA) neurons over the course of approximately 4 weeks. To improve the understanding of this model, gene expression changes in the striatum and substantia nigra, which contain the synaptic terminals and cell bodies for the DA neurons, respectively, were examined using an Agilent Technologies DNA microarray. Samples were collected from vehicle and toxin treated animals at 3 days, 1 week, 2 weeks and 4 weeks following treatment. Tissue DA content was determined, and only samples from animals which exhibited a significant depletion of striatal DA were included in the gene expression analysis. The functional consequences of the 6-OHDA treatment were evaluated at each time point using the forelimb asymmetry test and the stepping test. The results of the gene expression analysis indicate that 6-OHDA elicits a vigorous inflammatory response in the striatum at the earliest time point tested. In contrast, relatively few gene expression changes are observed in the substantia nigra at the 3-day time point. In both tissues examined there is evidence for an ongoing inflammatory response at the 1 and 2 week time points. Inflammation plays a prominent role in the 6-OHDA model of Parkinson's Disease.

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Cultured Neurons From Embryonic Tgcrnd8 Mice Display Cell Death, Increased Amyloid Beta Production, and Aberrant Calcium Regulation

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Murine models mimic a variety of classic Alzheimer's disease (AD) symptoms. The majority of these models have been studied in vivo, but lack characterization in vitro. The aim of this study was to characterize basal in vitro properties of the TgCRND8 mouse model of AD. This early-onset familial model exhibits Aβ brain deposits and deficits in cognition beginning at 3 months of age, reminiscent of classic signs of AD. We investigated APP and Aβ1-42 production, neuronal survival, neurite outgrowth, reactive oxygen species (ROS) generation, and calcium handling, in the TgCRND8

mouse. We found that primary cortical neurons from transgenic embryos produced excessive amounts of APP and secreted significantly more Aβ1-42 than WT littermates. Further, transgenic and WT cortical and hippocampal cells demonstrated similar initial survival and neurite outgrowth however, a significant decrease in survival beginning 11 days after culture in the transgenic group was observed. In addition, ROS and evidence of apoptosis were present in transgenic and WT cortical cells, but they were not significantly different. We observed a significant difference in glutamate-induced calcium release following pre-treatment with thapsigargin in transgenic cortical cells compared to WT littermates. However, no significant difference in glutamate-induced calcium release alone between the two groups was detected. Our data suggests that although initially no basal differences existed between transgenic and WT TgCRND8 primary neurons, after increased production of Aβ1-42, differences between the two groups became apparent.

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Impairments of Synaptic Functions Occur Prior to Neuronal Loss in a Mutant Tau Transgenic Mouse

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Filamentous tau lesions as exemplified by neurofibrillary tangles (NFTs) formed by tau filaments are characteristic hallmarks of Alzheimer's disease and associated neurodegenerative disorders termed tauopathies, while molecular events emerging early in tau pathogenesis are yet to be elucidated. To investigate alterations of neuronal functionalities anteceding loss of neurons, we conducted in vivo electrophysiological assays for transgenic (Tg) mice overexpressing mutant human tau, driven by a neuron-specific promoter. Deposition of fibrillary tau resembling human NFTs was observed in Tg mouse at around 6 months of age. MRI volumetric and histological analyses revealed progressive neuronal loss and consequent brain atrophy at or beyond 9 months of age. Notably, measurements of input-output curve and pared-pulse ratio in the hippocampi of 6-month-old Tg mice indicated perturbation of baseline synaptic transmission primarily by presynaptic dysfunction. Furthermore, substantial defects in relatively late phase of hippocampal long-term potentiation were observed in these Tg mice. Our findings provide explicit evidence that the functional deteriorations in hippocampal synapses are induced by pathological tau accumulation prior to neuronal death.

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Severe Behavioural Deficits and Distinctive Brain Pathology in a Transgenic Tau Mouse Model

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Background: TAU protein primarily expressed in neurons is an abundant microtubule-associated CNS protein, involved in the pathogenesis of Alzheimer's disease and related neurodegenerative disorders known as tauopathies. Their major neuropathological characteristics are neuronal and/or glial inclusions formed by aggregated paired helical and/or straight filaments composed of aberrantly phosphorylated TAU. Multiple TAU gene mutations are pathogenic for hereditary frontotemporal dementia and FTDP-17, with filamentous TAU aggregates as the major lesions in the CNS of these patients. Several FTDP-17 missense TAU mutations, including V337M and R406W have been demonstrated to reduce the ability of bacterially expressed recombinant TAU protein to bind to and promote the assembly of microtubules. Transgenic mice over-expressing mutated human TAU are thought to be a suitable model to study the influence of drugs on TAU phosphorylation, sequestration and deposition.

Methods: Mice over-expressing TAU441 bearing the missense mutations V337M and R406W under the control of the brain specific murine Thy-1 promoter were generated. Three transgenic mouse lines were evaluated in terms of behavior and brain pathology.

Results: The TMHT mice develop TAU pathology in early age, starting at 3 – 5 months. Mice showing no motor deficits but severe cognitive impairments in the Morris water maze task. TAU depositions were determined by staining with diverse anti-human PHF as well as TAU antibodies. Results showed numerous densely packed human tau-positive lesioned neuronal perikaria and neurites (axons and dendrites) in Hippocampus and Cortex.

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Comparison of Two Transgenic Mouse Models Expressing Truncated (1-120) Human Alpha-Synuclein in a Null Background and in the Presence of Endogenous Alpha-Synuclein in Dopaminergic Cells

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The presynaptic protein α-synuclein is a key player in the pathogenesis of Parkinson's disease (PD). Mutations or multiplication of its gene were identified in familial forms of PD, and overexpression in animal models reproduces several

features of the human disease. We have recently reported a transgenic mouse model in which a truncated (AA 1-120) form of the human protein is expressed under the control of the tyrosine hydroxylase (TH)-promoter, in a mouse α-synuclein null background (syn (1-120) mouse line). These mice show pathological α.synuclein inclusions and fibril formation in dopaminergic cells of the substantia nigra and olfactory bulb, as well as an increase in microglial numbers. In this mouse line striatal dopamine levels are reduced and spontaneous locomotion in the open field test is decreased. However, no significant dopaminergic cell death in the substantia nigra is observed in these mice. We have now produced another transgenic mouse model in which the truncated protein is expressed at higher levels under the control of the TH-promoter in a mouse expressing endogenous α-synuclein (syn (1-120) ENDO). Preliminary immunohistochemical data and stereological cell count of dopaminergic cells of the substantia nigra suggest an increase in α-synuclein pathology, which appears also at an earlier stage and is accompanied by cell death in syn (1-120) ENDO mice with endogenous α-synuclein compared to syn (1-120) mice in a mouse α-synuclein null background.

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The Human Parkin Mutation T240r Causes Selective Loss of Dopaminergic Neurons in a Drosophila Model

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Mutations of parkin have been identified in familial Parkinson's disease and in some sporadic cases. Heterozygous parkin mutations are an important determinant of age of onset. Current in vivo genetic models using parkin knockouts have not consistently produced loss of dopaminergic (DA) neurons. Expression of mutant T240R, but not wild type, human parkin in Drosophila causes age-dependent, selective degeneration of DA neurons, accompanied by an adult onset, progressive movement disorder. Overexpression or knockdown of the Drosophila vesicular monoamine transporter (DVMAT), which regulates cytosolic DA homeostasis, partially rescues or exacerbates, respectively, the degenerative phenotypes caused by mutant human parkin. These results support a model in which the vulnerability of DA neurons to parkin-induced neurotoxicity is due to the interaction of mutant parkin with cytoplasmic DA. The model we have established provides a novel platform for identifying pathways regulating survival of DA neurons. Supported by the American Parkinson's Disease Association and the Parkinson's Disease Foundation.

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C.Elegans Genetics as a Model for Parkinson's Disease

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Parkinson's disease (PD) is characterized by severe incapacitating motor symptoms primarily caused by the progressive and selective loss of the dopaminergic neurons of the substantia nigra. Exposure to toxins like 6-OHDA, MPTP and pesticides (Rotenone, Paraquat) is linked to PD. Independently, mutations affecting alpha-synuclein, parkin, UCHL1, DJ1, PINK1 and LRRK2 have been linked to familial forms of PD.

To further dissect mechanisms underlying dopaminergic neuronal death we aim to further develop the nematode C. elegans as the simplest possible experimental system. Our approach will take advantage of a system that allows for scoring the selective loss of the eight dompaminergic neurons of hermaphrodite worms as previously described by Nass et al., 2002. As most genes involved in PD are conserved in worms, we started by looking at the survival of dopaminergic neurons in PINK-1 (pink-1) and LRRK2 (lrk-1) knockouts upon 6-OHDA intoxication. While our preliminary studies haven't shown any overt worm phenotype or increased neurodengeneration, ongoing studies will explore further intoxication regimes.

In parallel, we will assess potential phenotypes of dominant PD genes in the worm dopaminergic system. Finally, we aim at setting up genetic screens to identify genes that protect worms form dopaminergic neurodegeneration, to analyze their role in the degenerative process and their link to known PD genes. We hope that our work will help to uncover genes and mechanisms involved in the dopaminergic cell death and that this, in the long term, will also allow to test and select for pharmacological drugs protecting from PD.

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Generation and Characterization of An in Vivo Model for LRRK2 Mutations in Parkinson's Disease

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Parkinson's disease (PD) is one of the most prevalent progressive neurodegenerative diseases in the world to date. PD is caused by degeneration of dopaminergic neurons in the substantia nigra, resulting in the typical devastating clinical features of PD. The LRRK2 gene has been identified only recently, but already turns out to be the most common genetic cause of familial but also sporadic PD. LRRK2 encodes a protein with multiple predicted functional domains. Point mutations leading to autosomal-dominant PD have been found in coding regions of all identified domains. Others have recently shown that LRRK2 has kinase activity, and that particular PD linked mutations modulating this activity, induce neuronal degeneration in cultured cells. In spite of this

exciting progress, it remains unclear what aspects of LRRK2 function are relevant toward PD progression under physiological conditions.

To address this issue, we have generated a model system for PD linked LRRK2 mutations, using the recombinase mediated cassette exchange (RMCE) insertion method, introducing any desired mutation of LRRK2 into a single parental ES cell line. The strength of this approach is that we use minimal changes in the LRRK2 genetic locus (not introducing artificial overexpression). Subsequently, we will use these ES cell lines to study LRRK2 function in vivo by generating 'knockin' mice and in vitro by differentiating ES cells into dopaminergic neurons. We hope that these studies will give us more insight into the role of LRRK2 in the molecular pathways of PD pathogenesis and identification of potential targets for therapeutic intervention.

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Diminished Nicotinic Receptor Density and An Impairment in Attention But Not Memory in the 192 IgG-Saporin Model of AD

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Background & Aims: 192 IgG-saporin (SAP) is a toxin used to create specific lesions of cholinergic neurons in the rat, which can model the known reduction of cholinergic cell density observed in the brains of AD patients (1). To further knowledge of this model, SAP was administered intracerebroventricularly (icv) to female Sprague-Dawley rats and subsequent changes in neuronal entities and cognitive functions were assessed.

Methods: Lesion efficacy was assessed with choline acetyltransferase (ChAT) immunohistochemistry. Neuronal nicotinic receptor (nAChR) and vesicular acetylcholine transporter (VAChT) densities were measured with autoradiography. Attention and mnemonic function were assessed with tasks measuring reaction time to vibrissal stimulation and exploration of a novel versus a familiar object, respectively.

Results: The lesion produced 63% - 74% reductions in ChAT neuron density. nAChR density, assessed with the specific radioligand [125]I-A-85380, was significantly reduced in the hippocampus and cerebellum whilst VAChT density was reduced in a known pattern (2). In cognitive tests, SAP animals displayed a significant, but mild, attentional impairment but no mnemonic impairment.

Conclusions: To our knowledge, this is the first study to observe a significant reduction of nAChRs in this model, likely due to the excellent properties of [125]I-A-85380. It is also the first time an icv SAP model has been assessed with these behavioural tests. The results found with these are consistent with the known behavioural profile of the SAP model (3).

(1) - Lancet, 2:1403

(2) - Nuc Med Bio, 27:23-31

(3) - Neurobiol Learn Mem 71: 325-52; Neurosci 132: 13-32

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Immunoreactivity Study of Neurotensin and Neurotensin Receptor Type 1 Among Globus Pallidus Neurones in Normal and Dopamine Depleted Conditions

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In rat Globus Pallidus (GP) neurotensin (NT) is believed to be released from striatal terminals. In normal conditions NT increases both GABA (form the striatum) and glutamate (from subthalamic nucleus) levels in the GP, through its receptor (NTR1)activation. Following dopamine (DA) depletion NT transmission is increased determining the neuroleptic-like effects of NT. Moreover, the toxin 6-hydroxydopamine, induces DA loss and increased synthesis of enkephalin and NT in GP neurones. These cells are a subset of GP neurones located in dorsal-lateral region of the GP, and also enriched in DA receptor D2. Here, we demonstrate using immunohistochemical techniques that: i) most of the GP neurones are immunoreactive for NTR1, ii) NTR1 is localised in soma and dendrites of these cells, iii) these neurones are located in dorsal-lateral GP a region that give rise to a pallido-striatal projection, iv) DA depletion did not change the distribution pattern, but all of the NTR1 neurones showed strong immunoreactivity for NT. Whether these changes correspond to an increased release of NT has to be however demonstrated. Our data suggest that in physiological conditions NT effects in the GP are related not only to striatal release (through the striato-pallidal terminals) but also to pallidal release, from neurones giving rise to local axon collaterals. The increased effects of NT in condition of dopamine depletion might be dependent not only on changes occurring in the striato-pallidal pathway, but also within GP neurones itself. All these changes has to be relevant to the neuroleptic-like effects of NT.

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Clinical Expressions of Experimentally Induced Alzheimer"S Disease (AD)

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Background: The restriction of Clinical Pathology in AD to Cognitive Functions limits the knoledge of this condition exlusive in Human.The State of Matters was challenged at the Meeting of Israel Society (2005)by publication of changes induced in laboratory animals by a novel antibody NGF-like(480T). The pathology produced was indistiguishable of the patholoy of genuine AD.

Objectives: Effects of anti 480 Ab may parallel the development of AD.The determination of the functional significance of the neuropsychiatric experimental findings

may enlarge the limitation of knoledge of AD to exclusive Cognitive Functions.

Methods: Study of the antigenic effect of 480 injected as an immunizant.

Results: As a working hypothesis the anti antibodies to 480 should be immune crossed with Abs of genuine AD if such Ab exist. The anti anti AB produce a series of neuro psychiatric conditions by active but also passive immunization.

Conclusion: The immune effect of 480T parallels the medical view of AD as an autoimmune condition and makes it possible to discriminate between the degenerative and autoimmune componentsof the experimental model

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Cerebroprotective Activity of Different Antihypertensive Drugs in a Model of Hypertensive Brain Damage

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Cerebrovascular disease contributes to vascular dementia (VaD) development. Hypertension is often associated with impaired cognitive function and accelerates cognitive decline in dementia disorders. Control of blood pressure protects brain from the development of cerebrovascular lesions and therefore from VaD. Spontaneously hypertensive rats (SHR) develop cerebral atrophy accompanied by increased volume of cerebral ventricles and neurodegeneration in comparison with age-matched Wistar Kyoto (WKY) rats. Lowering blood pressure protects brain from subclinical cerebrovascular lesions causing cognitive impairment. Dihydropyridine-type Ca2+ antagonists (DTCA) possess a favourable cerebrovascular profile compared to other classes of antihypertensive drugs.

The present study was designed to evaluate in the animal model of hypertensive brain damage offered by SHR, cerebroprotective effects of equi-hypotensive doses of different antihypertensive drugs. The beta-blocker carvedilol, the diuretic indapamine, the angiotensin (AT) converting enzyme inhibitor delapril, the AT-1 receptor blocker candesartan, the DTCAs nicardipine, nitrendepine and amlodipine and the non selective vasodilator hydralazine were used. Treatment of SHR started at the age of 16 weeks and lasted for 12 weeks. Parameters investigated using quantitative microanatomical techniques included frontal and occipital cortex as well as hippocampus volume, nerve cell number, astrogliosis and neurofilament breakdown.

Comparatively, DTCAs exerted the most pronounced cerebroprotective effect. Nicardipine was the most active in preventing cerebrocortical nerve cell loss. The observation of a neuroprotective activity of nicardipine in neocortex of SHR suggests a specific attention on the effects of this compound in the treatment of hypertension accompanied by brain damage and in cerebrovascular disease.

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Differential Co-Localisaton of Heparan Sulfate With Beta-Amyloid Deposits in APP-Transgenic Mice, Human Sporadic and Familial Variants of Alzheimer's Disease

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Background: Heparan sulfate (HS) is reported as a staple component of amyloid deposits, including the β-amyloid (Aβ) deposits of Alzheimer's disease (AD). Aβ40 and Aβ42 levels within individual Aβ deposits and more generally within different AD disease conditions are observed to differ significantly. However, little is known of heparan sulfate association with these Aβ species, particularly in the human APP transgenic mouse model and human familial variants of AD (FAD).

Objective: This study aimed to characterise the specific distribution of HS with Aβ40 and Aβ42 in β-amyloid deposits of Tg2576 mice, human sporadic AD and two variants of FAD.

Methods: Adjacent brain sections of Tg2576 mice were analysed in tandem with human hippocampal sections from cases with the following conditions; sporadic AD, presenilin-1 (PS1) mutation and the Swedish APP mutation (SweAPP). Co-localisation patterns were revealed through double fluorescent immunostaining with specific antibodies against HS, Aβ40 and Aβ42. Confocal microscopy provided detailed data on individual Aβ deposit construction and relevant HS distribution.

Results: While the exact form of HS distribution differed among the Tg2576 mice and human AD conditions the recurring pattern was that of a strong HS ring which was tightly associated with Aβ40. Aβ42 positive deposits demonstrated a clear trend of HS co-localisation; Tg2576 and sporadic AD cases showed strong Aβ42/HS co-localisation, SweAPP cases showed little Aβ42/HS co-localisation and PS1 cotton wool deposits showed no Aβ42/HS co-localisation.

Conclusion: AD disease type, deposit morphology and Aβ42 content may be limiting factors for HS co-localisation with Aβ deposits.

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Possible Functional Interaction of Dopamine and Acetylcholine on Learning and Memory at the Level of the Prefrontal Cortex and Hippocampus

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Parkinson's disease (PD) is characterized by a loss of dopamine (DA) producing neurons. The main clinical symptoms are motor deficits. However, in some PD patients a cognitive decline can occur. It is not clear whether this is due to a loss of DA, primarily in non-striatal forebrain areas, or can be explained by a co-existing dysfunction of the cholinergic system, or an interaction of both. In this study we have investigated if performance in learning and memory tasks require a convergence of DA and acetylcholine at the hippocampal or frontal cotical levels. For this purpose, we lesioned the cholinergic neurons with 192 IgG-saporin in these structure and the DA neurons in the ventral tegmental area (VTA) with 6-OHDA. The cholinergic neurons of the nucleus basalis magnocellularis (NBM) project mainly to the prefrontal cortex, while the neurons of the septum project mainly to the hippocampus. Dopaminergic neurons in the VTA have projections to both structures. Five weeks after surgery, the animals were assessed for deficits in the Morris water maze test. A significant increase in latency to find the platform was found in animals with a VTA lesion but not in the NBM or septum lesioned animals. All the double lesion groups had the same increase in latency as the VTA lesioned animals. Our findings suggest that the DA neurons in the VTA have an important function in learning and memory. However our data does not point to any clear indication whether an interaction between DA and acetylcholine neurons exists.

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Comparison of Single- and Repeated-Ischemia-Induced Changes in Expression of Flip and Flop Splice Variants of Ampa Receptor Subtypes GluR1 and GluR2 in the Rats Hippocampus CA1 Subregion

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In addition to their role in the physiological activities, ionotropic glutamate -amino-3-hydroxy-5-methyl-4-isoxazole propionate receptors (AMPARs) play an important role in neuronal death especially that following ischemic insults. In this study, we examined the effect of single (SI) and twice repeated (RI)-4-vessel occlusion-ischemia on rats performance in 8-armed radial maze test. Moreover, the effects of SI and RI on the AMPARs subunits GluR1 and GluR2 flip and flop variants composition in the CA1 subregion of hippocampus were investigated using RT-PCR, normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and expressed as their ratios to the latter. The results showed that SI and RI impaired the maze performance by decreasing correct choices and increasing the error choices, but RI increased error choices to a greater extent than the SI. The SI reduced only GluR1 flip/GAPDH on day 1. The SI did not alter ratios of GluR2 variants to those of GluR1. On the other hand, the RI decreased GluR2 flip and flop variants after 1 and 3 days respectively, whereas after 7 days increased the flip variant of both GluR1 and GluR2. Moreover, the RI reduced ratios of GluR2 variants to those of GluR1. These results reveal the differential effects of the SI and RI on memory and expression of the AMPARs subunits GluR1 and GluR2 and theirs flip and flop variants in the CA1.

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Novel Genetic Suppressors of Amylid-Beta Neurotoxicity

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Background and Aims: The most prominent pathologic hallmark in the Alzheimer's disease brain is the accumulation of the amyloid beta (Abeta) peptide. However, little is known about the molecular and biochemical events underlying neuronal dysfunction and death in Alzheimer's disease. Moreover, there is currently no cure for this dreadful disorder. The overall goal of this project is to identify key genes, pathways and molecular mechanisms involved in Abeta neurotoxicity. Methods: We generated transgenic flies expressing human Abeta that manifest age-dependent neurodegeneration. Importantly, expression of Abeta in the eye elicits a robust and sensitive eye phenotype, which motivated a screen of 5,135 insertions to block Abeta neurotoxicity in the eye. Results: We identified 10 molecularly defined insertions with robust suppressive activity. Interestingly, three of these insertions induced the expression of enzymes with known Abeta-degrading activity, a finding that validates the relevance of our genetic screen. The remaining protective insertions potentially express genes with no previous association to Abeta pathobiology. The most interesting protective insertion expresses a putative Phospholipid Acetyltransferase. This protein, potentially involved in fatty acid remodeling, is conserved in humans. We have also identified protective genes encoding a novel transcription factor, a microRNA and ubiquitin ligases. Conclusion: We hypothesize that these new genetic suppressors of Abeta neurotoxicity identified in flies will be also relevant in mammalian models of Alzheimer's disease and, ultimately, in the human patients. Our studies have the potential to, eventually, contribute to the design of more effective therapies directed towards halting or delaying pathogenesis in Alzheimer's disease.

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Limitations of Alzheimer's Disease Symptomatic Therapies in Pre-Clinical Cognition Models: Utilizing Multiple Recognition Memory Procedures to Evaluate New Treatment Options

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Current pharmacological treatments for the memory deficits associated with Alzheimer's Disease (AD) target glutamatergic or cholinergic systems through acetylcholinesterase inhibition or NMDA receptor antagonism. Pharmacological treatment with the cholinergic antagonist scopolamine or the NMDA antagonist MK-801, or an extended retention interval, induces deficits in a rat novel object recognition assay. Here, we investigate the ability of currently marketed therapies for cognitive deficits associated with AD to improve recognition memory in both time- and pharmacologically- induced deficit models. Finally, in an attempt to more closely mirror the deficits observed in AD, a dual-deficit model was developed in which impairments in memory were produced following co-treatment with both scopolamine and MK-801. Treatment with either donepezil or memantine was able to enhance retention in the time-induced deficit model. Significantly, however, in the pharmacologically-induced deficit models, each class of cognitive enhancer was only efficacious at reversing the deficits induced through the manipulation of it's respective transmitter system; acetylcholinesterase inhibition blocking scopolamine effects and NMDA antagonism blocking MK-801 effects, and neither was effective in the dual-deficit model. Evaluation of a novel mechanism of action, known to modulate both neurotransmitter systems, enhanced recognition memory in all three pharmacological deficit models. The neurotransmitter selective effects observed with current clinical standards may highlight limitations of existing therapies in the treatment of the cognitive deficits associated with AD while the combined glutamatergic/cholinergic deficit model may provide an improved system for evaluating novel treatment mechanisms.

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Orchestrating Signaling: in Silico Analysis of the Effects of Pseudophosphorylation on the Structure and Interactions of Tau Protein

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The emerging area of natively unfolded proteins and linear motifs prompts a re-examination of tau structure and function. This in silico study allows for an analysis of the entire human tau protein, a task difficult to perform in vitro.

Methods: The one letter code for the longest human isoform was derived from Entrez Protein, accession number NP_005901. Pseudophosphorylated constructs of tau were created by altering the one letter code by conversion of serine or threonine to glutamate. Codes were submitted to secondary structure prediction servers Psipred, SCRATCH and Jpred. Resultant secondary structures were visualized using Polyview. Location of linear motifs and sites of protein interactions were analysed by ELM and Scansite.

Results: Pseudophosphorylation caused changes in the secondary structure profile of α- helix and β- strand. Significantly pseudophosphorylation at specific sites caused changes throughout the entire molecule, even when no local change was observed. Analysis by ELM and Scansite revealed a large number of linear motifs including several SH3, WW, 14-3-3 and PDZ recognition motifs and some occur within areas sensitive to conformational change.

Conclusion: Tau contains many linear motifs which suggests that tau may play an active role in insulin signaling, in cell cycle control and DNA repair. Upon pseudophosphorylation the protein undergoes small but significant secondary structure changes that may affect its global structure and associations with other proteins. The data suggests that Tau is able to perceive and relay signals by coordinating the formation of signaling or transport complexes via phosphorylation induced conformational changes.

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Modification of Histone H3 and H4 in the Brain of Tg2576 Transgenic Mice -Implications for Alzheimer's Disease

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Alzheimer's disease (AD) is a progressive, neurodegenerative disorder. Excess of amyloid- (A) is postulated to be among the underlying pathological causes of AD. Long-term memory formation involves biochemical signaling cascades that lead to a change in neuronal gene expression. In the nucleus, DNA is tightly packaged into chromatin via histones. Regulation of histones, and thus chromatin structure, is an important step in modulating transcription and facilitating long-term changes in neuronal physiology and cell survival.

To test if persistent exposure to A can modulate changes in chromatin structure, and thereby consequently the gene expression, we investigated the methylation (Lys9), acetylation (Lys14) and phosphorylation (Ser10) of histone H3 as well as the acetylation of histone H4 (Lys5, 8, 12 and 16) in Tg2576 mice compared to WT controls.

We found an increase in histone H3 acetylation and phosphorylation in prefrontal cortex (PRF) and cortex. Methylation of histone H3 was increased in PRF but decreased in the striatum. There were no changes in acetylation of histone H4 in any of these regions. However, there was an increase in histone H4 acetylation in the CA1 of the hippocampus.

In conclusion, this strongly suggest that A triggers changes in chromatin structure in the CNS. Understanding these functions is of importance to further investigate the roles and mechanisms of persistent A exposure in the aging-related CNS dysfunction. This might hopefully provide new insights into this disease and into new treatment options by allowing us to rescue cells that otherwise would be destined to die.

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The AFFiRiS Mimotope Vaccine: A Novel Approach for the Treatment of AD

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Background: AD is characterized by the abnormal accumulation of Amyloid-ß peptides in the brain. Immunotherapeutic treatment targeting full length Aß led to reduced Aßburden and had beneficial impact on disease progression in animal models. However, the first phase II clinical vaccination trial using Aß42 as antigen had to be discontinued due to severe neuroinflammatory side effects including brain infiltration by autoreactive T-cells.

Objective: Therefore, a safe and effective AD vaccine has to avoid the formation of autoreactive T-cells and should exclusively induce an Aß specific antibody-based immune response. Consequently, we aimed at developing a novel and effective treatment approach avoiding the development of immunological complications.

Methods: The Mimotope technology of AFFiRiS has been developed to create antigens, lacking native Aß peptide sequences. Upon vaccination, antibodies are generated which are exclusively reacting with the pathological Aß molecules but not with parental structures like APP. Furthermore, Mimotopes do not contain potential T-cell epitopes and avoid induction of autoreactive T-cells. Mimotopes have been used to vaccinate transgenic mice overexpressing human APP (hAPP). Subsequently, amyloid burden, alterations of AD-like pathology and cognitive functions were analysed after repeated vaccination.

Conclusions: Mimotope vaccination of hAPP transgenic mice leads to significant reduction of amyloid plaque load and associated pathological alterations. Furthermore, there is evidence that successfully treated animals show improvement of cognitive functions following Mimotope vaccination. Additionally, no Aß specific T-cells are detectable in treated animals. Thus the AFFiRiS Mimotope technology could provide a novel treatment strategy with improved safety for use in AD patients.

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Intranasal Immunization With Phage-Single-Chain Antibodies Against EFRH, Improves Memory, Attenuates Hyposmia and Brain Pathology of PDAPP Tg Mice

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Amyloid based immunotherapy tested in transgenic mouse models of Alzheimer's disease has shown promising results in beta amyloid load reduction and the improvement in cognitive and memory functions. We have previously shown that site-directed monoclonal antibodies against the EFRH sequence (amino acids 3-6) of the N-terminal region of AβP can solubilize synthetic A P aggregates and suppress in-vitro formation of AβP. EFRH, located in the soluble tail of the N-terminal region, acts as a regulatory site controlling both solubilization and aggregation processes in the AβP molecule.

In the present study we used single-chain antibody (scFv) against the EFRH epitope, displayed on filamentous phages as a vaccine for intranasal immunization of PDAPP transgenic (Tg) mice. The effects of this treatment on object-recognition test performance, smell threshold toward unfavorable odor (menthol) and amyloid plaque load were investigated. The object recognition index was higher in Tg treated in comparison to Tg untreated mice. In addition, treated Tg mice showed a lower smell threshold than the Tg untreated mice. The menthol ERC50 (effective repulsion concentration of 50%) was 2,5 fold lower in Tg treated then in Tg untreated mice. Evaluation of the brain neuropathology showed the significant reduction in amyloid burden in Tg treated versus Tg untreated mice, measured after either thioflavin-S staining and 21F12 antibody labeling. These results suggest that an immunotherapy approach with phage-displaying recombinant anti-EFRH antibodies may serve as a potent tool for Alzheimer disease treatment

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A Novel Anti-Amyloid Beta Active Vaccine Approach for the Treatment of Alzheimer's and Related Disorders

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Previously, an anti-Aβ vaccine for the treatment of Alzheimer's disease (AD), using the full-length Aβ42 immunogen was shown to be efficacious in patients for plaque clearance and cognitive benefit. However, clinical trials were halted when some patients developed meningoencephalitis. It is widely held that Aβ mediated T-cell activation was central to this finding. Studies have suggested that the N-terminus of Aβ contains prominent B-cell epitopes while the C-terminus contains T-cell epitopes leading several investigators to suggest the use of N-terminal Aβ fragments as active vaccine constructs. To date, however, a systematic evaluation of immunogenic Aβ peptide fragments has not been performed. Accordingly, we evaluated Aβ peptide fragments $\leq$ 8 amino acids in length. This peptide length was purposely defined to minimize interaction with human MHC. Surprisingly, we found that the humoral immunogenicity of these Aβ fragments could not be predicted based on the relative position within the Aβ peptide. Further, vaccination of rhesus macaques with Aβ fragments conjugated to a protein carrier produced differential biological activity. For example, a multiple antigenic peptide (MAP) consisting of two peptide fragments (A and B) produced polyclonal immune sera that were reactive on human AD brain tissue. This A/B MAP also produced increases in plasma Aβ that were not produced by either the A or B vaccine constructs and decreased plaque deposition in plaque forming transgenic mice. As designed, the vaccine constructs produced robust humoral immune responses but did not induce Aβ-specific T-cell activation.

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Human Monoclonal Antibodies Against Specific Epitopes of A-Beta Peptide

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Active and passive immune therapy of AD has been shown in murine models to bear a significant therapeutic effect. In patients, treatment with xenogeneic anti A-beta antibodies would elicit serious complications. Therapy with in vitro engendered anti A-beta human monoclonal antibodies would override these problems. Anti A-beta antibodies are detected in the sera of all humans. In the peripheral blood of healthy individuals the frequency of B cells that produce these antibodies is very low. We employed sensitive selection methods to isolate those B cell that express anti A-beta antibodies on their surface. Infection of the antigen-specific cells with Epstein-Barr virus rendered them immortal. The production of the specific antibody is stabilized by fusion of the lymphoblastoid cells with a human-murine hetero-hybridoma. Two monoclonal antibodies studied in our laboratories were shown to bind to the N-terminal of the A-beta peptide. The affinity of the binding was similar to that of commercial anti A-beta mouse monoclonal antibodies. The antibodies bound specifically to A-beta but not to other amyloid proteins. In a murine model of AD there was a preferential penetration of one of the anti A-beta human monoclonal antibodies into the brain when compared to albumin and to a non-relevant human monoclonal antibody. The treatment of mice with that monoclonal anti A-beta antibody improved cognitive behavior. Human monoclonal antibodies against specific epitopes of A-beta molecule can be tailored by immortalization of specifically pre-selected B cells.

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Elucidation of T Cell Epitopes in Human Prion Protein: A Pre-Requisite to Safe Vaccine Design in Neurodegenerative Diseases

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Background: Cases of meningoencephalitis in the AN1792 Alzheimer disease trial are assumed to result from induction of a T cell response against β-amyloid. Elucidation of the human T cell repertoire against the intended self protein target is therefore essential prior to active vaccination in other neurodegenerative diseases. T cell epitopes within prion protein (PrP) have not previously been studied in humans.

Methods: We performed an in silico analysis to identify putative T cell epitopes in human PrP. We then cultured peripheral blood lymphocytes from 28 healthy humans with a panel of peptides spanning the human PrP sequence to assess proliferative and cytokine responses.

Results: In silico analysis of PrP reveals a number of putative T cell epitopes, principally around the position 129 polymorphism and in the C-terminus. Approximately 50% of donors made ex vivo responses to at least one PrP peptide. As predicted, responses were clustered around position 129 and in the C-terminus. Analysis of cytokine production revealed that some epitopes are associated with strong induction of IL-6 and IL-4, suggestive of a Th2 response, while others induce a limited Th1 or Th0 pattern. Most interestingly, substitution of methionine by valine at position 129 abrogated immunogenicity of PrP 121-134 and altered the cytokine response to PrP 128-141 from Th2 to Th1.

Conclusions: Our data indicate that the human T cell repertoire includes potential autoreactivity against PrP. Further modelling of these responses is required to ensure safe vaccine design for human prion disease.

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Identification and Characterization of Anti-ADDL Antibodies as Potential Therapeutics for Alzheimer's Disease

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Alzheimer's disease (AD) is characterized by an accumulation of amyloid beta plaques and tau tangles. While plaques were previously associated with the dementia of AD, a growing body of evidence indicates that amyloid beta-derived diffusible ligands (ADDLs) or oligomers may be primarily responsible. ADDLs are soluble, oligomeric species of amyloid beta and have been described as existing in multiple states of aggregation, including trimeric, tetrameric, dodecameric and higher order. ADDLs are abundant in AD, but not normal brain and induce deficits in rodent learning and memory. In previous studies we, and others, have defined the preparation of synthetic ADDLs and described their ability to bind to neurons. Further, we have demonstrated that the ability of antibodies to perturb the binding of ADDLs to neurons differentiates anti-ADDL antibodies from non-ADDL preferring antibodies (i.e., anti-amyloid beta antibodies). In the following, we characterize a panel of anti-ADDL antibodies using BIAcore and ELISA methods. These studies suggest that anti-ADDL antibodies preferentially bind to ADDL substrates over monomeric substrates. Further, this preferential binding correlates well with the ability of these antibodies to block ADDL binding to primary hippocampal neurons in culture. These studies collectively indicated that anti-ADDL antibodies can be identified that have favorable characteristics. Such antibodies may prove useful for therapeutic approaches where lowering ADDL levels would be expected to result in clinical benefit (e.g., Alzheimer's disease).

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Rasagiline in Relation to Parkinson's Disease Severity at Baseline - A Post-Hoc Analysis of the Tempo Study

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Background and Aims: Rasagiline shows safety and efficacy as initial monotherapy in early Parkinson's disease (PD) (TEMPO study). This post-hoc analysis investigated

whether baseline symptom severity influences rasagiline's magnitude of effect in patients with early PD.

Methods: The 26-week, multicentre, randomised, double-blind TEMPO study included patients (n=404) receiving once-daily rasagiline 1 mg, 2 mg, or placebo. Primary efficacy measure was change from baseline in UPDRS-Total score. Post-hoc, change from baseline in UPDRS scores was stratified by baseline UPDRS-Total score (>23, >27, >31), or presence of abnormal postural reflexes.

Results: In TEMPO, rasagiline 1 mg-treated patients showed a 4.20 unit change in UPDRS-Total score over placebo (adjusted means; $p<0.001$). Here, we demonstrate increased benefit of rasagiline 1 mg vs placebo, in patients with greater baseline symptom severity in the three analysed strata (>23, >27, >31), with treatment effects as follows: UPDRS-Total (3.80, 5.02, 6.02); UPDRS-Motor (2.52, 2.86, 3.17); UPDRS-ADL (1.21, 2.08, 2.65). Effects were statistically significant for all strata in UPDRS-Total and UPDRS-ADL scores ($p<0.01$), and for the >23 and >27-unit strata in UPDRS-Motor scores ($p<0.05$). UPDRS-Total treatment effects were also greater in a patient subgroup (22%) with abnormal postural reflexes at baseline, compared with patients showing normal baseline postural reflexes (6.74, 3.00, respectively).

Conclusions: In early PD patients, rasagiline monotherapy (1 mg once daily) provides symptomatic benefits that appear to be greater in patients with more pronounced baseline symptoms. In patients with either UPDRS-Total scores >31, or abnormal postural reflexes at baseline, improvement vs placebo was >6 UPDRS units.

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FK506-Binding Proteins Stimulate the Aggregation of Alpha-Synuclein

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Aggregation of alpha-synuclein plays a key role in Parkinson's disease. We have studied α-synuclein aggregation in vitro using fluorescence correlation spectroscopy (FCS) and turbidity measurements. The aggregation process was clearly accelerated by addition of FK506 binding proteins (FKBPs). FKBPs are members of the immunophilins, enzymes that bind to immunosuppressant drugs and have a peptidyl-prolyl isomerase (PPIase) or rotamase activity. The accelerated aggregation of alpha-synuclein was counteracted by FK506, a specific inhibitor of FKBPs. To further examine the in vivo physiological relevance of our findings, we have analyzed the effect of FKBPs in a SHSY5Y cell model of alpha-synuclein aggregation and after lentiviral vector-mediated overexpression of alpha-synuclein in mouse brain. In both model systems, FK506 decreased alpha-synuclein inclusion formation. Since FK506 and other non-immunosuppressive FKBP inhibitors are known to display neuroregenerative and neuroprotective properties in disease models, the observed inhibition of rotamase activity and alpha-synuclein aggregation, may explain their mode of action. Our results open perspectives for the treatment of Parkinson's disease with immunophilin ligands that inhibit a specific member of the FKBP family.

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Characterization of the Molecular Composition and Toxicity of Alpha-Synuclein Intermediate Aggregates

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Introduction: In both Parkinson's disease and dementia with lewy bodies, lewy bodies are present in the cytoplasm of surviving neurons. Lewy bodies consist mainly of an aggregated form of the presynaptic protein alpha-synuclein. In its native state alpha-synuclein lacks a defined tertiary structure whereas during the aggregation process a range of compact oligomeric intermediate species are formed, which eventually develop into insoluble beta-sheet-rich inclusions.

Methods: Recombinant forms of human alpha-synuclein, both wild type and mutant species (A30P, E46K, A53T and A30P/A53T), were either expressed and purified using the IMPACT-CN system or obtained commercially. A thioflavin T assay was used to characterize the fibrillation kinetics of the various alpha-synuclein variants. Next, the molecular composition of the generated aggregates was analyzed by size exclusion chromatography-HPLC (SEC-HPLC). Finally, preparations containing different aggregation states of alpha-synuclein, as defined by SEC-HPLC analysis, were added to HEK 293 cells and SH-SY5Y cells and the toxicity was assessed by a MTT cell viability assay.

Results: The following aggregation rate of the different alpha-synuclein species was observed in decreasing order: E46K, A30P/A53T, A53T, wt, A30P. The most pronounced cell toxicity was observed for soluble prefibrillar aggregates, with a molecular weight above 600 kDa, whereas low molecular weight near-native preparations and fibrillar material showed a more limited toxic effect.

Discussion: Characterizing the aggregation process of alpha-synuclein and determining the toxicity for its various molecular species provides us with an increased knowledge of the disease mechanisms behind disorders with lewy body pathology.

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Neuroprotective Effect of Dcpg, Mglur8-Selective Agonist, in a 6-Hydroxydopamine Rodent Model of Parkinson's Disease

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Glutamate-mediated excitotoxicity has been proven to be a contributory factor in the cell death of dopaminergic neurons in substantia nigra pars compacta (SNc) in Parkinson's disease (PD). Although the blockade of ionotropic glutamate receptors (iGluRs) provides neuroprotection against the dopaminergic cell death in vivo animal models, severe psychotomimetic side-effects have limited their clinical use in PD. Interest has now turned to the anti-parkinsonian and neuroprotective effect of the modulation of metabotropic glutamate receptors (mGluRs). From our previous reports, ligands of mGluRs,

especially the agonist of group III mGluRs, provide a significant neuroprotection against the 6-OHDA lesion in PD rat model (Vernon et al, 2005, 2006). In our previous studies we utilised the broad spectrum agonist L-AP4, however in this study to investigate which receptor subtype is responsible for the neuroprotective action of group III mGluRs we have utilised the mGluR8 agonist (S)-3,4-dicarboxyphenylglycine (DCPG). The neurotoxin 6-hydroxydopmine (6-OHDA) induced a significant loss of tyrosine hydroxylase-immunoreactivity (TH+) (53.62 ± 2.288 %; $p<0.01$), a marker of dopaminergic neurons, and the increase of striatal dopamine turnover ratio (61.00 ± 8.895%; $p<0.01$) in control male Sprague-Dawley rats. The 6-OHDA-induced loss of dopaminergic neurons and increase of metabolic turnover of dopamine were significantly attenuated by sub-chronic (7days) treatment with the agonist (DCPG), in a dose-dependent manner (4 doses). This finding is consistent to the reports of the expression of mGluR8 in SNc. The neuroprotective effect afforded by the DCPG suggest that the activation of mGluR8 may be a promising approach to provide effective treatment for PD.

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Effect of SMI on Dopaminergic Neurons of Central Nervous System

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Accumulating evidences suggest smilagenin (SMI), a derivative of the most potent ingredient sarsasapogenin from Rhizoa Anemarrhenae, has neuroprotective and neurotrophic action. The purpose of this study is to investigate whether SMI could protect dopaminergic neurons of central nervous system from neurodegeneration . The densities of dopamine receptor subtype 1 and 2 in the forebrain as well as the number of tyrosine hydroxylase (TH) positive cells in substantia nigra decreased in aged rats and SMI has the potential to prevent the decline. Immunocytochemistry(ICC) and MTT assay revealed that 6-hydroxydopamine or MPP+ had significant neuro-degeneration effect to primary culture of mesencephalic neurons as evidenced by decreased TH-positive cell number and decreased length of neurite outgrowth. SMI could protect the neurons from the damage induced by these neuro-intoxicants. The effect of SMI is closely related to GDNF, since both common RT-PCR and real-time quantitative RT-PCR showed that the mRNA of GDNF was markedly increased by addition of SMI to the culture medium. Furthermore, functional blocking anti-GDNF antibody and /or anti-GDNFRa antibody inhibited the neuroprotective action of SMI markedly although not completely. Taken together, we have shown that SMI has significant neuroprotective effect on dopaminergic neurons and this effect is at least partially mediated through the GDNF pathway

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The Hsp90/Cdc37 Chaperone System Regulates Pink1 Processing

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Mutations in the ubiquitously expressed gene PINK1 are associated with autosomal-recessive Parkinson's disease. PINK1 encodes a putative serine/threonine kinase with an N-tesminal mitochondrial leader sequence. The mechanism that leads to selective degeneration of dopaminergic neurons via PINK1 mutations is unknown.

Here, we report that endogenous Pink1 in cultured cells exists principally in two isoforms that are present in both mitochondrial and cytosolic fractions. We validated the specificity of the antibody used to detect endogenous Pink1 via lentiviral-mediated Pink1 shRNA expression. We next analyzed immuno-isolated Pink1 complexes by mass spectrometry in order to identify Pink1 binding partners. Here, we identified Hsp90 and its co-chaperone, Cdc37, as specific Pink1 binding partners. Hsp90/Cdc37 form a kinase-specific chaperone complex that stabilizes many kinases in their activated form. We then demonstrated that Pink1 is a bona fide Hsp90/Cdc37 client kinase by probing for the stability of exogenous and endogenous Pink1 in several cell lines aftes treatment with a specific Hsp90 ATPase inhibitor (Geldanamycin). In an attempt to identify the binding site between Cdc37 and Pink1, we found several Cdc37 truncations that show an increased binding affinity for Pink1. Intesestingly, ovesexpression of these truncations leads to altesed processing of Pink1, that is, they change the protein ratio between the two major Pink1 isoforms. We conclude that the Hsp90/Cdc37 chaperone system is involved in the physiological regulation of Pink1 processing.

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Rapamycin-Induced Autophagy Clears Intracellular Inclusions of Alpha-Synuclein Oligomers

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Background and Aims: Intracellular protein aggregates are characteristic of several neurological diseases, including the prion encephalopathies, Huntington's Disease, Alzheimer's Disease and Parkinson's Diseases. Autophagy is a mechanism for the clearance of bulk cytoplasmic contents, including such protein aggregates, and can be stimulated pharmacologically by treatment with the immunophilin ligand, rapamycin.

Methods: We induced cellular production of alpha-synuclein oligomers by transient expression of alpha-synuclein in CHO K1 cells followed by treatment with rotenone, or by transient co-expression of alpha-synuclein and synphilin in HEK293 cells. We also generated oligomers of alpha-synuclein by incubation of recombinant peptide with dopamine, and introduced those oligomers into cells using protein transfection techniques.

Results: Rapamycin treatment resulted in increased numbers of autophagic vacuoles as measured by monodansyl cadaverine (MDC) fluorescence and reductions in oligomeric alpha-synuclein as measured by western blotting and by

quantification of alpha-synuclein immunoreactive inclusions using Cellomics Array Scan technology. Rapamycin effects were inhibited by co-treatment with 3-methyl adenine.

Conclusions: These results suggest that the stimulation of autophagy is a potential approach to the clearance of intracellular alpha-synuclein oligomers. Moreover, autophagy may be a useful tool in elucidating the toxic form of alpha-synuclein.

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Studies on the Aggregation of An Amyloidogenic Alpha-Synuclein Peptide Fragment

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The aggregation of α-synuclein to form insoluble fibrillar inclusions is a possible key event in the onset of many neurodegenerative diseases. Previous studies have identified key regions within the α-synuclein sequence that are necessary for the aggregation of the protein, including a central hydrophobic region (encompassing residues 71-82). A peptide corresponding to this region (α-syn(71-82)) has been synthesised and used to study the structural characteristics of fibrils formed and to assess which residues form the critical self-recognition element. Solid-state nuclear magnetic resonance (NMR) structural studies on fibrils formed have revealed structural heterogeneity across the backbone of the peptide, with distinct ordered and disordered regions. One therapeutic strategy for prevention of amyloid diseases is to inhibit or reduce the rate of formation of amyloid fibrils. Our NMR results have been used to guide the synthesis of a range of unnatural peptides as inhibitors of α-synuclein aggregation. Preliminary screening has identified potential lead compounds for the treatment of neurodegenerative diseases such as Parkinson's.

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The E3 Ubiquitin Ligase Parkin Mediates Neuroprotection Through Activation of Ikk/Nf-Kappa B Signaling

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The discovery of monogenic familial variants of Parkinson's disease (PD) marked a new era in PD research, making it possible to study the role of specific proteins in the pathogenesis of this disease. Among the genes which are responsible for familial PD, the parkin gene seems to play a major role. Parkin, a 465 amino acid protein, is characterized by an ubiquitin-like domain (UBL) at the N-terminus and a RING box close to the C-terminus, consisting of two RING finger motifs which are separated by a cysteine-rich in-between RING domain. Parkin shows an E3 ubiquitin ligase activity and has therefore been connected with the ubiquitin-proteasome system.

Parkin protects neurons against diverse cellular insults in different model systems, indicating that it may play a central role in maintaining neuronal integrity. In order to gain insight into the mechanism underlying the broad neuroprotective capacity of parkin, we investigated a possible role of parkin in different stress response pathways. Our analysis revealed that activation of the IKK/NF-κB signaling cascade is causally linked to the neuroprotective potential of parkin. Inhibition of NF-κB activation by an IκB superrepressor or a kinase-inactive IκB kinase β (IKKβ) interferes with the neuroprotective activity of parkin. Furthermore, pathogenic parkin mutants with an impaired neuroprotective capacity show a reduced ability to stimulate NF-κB-dependent transcription. In support of a role of parkin in ubiquitin signaling, we present evidence that parkin can stimulate the degradation-independent ubiquitylation of specific components of the NF-κB cascade.

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Effect of the C-Terminal Chain of Tetanus Toxin on the Motor Behaviors in Two Models of Dopaminergic Lesion in Rats

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Recently, it has been shown that C-terminus fragment of the tetanus toxin (Hc-TeTx) has neuroprotective effects against oxidative stress and 1-methyl-4-phenylpridinium (MPP+) toxicity. The aim of this work was to evaluate the effect of Hc-TeTx in 6-OHDA or MPP+ treated rats using rotation and motor behavioral tests. Male Wistar rats (250-300gr) were injected with 2µl (8 µg/µl) of 6-OHDA into nigrostriatal pathways, for the first experiment. Previous to dopaminergic lesion different groups received the Hc-TeTx fragment at following doses (2, 10, 20 y 50 µM). In the second experiment, animals were injected with 2 µM of Hc-TeTx previous to MPP+ 1 µl (10 µg/µl), all groups were evaluated respect their control group. All the animals were evaluated on the rotational behavior test using methamphetamine, the motor behavior in the close field boxes was evaluated at the 4th and 20th day post-surgery, respectively. The rotational behavior of groups with Hc-TeTx/6-OHDA (2, 10, 20 y 50 µM) displayed a decreased number of ipsilateral turns (81%, 85%, 70% y 78%), whereas in the motor behavioral test, Hc-TeTx/6-OHDA groups demonstrated an improvement of 20%, 77%, 11% and 12% at the different doses. The group Hc-TeTx/MPP+ had significantly lower rotations than controls of 50%, whereas in motor behavior the groups with MPP+ and Hc-TeTx/MPP+ was not affected. The fragment Hc-TeTx improved rotational behavior in both models, probably by their action on Trk pathways that increase the cell's survival and improve dopaminergic neurotransmission. Our results strongly favor the hypothesis of neuroprotective properties of Hc-TeTx fragment.

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Neuroprotective Effects of Wogonin Against 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydridine (MPTP)-Induced Dopaminergic Toxicity in C57bl/6 Mice Through Inhibition of Matrix Metalloproteinase-3 and TNF-A

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Parkinson's disease (PD) is the neurodegenerative disorder affecting dopaminergic neurons in substantia nigra pars compacta. Although exact cause for the PD remains unknown, the presence of oxidative stress and inflammation is one of the significant pathological features of PD and the levels of cytokines are elevated in the SN of PD patients. Recently we have demonstrated that active matrix metalloproteinase-3(MMP-3) led to production of microglial inflammatory cytokines including TNF-α, which in turn exacerbated dopaminergic (DA) neuronal degeneration in MPTP-treated mouse model of PD. Moreover, MPTP-mediated DA degeneration and microglial activation were largely attenuated in MMP-3 KO mouse.

Based on this finding, we're screening various compounds either synthesized or extracted from plants that have MMP-3 inhibitory effect. Wogonin (5,7-dihydroxy-8methoxyflavone) originated from the Scutellaria baicalensis Georgi is known as anti-inflammatory drug. Synthesized wogonin is the one showing strong MMP-3 inhibition. Wogonin also significantly reduced lipopolysacchride (LPS)-mediated tumor necrosis factor-α (TNF-α) release from BV2, microglial cells. In this study, wogonin was investigated for their neuroprotective effects using both MPTP treated mouse PD model and SH-SY5Y cells treated with MPP+. Wogonin showed significant protective effect against MPP+-induced SH-SY5Y cell death. Pretreatment of mouse with Wogonin (i.p) 2 hrs prior to MPTP injection (30 mg/kg, once a day for 5days) also demonstrated significant reduction in microglial activation, striatal TH-positive fiber loss and DA neuronal degeneration. These findings suggest that the neuroprotective effects of wogonin might mediate through an inhibition of MMP-3, and diminish of TNF- α release from microglia.

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Role of CGMO Signaling in Alteration of Phospholipase A2 and Arachidonic Acid Release in Experimental Model of Parkinson Disease

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Previous studies have shown that nitric oxide (NO)/cGMP pathway is up-regulated in mice model of Parkinson Disease (PD) induced by1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Since NO is known to be involved in cPLA2 regulation (Chalimoniuk et al., 2006), we investigated if cGMP/PKG signaling participate in MPP+-induced cPLA2 activation and death of PC12 cells in culture.

Our results indicated that MPP+ causes time-dependent enhancement of arachidonic acid (AA) release into medium from [3H]AA prelabeled PC12 cells. Moreover, AA release was significantly decreased (60%) in time dependent manner (1-24 h) by inhibitor of cPLA2 (ACOCF3). Inhibitor of Ca2+-independent PLA2 (BEL) decreased by 30% MPP+-induced AA release 24h after treatment of PC12 cells. The enhancement of AA release was accompanied by significant increase of cPLA2 total protein level and its phosphorylated form. PKG inhibitor (KT5823) decreased significantly cPLA2 activity and also total and phosphorylated cPLA2 protein level in MPP+ treated PC12 cells. The same effect was observed in the presence of other protein kinase inhibitors: PKC (GF109203X) and ERK1/2 (U0126). We also observed dose- and time-dependent decrease of viability of MPP+ treated PC12 cells. The cPLA2 and PKG inhibitors prevented against MPP+-induced enhancement of free radicals production and PC12 cells death. These results indicate that up-regulated cGMP/PKG signaling pathway is involved in activation of cPLA2 in PC12 cells treated with MPP+.

Supported by the statutory theme No13 of Medical Research Centre.

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Cognitive Dysfunction in PD With Dementia, Distinct From Amnestic Mci: Observations From the Cantab Test

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Background: Cognitive dysfunction in PD is mediated by monoamine or cholinergic deficits and/or cortical Lewy or Alzheimer pathology. CANTAB Paired Associates Learning (PAL) is useful in detecting Alzheimer type cognitive dysfunction whereas the 5-choice serial reaction time task (RTI) is more sensitive to cognitive dysfunction mediated by cholinergic and monoaminergic systems in the brain. We tested if CANTAB could assess non-DA mediated cognitive dysfunction in PD.

Methods: CANTAB PAL and RTI tasks were performed in 17 subjects with amnestic MCI and age-matched 32 PD patients (18 PD without dementia and 14 PD with dementia). UPDRS motor scores were used as an approximate indicator of DA depletion in the striatum.

Results: (1) In the MCI group, MMSE scores showed a significant correlation with PAL (r=0.649, p=0.005), but not with RTI scores. (2) In the PD group, MMSE scores showed significant correlation with PAL (r=0.450, p=0.010) and RTI scores (r=0.454, p=0.012), but not with UPDRS motor scores. (3) In PD patients without dementia, MMSE scores showed significant correlation with UPDRS motor scores (r=0.756, p=0.002).

Conclusions: Findings of significant correlation between MMSE and UPDRS motor scores in the PDND but not in the

whole PD group, suggest that dementia in PD patients is mediated by non-DA mechanisms. Our data suggest that CANTAB may be useful to detect non-DA-mediated cognitive dysfunction in PD patients with dementia. In particular, PAL and RTI tasks seem to detect cognitive dysfunction in PD patients that is mediated by different mechanisms.

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Neuronal Microtubule Dynamics as a Novel Target for the Parkinsonism Producing Neurotoxin MPTP

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Dysfunction of microtubule system is emerging as a novel contributing factor in Parkinson's disease (PD). Although tubulin, the protein which makes up microtubules, has been described to be a component of Lewy bodies long time ago (Galloway et al., 1992), only recent evidence indicates that the interaction with and the subsequent dysfunction of the microtubule cytoskeleton could be common to toxins known to cause PD and to proteins mutated in PD.

We reported earlier that MPP+, the toxic metabolite of MPTP, binds specifically to tubulin and affects microtubule dynamics by acting as a destabilising factor in vitro (Cappelletti et al., 2005). Our current work is focused on the study of the dynamic behaviour of the microtubular cytoskeleton in NGF-differentiated PC12 cells exposed to MPP+. Here we investigate: i) post-translational modifications occurring on tubulin and correlating with stability of microtubules, and ii) tubulin dynamics in live cells. By immunofluorescence and confocal microscopy analysis we found that MPP+ heavily affects microtubule organization and induces the loss of highly labile microtubules at the neuronal tips. By FRAP (fluorescence recovery after photobleaching) experiments of YFP-tubulin in live PC12 cells we examined tubulin dynamics and found that MPP+ induces a significant reduction of tubulin movement at the neuronal tip and along the axon.

Since dynamics is crucial in microtubule biological functions, we hypothesise that the altered dynamic behaviour of microtubules caused by MPP+ could profoundly affect the functionality of neurones and, consequently, represent a novel pathogenetic pathway triggering neuronal cell death.

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Correlating Efficacy in Rodent Cognition Models With in Vivo 5-HT1A Receptor Occupancy by a Novel Antagonist, Way-101405

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5-HT1A receptors play an important role in multiple cognitive processes and compelling evidence suggests that 5-HT1A antagonists can reverse cognitive impairment. We have examined the therapeutic potential of a potent (Ki = 1.1 nM), selective (> 100-fold) 5-HT1A receptor antagonist (KB = 0.4 nM), WAY-101405 ((R)-N-(2-methyl-(4-indolyl-1-piperazinyl)ethyl)-N-(2-pyridinyl)-cyclohexane carboxamide). The selective radioligand [3H]WAY-100635, administered i.v., was used for in vivo receptor occupancy studies, where WAY-101405 occupied 5-HT1A receptors in the rat cortex with an ED50 of 0.1 mg/kg p.o. Oral administration of WAY-101405 was shown to be effective in multiple animal models of learning and memory. In a novel object recognition paradigm, 1 mg/kg enhanced retention for previously learned information and was able to reverse the memory deficits induced by scopolamine. WAY-101405 (1 mg/kg) also reversed scopolamine induced deficits in a rat contextual fear conditioning model. In the Morris water maze WAY-101405 (3 mg/kg) significantly improved learning in a paradigm of increasing task difficulty. In vivo microdialysis studies in the dorsal hippocampus of freely moving adult rats demonstrated that acute administration of WAY-101405 (10 mg/kg) increased extracellular acetylcholine levels. Collectively these studies demonstrate that WAY-101405 is a potent and selective, brain penetrant, orally bioavailable 5-HT1A receptor 'silent' antagonist which is effective in pre-clinical memory paradigms at doses where approximately 80% of the post-synaptic 5-HT1A receptors are occupied. These results further support the rationale for use of this compound class in the treatment of cognitive dysfunction associated with psychiatric and neurological conditions.

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Calcium Modulates the Formation of Dopamine: Alpha-Synuclein Oligomers

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The α-synuclein protein is a major component of the intracellular inclusions called Lewy bodies found in Parkinson's disease (PD) patient brains. α-Synuclein is a 140 amino acid presynaptic protein with a natively unfolded structure. The exact form of the protein responsible for neuronal dysfunction and development of disease is unknown. Dopamine and α-synuclein are two key molecules associated with PD. We and others have shown that dopamine converts α-synuclein into soluble, non-amyloidogenic, SDS-resistant oligomers (Cappai et al FASEB J (2005); Norris et al JBC (2005); Li et al FASEB J (2004)). The YEMPS sequence located in the α-synuclein C-terminal domain (residues 125-129) is necessary for dopamine:α-synuclein oligomer formation (Norris et al JBC (2005)). Calcium binds α-synuclein via residues 126-140 and promotes α-synuclein aggregation (Nielsen et al JBC (2001; Lowe et al Prot Sci (2004)). Given the overlap of the calcium binding site to YEMPS we investigated if calcium affects dopamine:α-synuclein oligomers. METHODS: We have studied the

aggregation of recombinant dopamine:α-synuclein oligomers in different buffers and co-factors. RESULTS: This process is buffer dependent. Both the pH and buffering agent affect dopamine:α-synuclein oligomer formation. Calcium inhibits dopamine:α-synuclein oligomer formation in a dose dependent manner and acts at all parts of the dopamine:α-synuclein aggregation pathway. This is specific for calcium as magnesium is significantly less effective. CONCLUSION: Calcium is a potent inhibitor of dopamine:α-synuclein oligomer formation and the close proximity of the calcium binding and YEMPS sites indicates an interplay between dopamine and calcium on α-synuclein aggregation.

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Examination of Cardiovascular Autonomic Function in Lewy Body Dementia

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Objectives: To evaluate cardiovascular autonomic function in Lewy body dementia(LBD) in comparing to Parkinson's disease(PD) and Parkinson's disease dementia(PDD) and try to discover the reasons and mechanisms of orthostatic hypotension(OH) in LBD.

Patients and methods: 20 patients with LBD, 26 patients with PD and 8 patients with PDD were included. We used heart rate variability(HRV) with power spectral analysis of 5-min beat-to-beat RR interval data. Baroreceptor reflex sensitivity(BRS) also was measured.

Results. Autonomic disorders were detected in all groups but more severe - in LBD and PDD patients. In LBD and PDD groups the autonomic disorders were presented as orthostatic hypotension, body weight lowering, urination problems, constipation and hypohidrosis and in PD group - as seborrhea, hyperhidrosis, salivation and constipation. Orthostatic hypotension was observed in 50%LBD, in 67%PDD and 30%PD patients. According HRV data LBD patients show the decrease of sympathetic influences on heart function in distinction from PD and PDD patients (HF/LF index=0,91, 1,09, 1,67 respectivetely). Power spectral analysis showed additional decrease of sympathetic influences in patients with OH, especially in LBD. Actually LBD patients showed severe adaptation disorders with inversion of HF/LF index to the prevalence of parasympathetic reactions. Tilt-test showed that BRS is decreased in all examined groups on the first minute. On the third minute BRS restores in all groups except LBD patients.

Conclusions. Autonomic disorders are typical for LBD and PDD patients. Severe disorders of sympathetic innervation and decreasing of sympathetic influences on heart function are observed in LBD patients especially in patients with OH.

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Evaluation of Heart Rhythm Disturbances in Lewy Body Dementia

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Objectives: To evaluate heart rhythm disturbances in Lewy body's dementia (LBD) in comparing to Parkinson's disease (PD) and Parkinson's disease dementia (PDD).

Patients and methods: 20 patients with LBD, 26 patients with PD and 8 patients with PDD underwent clinical neurological examination and clinical evaluation of autonomic disorders with heart rate variability (HRV) in supine position and during tilt-test.

Results. The study showed that auricular extrasystoles are observed in LBD patients more frequently than in other groups. The frequency of extrasystoles increases during job (tilt-test). The comparing of patients with extrasystoles and without them showed that LBD patients with extrasystoles have more severe sympathetic deficit.

Conclusions. The frequency of auricular extrasystoles is significantly higher in LBD patients. The appearance of extrasystoles may be explained by insufficient sympathetic traffic during tilt-test and additional action of heterogeneous humoral factors on desympathetic heart.

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CNI-1493 Inhibits Aß Production and Prevents Plaque Formation in An Animal Model of Alzheimer's Disease

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Alzheimer's disease (AD) is characterized by neuronal atrophy due to soluble Ab peptide "oligomers" and a microglial-mediated inflammatory response elicited by extensive amyloid deposition in the brain. Nonsteroidal anti-inflammatory drug treatment reduces AD risk, slows disease progression, and reduces microglial activation. In our screen, CNI-1493, an anti-inflammatory agent, was discovered to interfere with the Ab assembly as well as protecting neuronal cells from the toxic effect of soluble Ab oligomers.

We next found that the treatment of 4-month-old TgCRND8 mice overexpressing human amyloid precursor protein (APP) with CNI-1493 for a treatment period of only 8 weeks resulted in the dramatic reduction of Aβ deposition. CNI-1493 treatment resulted in 70% reduction of amyloid plaque area in the cortex and 87 % reduction in the hippocampus of these animals. In addition, CNI-1493 treatment resulted in a significant reduction in microglial activation in the TgCRND8 mice, as measured by F4/80 expression.

Our in vitro analysis of CNI-1493 treatment on APP processing in an APP overexpressing cell line suggests a profound dose-dependent decrease of total Aß accumulation. This effect appears to be completely unrelated from both the production of APP and changed β- or γ-secretase activities.

This study identifies the anti-inflammatory agent CNI-1493 as a very promising candidate for the treatment and prevention of AD in clinical trials.

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Pyrroloquinoline Quinone: A Potential Anti-Fibrillation Reagent Against Alpha-Synuclein

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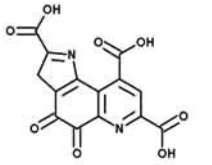
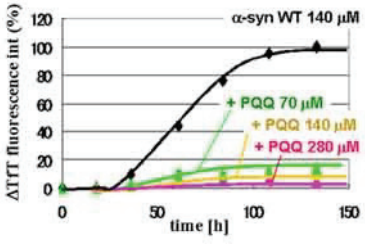
Pyrroloquinoline quinone (PQQ) is a noncovalently bound cofactor in the bacterial oxidative metabolism of alcohols. PQQ also exists in plants and animals. Due to its inherent chemical feature, namely its free-radical scavenging properties, PQQ has been drawing attention from both the nutritional and the pharmacological viewpoint. α-Synuclein, a causative factor of Parkinson's disease (PD), has the propensity to oligomerize and form fibrils, and this tendency may play a crucial role in its toxicity. Considering that several neurodegenerative diseases and also the fibrillation of causative proteins have been reported to be triggered by the radical formation, the inherent property of PQQ molecules inspires us to investigate its potential as the inhibitor which acts as the anti-fibril forming reagent against human α-synuclein. We show that PQQ prevents the amyloid fibril formation and aggregation of α-synuclein in vitro in a PQQ-concentration-dependent manner. We also found that the presence of PQQ reduces cytotoxicity possible caused by the prevention of the protofibril formation. Moreover, PQQ forms a conjugate with α-synuclein, and this PQQ-conjugated α-synuclein is also able to prevent α-synuclein fibrillation. This is the first study to demonstrate the characteristics of PQQ as an anti-amyloid fibril-forming reagent. Agents that prevent the formation of amyloid fibrils might allow a novel therapeutic approach to PD. Therefore, together with further pharmacological approaches, PQQ is a candidate for future anti-PD reagent compounds.



Pyrroloquinoline quinone (PQQ)



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Cholinesterase Inhibitors in Dementia With Lewy Bodies-Which one to Use?

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Background and Aims: Dementia with Lewy bodies (DLB) is a common neurodegenerative disease of the elderly accounting for 15-25% of all cases of dementia. There is a significant cholinergic deficiency in patients with DLB thus making cholinesterase inhibitors (ChEIs) a rational treatment option. The aim of the study is to determine if there is evidence supporting greater efficacy of one ChEI over another in treating patients with DLB.

Design /Methods: Retrospective comparison of three independent clinical studies of ChEI treatment using donepezil, galantamine or rivastigmine in patients with DLB. Data was obtained from open label trials of donepezil and galantamine and a placebo controlled randomized trial of rivastigmine in DLB. Changes in the effect size upon Mini Mental State Examination (MMSE), Neuropsychiatric Inventory (NPI) and United Parkinson's Disease Rating Scale (UPDRS-III) were compared between the three treatments at 12 and 20 weeks.

Results: All three ChEIs significantly improved cognitive and neuropsychiatric measures. Reduction in the total NPI score appeared significantly greater after donepezil treatment as compared to galantamine and rivastigmine. There was no significant increase in UPDRS-III scores with any of the drugs.

Conclusions: It is unclear to what extent these findings reflect true differences between ChEIs or are due to methodological artefacts of comparing different studies. There is so far no compelling evidence that any one ChEI is better than the other in treating DLB but head to head comparative studies of different ChEIs are warranted to clarify this.

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LRRK2 Regulates Neurite Morphology

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Mutations in LRRK2 underlie an autosomal dominant, inherited form of Parkinson's disease (PD) that mimics all of the clinical features of the common 'sporadic' form of PD. The LRRK2 protein includes putative GTPase, protein kinase, WD40 repeat, and leucine-rich repeat (LRR) domains of unknown function. Here we show that PD-associated LRRK2 mutations display disinhibited kinase activity and induce a progressive reduction in neurite length and branching both in primary neuronal cultures and in the intact rodent CNS. In contrast, LRRK2 deficiency leads to increased neurite length and branching. Neurons that express PD-associated LRRK2 mutations additionally harbor prominent phospho- Tau-

positive inclusions with lysosomal characteristics and ultimately undergo apoptosis.

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A Novel Transcriptional Regulation of Oxidative Stress Sensitive Kinase Pkcdelta Expression by Alpha-Synuclein Via NF- kB in Parkinson's Disease Models

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Recently, we demonstrated that protein kinase Cδ (PKCδ) is an oxidative stress sensitive kinase which plays a causal role in apoptotic cell death in Parkinson's disease (PD) models (Kaul et al., 2004; Yang et al., 2004). Herein, we report that α-synuclein regulates the proapoptotic gene PKCδ transcription via the redox sensitive transcription factor NF-kB. Overexpression of wild-type human α-synuclein in the mesencephalic dopaminergic neuronal cell line (N27 cells) significantly reduced PKCδ protein and mRNA levels in an isoform specific manner without altering other PKC isoform levels (alpha, beta1, epsilon and zeta). Pulse-chase analysis revealed that the PKCδ protein degradation rate was unaltered in α-synuclein-expressing cells. Additionally, results from nuclear run-on assay and mRNA stability experiments revealed that α-synuclein directly alters the PKCδ transcription but not the stability of the transcripts. PKCδ promoter analysis and EMSA revealed the presence of two NF-kB (p50/p65) binding sites in the proximal region of the PKCδ promoter. siRNA directed against the p65 NF-kB subunit decreased the PKCδ expression. Notably, overexpression of α-synuclein attenuated NF-kB binding to PKCδ promoter sites, whereas siRNA directed against the p65 NF-kB subunit decreased the PKCδ expression and siRNA directed against α-synuclein abolished the α-synuclein-mediated attenuation of NF-kB binding to the PKCδ promoter. Together, these results suggest that α-synuclein specifically suppresses the proapoptotic kinase PKCδ gene at the transcriptional level via the NFkB signaling pathway, and these findings provide new insights into α-synuclein's role in regulating oxidative cell death signaling in PD (supported by NIH grants NS45133 and ES10586).

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Long-Term Safety and Tolerability of Rosiglitazone XR in Alzheimer's Disease

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Background and Aims: Rosiglitazone, a peroxisome proliferator-activated receptor-gamma agonist used to treat type 2 diabetes, may increase brain glucose metabolism and provide benefits to patients with Alzheimer's disease (AD). The long-term safety and tolerability of an unmarketed formulation of rosiglitazone was evaluated in an open-label extension of a previously reported double-blind parent study in subjects with AD.

Methods: This multicentre, open-label study (AVA100468) enrolled subjects who completed a 24-week, randomised, double-blind, placebo-controlled trial of rosiglitazone XR (AVA100193). All subjects entering the 48-week extension received rosiglitazone XR 4 mg/day for 4 weeks, then 8 mg/day. Safety and tolerability assessments included: adverse events (AE), serious AE (SAE), vital signs, clinical chemistry, haematology, fasting insulin levels, and fasting plasma glucose levels.

Results: Of the 511 subjects who completed the double-blind study, 337 entered the open-label extension and are included in the safety analyses. 82% completed the open-label study; 7% withdrew due to AE. 48% of subjects experienced ≥1 AE, most commonly peripheral oedema (6%) and nasopharyngitis (5%). 9% experienced ≥1 SAE; each SAE except fractures (2%) occurred in ≤1% of subjects. Few subjects exhibited clinically significant changes in heart rate (<1%), or clinically significant abnormal ECG readings (2%) during the study. Changes in fasting insulin levels and fasting plasma glucose levels were within expected ranges for this population.

Conclusions: Rosiglitazone XR 8 mg/day appears to be generally safe and well tolerated for up to 72 weeks in subjects with AD, and has a safety profile comparable to that established in type 2 diabetes.

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Effects of Monomeric and Oligomeric Alpha-Synuclein on Activity of Kinases as Assessed on High-Density Kinome Arrays

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Co-immunoprecipation led to the identification of several kinases that interact with α-synuclein. Overexpression of α-synuclein in cell culture affects protein kinase C signalling. However, it is not known whether natively-unfolded or aggregated of α-synuclein is underlying these findings. In the present study we have incubated high-density arrays spotted with 179 kinases with monomeric and oligomeric recombinant Alexa488 labelled α-synuclein. Alpha-synuclein binding was detected by Alexa488 fluorescence and kinase activity was monitored by autophosphorylation using 33P-ATP.

Our preliminary results indicate that Alexa488-labeled oligomeric α-synuclein binds to several kinases. Kinase activity is differentially modulated by α-synuclein monomers and oligomers, respectively. Validation in neuronal cell lines overexpressing wildtype and mutant α-synuclein is ongoing.

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Dynamics of Alpha-Synuclein Aggregation and Inhibition of Pore-Like Oligomer Development by Beta-Synuclein

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Background And Aims: Accumulation of α-synuclein resulting in the formation of oligomers and protofibrils has been linked to Parkinson's disease and Lewy body dementia. In contrast, β-synuclein—a close homologue—does not aggregate and reduces α-synuclein related pathology. Although considerable information is available about the conformation of α-synuclein at the initial and end stages of fibrillization, less is known about the dynamic process of α-synuclein conversion to oligomers and how interactions with anti-aggregation chaperones such as β-synuclein might occur.

Methods: We utilized molecular modeling, in vitro cell-free, and in vitro electrophysiology approaches to investigate the oligomerization of α-synuclein, its electrophysiological consequences, and the effects of β-synuclein on this process.

Results: Molecular modeling and molecular dynamics simulations showed that α-synuclein homodimers can adopt non-propagating (head-to-tail) and propagating (head-to-head) conformations. Propagating α-synuclein dimers on the membrane incorporate additional α-synuclein molecules, leading to the formation of pentamers and hexamers forming a ring-like structure. In contrast, β-synuclein dimers do not propagate and blocked the aggregation of α-synuclein into ring-like oligomers. Under in vitro cell-free conditions, α-synuclein aggregates formed ring-like structures that were disrupted by β-synuclein. Similarly, cells expressing α-synuclein displayed increased ion current activity consistent with the formation of Zn2+-sensitive non-selective cation channels.

Conclusions: These results support the contention that in Parkinson's disease and Lewy body dementia α-synuclein oligomers on the membrane might form pore-like structures, and that the beneficial effects of β-synuclein might be related to its ability to block the formation of pore-like structures.

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Non-Covalent Binding of SUMO-1 Modulates Parkin E3 Ubiquitin Ligase Activity and Its Subcellular Localization: Pathogenic Implication to Parkinson Disease

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Parkinson disease (PD) is the second most common neurodegenerative disorder characterized by the extensive and progressive loss of dopaminergic neurons in CNS substantia nigra pars compacta region. Mutations in the parkin gene, which encodes for E3 ubiquitin ligase, have been implicated in autosomal recessive juvenile Parkinsonism, an early onset and common familial form of PD. Although several parkin substrates have already been identified, the molecular mechanism underlying the regulation of enzymatic activity of parkin has yet to be clarified. In the previous study we demonstrated that RanBP2 becomes a new target for parkin E3 ubiquitin-ligase, and is processed via parkin-mediated ubiquitination and subsequent proteasomal degradation. RanBP2, which is localized in the cytoplasmic filament of the nuclear pore complex, belongs to the small ubiquitin-related modifier (SUMO) E3 ligase family. Presently we show that parkin appears to selectively bind to the SUMO-1 in vivo and in vitro. Moreover, the physical association of SUMO-1 with parkin results in an increase in the nuclear transport of parkin as well as its self-ubiquitination. Our findings suggest that the E3 ubiquitin ligase activity of parkin and its intracellular localization may be modulated through the SUMO-1 association.

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Down Syndrome-Linked Dual-Specificity Tyrosine-Regulated Protein Kinase Enhances Intracellular Alpha-Synuclein Aggregates

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Lewy bodies (LBs) are pathological hallmarks of Parkinson disease (PD), but also occurs in Alzheimer disease (AD), and dementia of LBs. α-Synuclein, the major component of LBs, is observed in the brain of Down Syndrome (DS) patients with AD. Dyrk1A, a dual-specificity tyrosine-regulated kinase (Dyrk) family member, is the mammalian ortholog of the Drosophila minibrain (Mnb) gene, essential for normal postembryonic neurogenesis. The Dyrk1A gene resides in human chromosome 21q22.2 region, which is associated with DS anomalies including mental retardation. In this study, we examined whether Dyrk1A interacts with α-synuclein and subsequently affects intracellular α-synuclein inclusion formation in immortalized hippocampal neuronal (H19-7) cells. Dyrk1A selectively binds to α-synuclein in transformed and primary neuronal cells. α-Synuclein overexpression, followed by bFGF-induced neuronal differentiation resulted in cell death. We observed that accompanying cell death was increased α-synuclein phosphorylation and intracytoplasmic aggregation. In addition, the transfection of kinase-inactive Dyrk1A or Dyrk1A siRNA blocked α-synuclein phosphorylation and aggregate formation. In vitro kinase assay of anti-Dyrk1A immunocomplexes demonstrate Dyrk1A could phosphorylate α-synuclein at Ser-87. Furthermore, aggregates formed by phosphorylated α-synuclein have a distinct morphology and are more neurotoxic, compared with aggregates composed of unmodified wild type α-synuclein. These findings suggest α-synuclein inclusion formation regulated by Dyrk1A, potentially affecting neuronal cell viability.

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Dj-1 Protects Sk-N-Be Cells From Oxidative Stress and Modulates Alpha-Synuclein Toxicity

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Background And Aims: DJ-1 and alpha-Synuclein (a-syn) are involved in family forms of Parkinson's disease (PD). DJ-1 displays antioxidant properties, while many data suggest that a-syn becomes neurotoxic after aggregation. To clarify DJ-1 function and possible interaction with a-syn, we performed in vitro studies in SK-N-BE cells exposed to oxidative challenge. DJ-1 and a-syn expression was modulated by siRNA and exogenous addiction of non-toxic quantity of TAT-fused proteins.

Methods: Cell treatments. DJ-1 silencing was performed using predesigned siRNA (Ambion). Oxidative challenge was done by H2O2 or 6-OHDA treatment (50-75 uM, 24h). Western Blotting. 20 µg of protein extract was separated on a 5-12% SDS/PAGE gradient gel, transferred to a nitrocellulose membrane and incubated with a proper primary antibody prior of ECL. RT-PCR. Semiquantitative RT-PCR was performed starting from 100ng of total RNA (internal standard: aldolase).

Results: We found that DJ-1 silencing made cells more susceptible to oxidative stress, while DJ-1 overexpression showed a protective action. TAT-a-syn 0.5 uM was protective as well, but DJ-1 siRNA prevented this positive effect, and in this condition TAT-a-syn was toxic. Finally, TAT-DJ-1 prevented TAT-a-syn toxicity in DJ-1 silenced cells.

Conclusions: Our results confirm that DJ-1 acts as an antioxidant molecule in a dopaminergic context. Moreover, the experiments with TAT-a-syn indicate that DJ-1 may inhibit a-syn aggregation. This observation might explain how DJ-1 mutations lead to a-syn toxicity and PD onset. The development of an in vivo PD model will verify whether TAT-DJ-1 can prevent nigrostriatal degeneration in an animal model.

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Synucleins and Metal Binding

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Alpha-synuclein aggregation has been implicated in the pathogenesis of human neurodegenerative disorders. Aggregation of alpha-synuclein is accelerated by the presence of metals. Recent evidence suggests that alpha-synuclein binds copper at much lower concentrations than those associated with inducing aggregation. Further understanding of the effects of metals on alpha-synuclein is required to elucidate its function and its role in disease. The synuclein family also includes the homologues beta- and gamma-synuclein. Given overlapping expression patterns and the observation that beta-synuclein inhibits alpha-synuclein aggregation, it is clear that greater understanding of the function of the synuclein family is essential. Isothermal calorimetry has enabled us to assess the affinity of recombinant synucleins for metals. Clear differences in affinity for each different synuclein were identified. These differences suggest similar but different roles for the three synucleins in the homeostasis of metals in cells. We aim to further characterise metal binding by the synuclein family to enable a greater understanding of synuclein function and the ability to regulate alpha-synuclein to combat neurodegenerative disease.

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Regulation of Alpha-Synuclein Expression

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Accumulation of alpha-synuclein is implicated in the progression of many human neurodegenerative diseases, including Parkinson's and Alzheimer's disease. While many pathways regulate the levels of alpha-synuclein, it is clear that regulation of alpha-synuclein expression is essential to combat disease. Very little is understood regarding the signals and transcription factors regulating alpha-synuclein expression. There is some evidence that oxidative stress increases alpha-synuclein expression. Given the relationship between metals, oxidative stress, alpha-synuclein and neurodegenerative diseases, we have begun investigating their links to the regulation of alpha-synuclein expression. Regions of the alpha-synuclein promoter have been cloned into reporter systems and promoter activity assessed upon transfection into SH-SY5Y cells. By monitoring changes in reporter activity, regions of the alpha-synuclein promoter that regulate transcription have been defined. Comparison of basal reporter activity to reporter activity stimulated with metals and oxidative stressors will enable definition of the regions of the alpha-synuclein promoter that are regulated by these signals. We aim to identify the regulators of synuclein expression, which is essential to elucidate synuclein function and to enable manipulation of synuclein expression. Such information could eventually be of value in limiting disease progression in PD and other Lewy body dementias.

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Specific Cell-Derived Soluble Alpha-Synuclein Oligomers Are Degraded by, and Impair, The26s Protesome

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Proteasomal dysfunction may play a role in neurodegenerative conditions, and protein aggregation. Studies have shown that overexpression in neuronal cells of a-synuclein, a molecule linked to Parkinson's Disease, may lead to proteasomal dysfunction, via unknown mechanisms. Using PC12 cells stably expressing mutant a-synuclein, we demonstrate that a-synuclein co-elutes with the 26S proteasome in high molecular weight fractions obtained by gel filtration. Expression of mutant a-synuclein caused a significant inhibition of all proteasomal activities without affecting the levels or assembly of the 26S proteasome. Importantly, the species of a-synuclein responsible for this inhibition were soluble oligomers of intermediate size. Pharmacological dissociation of these oligomers restored proteasomal function. Soluble a-synuclein oligomers, but not total monomeric a-synuclein, increased following treatment

with proteasomal inhibitors, indicating specific degradation by the 26S proteasome. Our findings suggest a model where specific soluble cell-derived a-synuclein oligomers are targeted to the 26S proteasome for degradation, inhibiting proteasomal function by impeding degradation of other substrates. This is the first demonstration that specific soluble oligomeric species of a-synuclein impair the 26S proteasome in a cellular context.

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E46K Alpha-Synuclein Associated With Early-onset Parkinsons Disease Modulates SECRA2b Expression

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Three mutations (A30P, A53T and E46K) in the gene encoding the pre-synaptic protein alpha-synuclein are associated with inherited early-onset Parkinson's disease. The E46K alpha-synuclein was discovered in a large Spanish family, with complete segregation for disease onset when in the heterozygous configuration. We performed heterologous expression experiments to elucidate characteristics of E46K alpha-synuclein that may contribute to pathological mechanisms associated with neuron loss. Although lacking a signal sequence, E46K alpha-synuclein associates with the endoplasmic reticulum (ER) membrane, as well as the cell surface membrane, as detected in immunofluorescent microscopy images. Association with mitochondria and Golgi were not detected. Our data also indicate that E46K alpha-synuclein increases cell surface membrane permeation, and depletes intracellular calcium stores, as detected by Fura-2 calcium imaging technology. E46K alpha-synuclein also appears to reduce steady-state expression levels of the SR/ER calcium ATPase SERCA2b, which is involved in replenishing the ER with calcium. Based on these experimental observations, a pathological mechanism associated with E46K alpha-synuclein may, at least it part, be due to dysregulated calcium fluxes between ER and cytosol.

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Proteomics Investigations of Protein Degradation Arrest by Selective Proteasome Inhibitors

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Late-onset neurodegenerative diseases are often associated with the formation of toxic intracellular aggregates which should be substrates of cell degradation pathway such as the ubiquitin-proteasome system. In fact, aggregates of ubiquitinated proteins have been observed in the CNS of patients affected by Alzheimer's, Parkinson's and Huntington's disease.These aggregates might be suggestive that dysfunctions in the ubiquitin-proteasome system might contribute to the pathology of various neurodegenerative disorders.

In order to investigate the early molecular events in the accumulation of ubiquitinated proteins we have employed selective proteasome inhibitors such as epoxomicin and PSI on an in vitro human cell line model of neuroectodermic origin (Neuroblastoma, SH-5YSY). A combination of flow citometry and proteomics experiments by 2D-PAGE protein separations coupled to MALDI-TOF-MS and nLC-Q-TOF-MS/MS were applied to characterise the differential protein profiles.

Increased levels of poli-ubiquitinated proteins were found associated to the activation of the drugs induced apopotosis as shown by western blot analysis of caspase-8, p21 and p53. Nevertheless a sub-population of the cell lines is capable to overcome cell death by the proteasome inhibitor toxicity. We have performed a proteomics investigation on the different cell populations before and after exposure to epoxomycin by employing cell citometry analysis combined with cell sorting harvesting. Our results identified a direct involvement of various intracellular pathways such as the heat shock protein machinery (HSP60, 70), of Trans Golgi Network (TGN) and of nucleotide metabolism. Interestingly the resistant cell population shows higher level of VGF inducible factor.

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Thermodynamics Imprinting Reveals Differential Binding of Metals to Alpha-Synuclein in Relevance to Parkinson's Disease

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Background: The aggregation of alpha-synuclein is a hallmark feature of Parkinson's disease (PD) and other synucleinopathies. Metals are the significant etiological factors in PD, and their interaction with synuclein affect dramatically the kinetics of fibrillation in vitro and are proposed to play a potential neurodegenerative role in vivo. To date, there is no data available on the binding constants and the thermodynamic parameters on the effect of metals on synuclein fibril formation in PD.

Aims: The present study, for the first time investigates the stoichiometry of binding of Copper [Cu (II)] and Iron [Fe (III)] with synuclein (wild recombinant type and A30P, A53T, E46K mutant forms) using Isothermal Titration Calorimetry (ITC).

Results: Titration of Cu (II) with synuclein monomer (wild and mutant forms) showed i) two binding sites, with an apparent Kd of 10-5 M and 10-4 M respectively, ii) the high affinity site has a smaller enthalpic contribution but a larger entropic contribution than the low-affinity binding site. In contrast, titration of Fe (III) with synuclein (wild type and mutant forms) yields exothermic values with a Kd of 10-5 M with single binding site. We have also observed that delta-G is negative in case of Cu (II), while with Fe (III) it is positive.

Hence compared to Cu (II), Fe (III) saturates the binding sites faster, thereby enhances self-oligomerization quicker than Cu (II).

Conclusion: The present investigation on ITC uncovers the detailed binding propensities between metals and synuclein and its biological implications in PD.

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Amino Acid Coupled Liposomes for Effective Management of Parkinsonism-A Novel Approach

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The Blood Brain Barrier restricts the brain uptake of many valuable hydrophilic drugs and limits their efficacy in the treatment of brain diseases.Parkinson€™s disease is a brain disorder caused due to deficiency of dopamine-HCl (Neurotransmitter). In the present project, amino acid coupled liposomes bearing dopamine-HCl were prepared to deliver the drug to the brain for the management of Parkinsonism utilizing receptor-mediated transcytosis. Uncoupled liposomes were prepared by cast film method using phosphatidylcholine and cholesterol whereas coupled liposomes were prepared using phosphatidylcholine, cholesterol and glutamine stearylamine conjugate (GSC) in the film. These liposomes were characterized for entrapment efficiency, vesicle size, shape, in vitro drug release and in vivo studies. The vesicle size was found to increase upon coupling of liposomes, whereas percent entrapment efficiency was reduced from 38.89±1.94% to 34.15±1.70% after coupling of liposomes. The in vitro percent cumulative drug-release studies exhibited 51.6% drug-release for uncoupled liposomes and 37.9% drug-release for coupled liposomes at the end of 24 hours. These selected formulations were subjected for in vivo performance, which was assessed by periodic measurement of drug (Chlorpromazine) induced catatonia in albino rats (Wistar strain) and fluorescence microscopy studies of the rat brain. The results were compared with plain dopamine-HCl solution. Studies revealed that dopamine-HCl can be effectively delivered to the brain via glutamine-coupled liposomes and exhibited superiority of coupled liposomal formulation over uncoupled formulation.

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Colostrinin Increases the Life-Span and Neurological Performance in Senescence Accelerated Mice

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Background and Aims: ColostrininTM (CLN), a uniform mixture of low-molecular weight proline-rich polypeptides extends the life-span of diploid fibroblasts, induces neurite outgrowth of pheochromocytoma cells, decreases mutation frequencies in both Chinese hamster and human cells and inhibits beta amyloid-induced apoptosis in human neuroblastoma cells. Most importantly, oral administration of CLN has shown a stabilizing effect on cognitive function in Alzheimer's patients measured by the Alzheimer's disease Assessment Scale-cognitive (ADAS-cog) and in Instrumental Activities of Daily Living (ILDL). In this study, we investigated the effects of oral administration of CLN on the life-span and various behavior characteristics in senescence-accelerated mice.

Methods: The battery of behavioral tests included: swim maze, locomotor distance, rotorod running, walking initiation, alley turning, bridge walking, wire suspension, and discriminated active avoidance tests.

Results: Here we show that CLN administration to mice prolongs life-span (34% increase), improve age-associated locomotion, motor coordination, and learning/memory capacities. Increase in life-span and improved neurological performance correlated well with the levels of oxidative stress markers measured in various organs. In particular, we demonstrate an improved mitochondrial function, decrease in levels of 8-oxoguanine in nuclear and mitochondrial DNA and significantly reduced oxidative damage to proteins in brain and liver.

Conclusions: These results support the view that this newly discovered characteristic of CLN underlines its utility in age-related neurodegenerative diseases, and the quality life improvement in elderly. Supported by a fund from ReGen Therapeutics, Plc. London, England.

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Cellular Study of LRRK2 Domains

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Mutations in the leucine rich repeat kinase 2 gene (LRRK2) have been shown to segregate with Parkinson's disease in an autosomal dominant fashion. The LRRK2 gene product is a very large protein (2527 amino acids) encompassing several functional domains: a leucine rich repeat domain, a domain homologous to small GTPases of the Ras family termed Roc (for Ras of complex proteins), a COR domain (for C-terminal of Roc) of unknown function, a kinase domain of the non-receptor tyrosine kinase family as well as WD40 repeats. In vitro studies have confirmed a kinase activity for LRRK2, and this activity is increased in mutant forms of LRRK2, suggesting that increased kinase activity is the pathogenic mechanism. Cellular studies show a role of LRRK2 in pathogenicity by demonstrating that transiently transfected mutant LRRK2 causes neurodegeneration in SHSY5Y neuroblastoma cells and in primary neurons. In order to further characterize the function of LRRK2 in cells we decided to overexpress the separate LRRK2 domains in SHSY5Y neuroblastoma cells via transient transfection as well as via stable transduction using lentiviral vectors. Using GFP and flag tags, we found that most of the separate LRRK2 domains were localized to the cytoplasm, as is full length LRRK2. Overexpression of the GFP tagged LRRK2 domains also lead to the formation of GFP-positive cytosolic aggregates, to varying degrees. We are currently investigating the effect of domain overexpression on cell viability.

Neuro–degenerative Diseases

Neurodegenerative Dis 2007; 4(suppl 1):1-350

Sunday, March 18, 2007

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Predicting Dementia in Parkinson's Disease: Clinical Risk Factors

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Cumulative incidence of dementia in Parkinson's disease (PD) may be up to 80%. When disease-modifying treatments become available for cognitive failure in PD, prompt identification of individuals at high risk of dementia will assume great importance. Increasing age is the most important risk factor for incident dementia in PD (PDD). Increased motor disability, symmetric motor presentation and axial motor impairment with reduced tremor, longer disease duration and male gender have been associated with increased risk of PDD. Less well established risk factors include low educational attainment, current smoking habit, depression, excessive daytime somnolence, REM sleep behaviour disorder and orthostatic hypotension. Anticholinergic drug use may be a risk factor for PDD, and prolonged use of these agents has been associated with an increased frequency of cortical plaques and tangles in non-demented PD patients. Poor response to L-dopa and hallucinations on dopaminergic treatment may predict dementia. L-dopa-induced hyperhomocysteinaemia could contribute towards cognitive failure while amantadine may delay and attenuate dementia. Visual hallucinations in PD predict more rapid cognitive deterioration. Psychosis requiring antipsychotic therapy has been associated with development and progression of dementia, while the use of atypical antipsychotics may have adverse disease modifying effects. Deficits in auditory verbal learning and nonverbal reasoning, picture completion, Stroop interference and verbal fluency have all been independently associated with an increased risk of cognitive failure in PD. There is an association between apathy and cognitive dysfunction, particularly executive impairment, but it is unknown whether apathy is independently predictive of dementia.

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The Neuroinflammatory Response in Plaqes and Amyloid Angiopathy in Alzheimer's Disease: Therapeutical Implications

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The cerebral Abeta deposition in parenchyma (plaques) and walls of vessels (amyloid angiopathy) is the consequence of an impaired balance between the production and removal of the Abeta peptide. The amyloid plaques are closely associated with a locally induced chronic inflammatory response but not with the majority of the amyloid deposits in the walls of meningeal and medium-sized arteries in contrast to the microcapillary amyloid angiopathy. However, the Abeta amyloid angiopathy in the larger vessels of patients with hereditary hemorrhage with amyloidosis-Dutch type (HCHWA-D) is associated with a strong monocyte/macrophage reactivity. The immunohistochemical findings in HCHWA-D brains are in line with transgenic mice models harboring vasculotropic Abeta mutants showing a prominent deposition of vascular amyloid associated with microglial activation and (micro)hemorrhages. The present view is that the neuroinflammatory response has both beneficial and deleterious effects on the progression of the disease process. Inflammatory mediators are involved in the dynamic proces of Abeta deposition, removal and transport. Studies with passive immunotherapy in transgenic APP mice with anti-Abeta antibodies have shown a decrease of parenchymal Abeta deposits but an increase of vascular amyloid and of the number of microhemorrhages. With respect to an inflammation-based therapy an option for further research could be the combination of immunotherapy to stimulate Abeta removal and anti-inlammatory drugs to reduce the increased levels of pro-inflammatory cytokines. Recently we have found that minocycline reduces the fibrillar Abeta induced secretion in human microglial cell cultures but does not influence Abeta phagocytosis.

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Amyloid Beta Peptide: Some New Ideas About Where It Comes From and Where It Goes

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Background And Aims: There is abundant dogma but little definitive data concerning the origin of brain amyloid β peptide (Aβ) deposits or the fate of such Aβ. Two novel alternatives were explored.

Methods: Multidisciplinary.

Results: Sources of Aβ deposits: 1) Platelets contain substantial amounts of Aβ, and release it when activated. 2) Pathology of the AD cerebral vasculature is conducive to platelet activation. 3) Genes and gene products for platelet activation are upregulated in AD cortical samples. 4) Microvessels that are immunoreactive for platelet activating factors can virtually always be found within brain Aβ deposits. 5) Micro-injection of platelet activating factors into rabbit cortex produces multiple parenchymal Aβ deposits distal to the injection site.

Results: Clearance of Aβ: 1) Human plasma Aβ is rapidly bound by complement opsonins. 2) The complement/Aβ complexes are, in turn, rapidly bound by complement receptors on erythrocyte membranes. 3) Such complement/pathogen complexes are known to be stripped off erythrocytes and degraded by Kuppfer cells of the liver, and a previous study has shown that the majority of spiked Aβ is cleared to the liver. 4) AD patients appear to exhibit deficiencies in this mechanism compared to non-demented elderly subjects, with intermediate values in mild cognitive impairment patients.

Conclusions: Platelet mechanisms may provide a link between the increased vascular pathology of AD and brain Aβ deposition. Complement mechanisms for Aβ clearance may help explain why Aβ immunization strategies work, despite miniscule anti-Aβ antibody penetration: the availability of peripheral anti-Aβ antibodies should enhance complement opsonization of Aβ.

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Increased Vascular Amyloid and Microhemorrhage is Observed With Amyloid Reductions Caused by Active Immunization Against Aß But Not With Nitro-NSAIDS

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Passive immunotherapy of aged APP transgenic mice causes dramatic reductions in amyloid load, but an increase in vascular amyloid and extravasation of blood associated with the vascular amyloid. We treated APP+PS1 transgenic mice between 10 and 20 mo of age with either a) vaccination against the Aß peptide, b) the nitro-flurbiprofen derivative NCX-2216 or c) both agents. Vaccination reduced total Aß immunostaining (largely diffuse deposits) by 20% and Congo red deposits (mostly compacted plaques) by approximately 35%. When the Congophilic deposits were subdivided into parenchymal and vascular locations, there was a 2 fold increase in the vascular deposits and a 3 fold increase in the number of microhemorrhages. The NCX-2216 treatment, possibly acting by modifying gamma secretase cleavage towards shorter forms of Aß, caused a comparable 20% reduction in total Aß immunostaining and a 30% reduction in Congophilic deposits, but no increase in vascular amyloid or the number of microhemorrhages. The combination of NCX-2216 plus the anti-Aß vaccine were identical to the effects of vaccination alone. These data show 1) that active immunotherapy increases vascular amyloid and microhemorrhage, 2) that NCX-2216, does not cause these changes in spite of a comparable reduction in amyloid and 3) that the nitro-NSAID does not modify the actions of active immunization on vascular build-up of amyloid. Supported by AG-15490 and AG 18478.

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Eta Amyloid Imaging With a Novel F-18 Labelled PET Ligand

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Objective: We demonstrated cortical A deposition using 11C-PIB PET in 100% of 45 subjects with Alzheimer's disease (AD), 80% of 12 subjects with Dementia with Lewy Bodies, 60% of 44 subjects with mild cognitive impairment, none of 10 subjects with fronto-temporal dementia and 23% of 34 healthy elderly subjects. Thus A PET imaging shows promise for differential diagnosis of dementia, early detection of AD and monitoring anti-A therapy. However, the 20-minute half-life of C-11 restricts the use of PIB to centres with an on-site cyclotron. The aim of this study was to assess the lead compound from a novel series of F-18 labelled amyloid imaging ligands in AD patients and age-matched healthy controls (HC).

Methods: Five mild AD subjects (MMSE 24±3, CDR 1) and 6 HC (MMSE >28) underwent PET imaging over 3 hours after injection of 300 MBq of the 18F-ligand. Distribution volume ratios (DVR) were calculated through graphical analysis using the cerebellum as input function.

Results: All AD subjects showed neocortical binding, greatest in the precuneus/posterior cingulate and frontal cortex, followed by lateral temporal and parietal, with relative sparing of sensorimotor cortex. HC showed no binding in cortical or subcortical grey matter and their scans were clearly distinguishable from AD subjects. Cerebellar cortex showed no retention in either group. Significantly higher neocortical DVRs were observed in AD (1.84 ± 0.20) when compared with HC (1.2 ± 0.17, $p = 0.009$). Cortical uptake to cerebellar cortex ratio (SUVR) at 90-120 minutes post-injection gave similar results to DVR with a Cohen's effect size of 3.55 for AD vs HC.

Conclusions: Our results show that A burden can be quantified in AD with a novel F-18 labelled PET ligand. The pattern of binding closely matches that reported with 11C-PIB. This ligand may permit wide application of amyloid imaging by centralized production and distribution not possible with a C-11 labelled A ligand.

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Imaging of Amyloid Pathology in Alzheimer´S Disease

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The critical role of different forms of Aß, soluble Aß oligomers and fibrillar Aß deposits is still not yet fully known. It is a great challenge to try to detect Aß in AD in its earliest stages. In vivo amyloid imaging technology may contribute to a further understanding of Aß protein aggregation and plaque formation. A robust difference in 11C-PIB retention has been observed in cortical brain but also subcortical brain regions of AD patients compared to age-matched controls1,2 The cortical retention of 11C-PIB inversely correlates with deficits in cerebral glucose metabolism. When the AD patients underwent repeated PET studies with 11C-PIB and 18F-FDG 2 year later the PIB retention remained quite unchanged despite a further impairment in cerebral glucose metabolism (18F-FDG uptake) especially in AD patients with progression in cognitive impairment2. The unchanged PIB retention suggest a difference in time course between amyloid brain deposition and cerebral glucose metabolism and cognitive function. PIB studies in patients with mild cognitive impairment (MCI) show both high and low PIB retention retention3. Further studies in patients at risk for developing AD will provide understanding of the evolution of amyloid in brain.We have found that phenserine treatment to AD patients improves cerebral glucose metabolism and cognitive performance after 3 months treatment but also seem to change the relation in Aß levels in CSF and brain4.

1 Klunk et al. Ann Neurol 2006: 55:306-319,2 Engler et al Brain 2006 doi:10,1093

3 Forsberg et al. submitted, 4 Kadir et al.in preparation

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Proteoglycans/Glycosaminoglycans in the Pathogenesis of Alzheimer's Disease and Parkinson's Disease and the Development of Novel Small Molecule Amyloid Disease-Modifying Therapeutics

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Over the last 15 years we demonstrated the importance of heparan sulfate proteoglycans (HSPGs) in the pathogenesis of beta-amyloid protein (Aβ) fibrillogenesis in Alzheimer's disease (AD) and other amyloidoses. HSPGs and other highly sulfated glycosaminoglycans (GAGs) aid in the induction and acceleration of amyloid fibril formation in AD and Parkinson's disease (PD), and protect such fibrils from protease degradation. Specific highly sulfated GAGs can induce Aβ 1-40 to spontaneously form maltese cross amyloid-plaques that are structurally similar to amyloid plaques present in human AD brain. HSPG/ highly sulfated GAG accumulation in tissues is an early event in APP transgenic models of AD and in experimental AA amyloidosis. Highly sulfated GAGs require a specific minimum repeating disaccharide chain length to accelerate amyloid fibril formation for both AD (i.e. Aβ) and PD (i.e. alpha-synuclein). Increased sulfation of GAG chains that may occur during production of PG splice variants during aging and disease may increase the susceptibility of individuals to amyloid fibril formation. On the other hand, short chain length GAGs (i.e. disaccharides) may serve as inhibitors of amyloid fibril formation. Thus, GAG chain length and structure play important roles in the modulation of fibrillogenesis in both AD and PD. Evidence will be presented confirming the role of PGs/GAGs in amyloid pathogenesis and demonstrating the efficacy of promising pre-clinical compound candidates being developed by ProteoTech that show remarkable in vitro and animal model effects for the reduction and clearance of amyloid proteins and associated fibrils for both AD and PD.

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Engineered Heparins: Novel β-Secretase Inhibitors as Potential Alzheimer's Disease Therapeutics

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Background: Cleavage of amyloid β-protein precursor (APP) by the protease beta-secretase (BACE1) is a key step in Aβ peptide processing. We recently described a novel role for heparan sulfate polysaccharides in AD pathology as one of the first naturally occurring inhibitors of β-secretase (BACE1) (Scholefield et al., J. Cell. Biol, 2003).

Aims: evaluate "engineered" (chemically modified) heparin analogues as novel BACE1 inhibitors in vitro and in vivo

Methods & Results: We have developed and extensively tested a number of engineered heparin analogues for their ability to inhibit BACE1 and also their activity as anticoagulants and as inhibitors of other proteases related to BACE1 (Patey et al, J Med Chem 2006). Several lead compounds have been identified that are effective BACE1 inhibitors, but have negligible activity as anti-coagulants or on other proteases related to BACE1. They are effective at lowering Aβ production and do not show cytotoxicity in organotypic brain cultures. We have gone on to test these novel compounds in the Tg2576 transgenic mouse model of AD. Initial assessments have been made of the bioavailability, pharmacodynamics and toxicity of both high and low molecular weight types of these compounds in vivo. We have tested the efficacy of the compounds by measuring brain A? levels as well as by behavioural testing.

Conclusions: These data provide crucial new insights into the in vivo efficacy of selective engineered heparins as BACE1 inhibitors, and could underpin the development of new therapeutic strategies for human AD and other neurodegenerative disorders.

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Neuroprotective Properties of Glycosaminoglycans (GAGs): Potential Treatment for Neurodegenerative Disorders?

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Previous studies suggest that proteoglycans (PG) and glycosaminoglycans (GAGs) may play an important role in the pathogenesis and/or alleviation of neurodegenerative disorders, including Alzheimer's disease (AD). Proteoglycans increase the formation of neurofibrillary tangles, and stimulate the aggregation of amyloid-β. This effect, on the other hand, is believed to be competitively inhibited by certain GAGs.

Over the past few years we have examined the neuroprotective properties of C3, a low molecular GAG, on animal models of lesions characteristic for AD. C3 is composed of 4-10 oligosaccharides, and it is a low molecular weight (~ 2.1 K) derivative of heparin, extracted from heparin fragmented by gamma radiation.

In our experiments, C3 protected against cholinergic lesions induced by intracerebroventricular injection of the specific cholinotoxin, AF64A, in rats. Administration of C3 also attenuated AF64A-stimulated, low-affinity nerve growth factor receptor immunoreactive axonal varicosities in the rat septum. Moreover, C3 reduced tau-2 immunoreactivity in rat hippocampus, stimulated by intra-amygdaloid injection of Aβ(25-35). These findings are in good agreement with our previous data indicating the neuroprotective role of GAGs.

These results, plus others to be discussed at the conference, all suggest that C3 may possess neuroprotective properties against many of the characteristic neural lesions in AD. Since our pharmacokinetic studies revealed that C3 is capable of crossing the blood-brain barrier, C3 may, conceivably, open an entirely new avenue in the treatment of neurodegenerative disorders. Indeed, Phase I studies have proven to be extremely supportive in that regard.

Supported by:NIA/STTR Grant#1-R41-AG15740-02

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Role of Glycosaminoglycans in the Pathogenesis of Neurodegenerative Diseases

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Glycosaminoglycans (GAGs) are a group of highly sulfated sugars found on proteoglycans that regulate a number of important trophic mechanisms in the brain and peripheral nervous system. GAGs have also been implicated both in the aggregation and deposition of amyloid, as well as in other biochemical mechanisms involved in neurodegeneration. Our studies show that the interaction between Abeta and basement membrane proteoglycan may be important for the toxic effects of beta-amyloid on vascular smooth muscle cells in amyloid angiopathy. Recent studies suggest that the beta-secretase (BACE1) of Alzheimer's disease can bind strongly to GAGs. We have identified a high affinity heparin-binding site within the prodomain of the BACE1 zymogen. Binding of GAGs and GAG analogues to the prodomain can stimulate BACE1 activity by easing steric hindrance to the active site of the enzye. GAGs may also play an important role in other amyloidoses as well, as they are common constituents of amyloid deposits. Familial amyloidotic polyneuropathy is caused by deposition of TTR (amyloid P) around peripheral nerves. Our studies show that heparin can increase the rate of TTR nucleation, leading to an increase in the rate of amyloid fibril formation. These studies raise the possibility that GAGs or GAG analogues could have value in the treatment of certain neurodegenerative diseases.

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Neuroparin (C3), a Low Molecular Weight Glycosaminoglycan, Lowers Formic Acid Extractable Ab1-40 and 1-42 in Appswe/Psen1de9 Male Mice

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Background and Aims: Proteoglycans (PGs) and their glycosaminoglycan (GAG) side chains are colocalized with Ab in senile plaques and can stimulate fibril nucleation and stability of amyloid plaques. This suggests that PGs may act as "seed molecules" for Ab fibril formation and hence promote senile plaque deposition seen in Alzheimer's disease (AD). Conversely, GAGs which comprise the side chains of PG's can inhibit Ab's neurotoxic effects. Therefore, it was hypothesized that low molecular weight GAGs (neuroparin/C3; 2200 MW) act as pharmacological antagonists of endogenous proteoglycan compounds and could prevent AD associated neuropathology.

Methods: Beginning at 4 months of age, the effects of orally administered C3 (100 mg/kg/day) in an AD mouse model of increased Ab burden was studied (APPswe, PSEN1dE; WT/V n=7, WT/C3 n=7, Tg/V n=9, Tg/C3 n=10). Previously reported behavioral testing done between 8 and 9 months showed improvement in memory in the C3 treated group and decreases in amyloid plaque counts in males but not females.

Results: In these animals, recent analysis demonstrates there are decreases in formic acid extractable ("insoluble") Ab 1-40 (-51%; p=0.02) and 1-42 (-34%; p=0.02) in C3 treated males but not females. However, C3 did not alter TBS-extractable ("soluble") Ab 1-40 or 42 in either males or females.

Conclusions: Given there is a trend toward an increased plaque count in female compared to male mice at 8-9 months (+58%), these data suggest that C3 needs to be given earlier and/or prior to plaque formation to be an effective treatment for lowering amyloid burden.

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Characterization of Rabbit Antibodies Specific for Human Fibrillar a 1-42 (Fa) Peptide

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Objective: To produce and characterize rabbit antibodies specific for human fA peptide, and determine if they detect fibrillar amyloid deposits in brain from patients with Alzheimer disease (AD).

Background: In animal models of AD, immunization with fibrillar human A lowered the level of A deposits in their brains. However, vaccination of human A in AD resulted in

the development of meningoencephalitis in some patients, and their sera showed binding to vascular amyloid deposits. Since the antibodies were not isolated or characterized, we hypothesized that antifibrillar antibodies possess higher affinity to bind fibrils than those produced with nonfibrillar A peptides. Here we characterized the immune response of rabbits to human fA , since the A sequences of human and rabbit are identical.

Design/Methods: Human fA peptide was prepared by solubilizing and disaggregating A 42 peptide in .05 M ammonia. Rabbits were immunized with fA , and the antibodies were purified by affinity chromatography. Their binding properties were characterized using sedimentation assay, ELISA, and immunohistochemical methods.

Results: Rabbit antibodies to fibrillar A showed moderate titers against A 42 in an ELISA. However, they did not react with A 42 in Western blotting at o.1 pmol concentrations, which could be readily detected by other polyclonal or monoclonal anti-A sera. The data suggested that anti-fibrillar antibody recognized an epitope unique to the fibrils. Electron microscopy confirmed the binding of anti-fibrillar antibodies to fibrils. Further characterization showed that the anti-fibrillar antibodies bound to an epitope in the A 1-8 sequence, and required a free amino terminus on Asp-1. Fibril binding assay and ELISA data showed that anti-fibrillar antibodies were about 500 times more effective in binding to fibrils than nonfibrillar peptides. In addition, antibodies raised to four different nonfibrillar peptides corresponding to internal A sequences did not exhibit high affinity for fA peptide. Immunohistochemical studies showed that fibrillar antibodies had greater affinity for amyloid deposit in cerebellar blood vessels, than nonfibrillar antibodies.

Conclusions: The meningeal inflammation recently seen in several AD patients immunized with aggregated A might be due to the higher binding of anti-fibrillar antibodies to A fibrils than with endogenous APP or monomer A sequence. Since vascular amyloid deposits occur in meningeal vessels, the antibodies may react with cerebral A fibrils and trigger an inflammatory response. Passive immunization of humans with an antibody directed to an epitope of the C-terminal sequence of A 42 (MVGGVVIA) hidden in fA might be a better choice to remove A monomers without provoking an inflammatory response.

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Relationship Between Neprilysin and Ab Levels During Aging in Normal and AD Brain

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Brain deposition of b-amyloid (Ab) is not exclusive to AD but Ab is also detected in non-demented elderly individuals.. It has been suggested that the onset of sporadic AD may in many cases be attributed to an impaired clearance of Ab. Neprilysin has been shown to be an important enzyme to degrade Ab in brain, and to be reduced with aging in mouse brain. To assess the potential role of neprilysin in the clearance of Ab, we analyzed neprilysin and Ab levels during aging in normal and AD brain. Protein levels of neprilysin were reduced with age in the temporal and frontal cortex of both AD and normal brain. There was a significant positive correlation between insoluble Ab 40 and Ab 42 with age in cortex of normal brain whereas in AD brain the correlation was weaker. An inverse correlation between neprilysin and insoluble Ab levels was observed in both groups, suggesting that neprilysin is involved in the clearance of Ab. No differences in neprilysin protein and mRNA levels were found between AD patients and age-matched controls. The observed age-dependent decline in neprilysin may be related to the increased Ab levels during normal aging. However, the similar rate of decline in neprilysin with age may not be the major cause of the high levels of Ab associated with AD but is likely to be a trigger of AD pathology.

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Pathological and Physiological Roles of Intramembrane Cleavage Precision by Presenilin/Gamma-Secretase

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Background and Aims: Many type-1 receptors undergo dual-cleavage by PS/gamma-secretase within their transmembrane regions. PS/gamma-secretase hydrolyzes betaAPP and Notch-1 near the middle of transmembrane domain (TM) at the gamma and S4 sites, respectively, and at the border of the TM and the cytosol at sites epsilon and S3, respectively. In some cases, there is diversity in the specific sites of cleavage. How this change in precision occurs has been the focus of intense study because gamma-cleavage of the betaAPP increases the production of Alzheimer disease-associated longer Abeta42.

Results: We identified that Nbeta and other Abeta-like peptide species are secreted. The results demonstrate that the characteristics of C-terminal elongation of Nbeta and Abeta are similar. We suspect that the release of Abeta- or Nbeta-like peptides is a common feature of the proteolysis during RIP signaling, and anticipate that our data may open the door to searches for surrogate markers for Abeta42 production. Moreover, we found that, due to diversity in the site of S3 cleavage, intracellular domain of Notch-1 is cleaved into two distinct NICD species.

Conclusions: Our results suggest a novel aspect of intracellular Notch signaling, and they support the idea that a change in the precision of cleavage by PS/gamma-secretase is physiologically important

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Addls Trigger AD Memory Deficits Via Post-Synaptic Signaling and LTP Compromise

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Key research findings over the past decade have established a clear connection between ADDLs, Alzheimer's disease and AD-related memory deficits. ADDLs affect memory by binding to post-synaptic receptors on specific subsets of neurons, leading to disruption of long-term potentiation (LTP). Surprisingly, this LTP inhibition does not involve compromise of normal neurotransmission, but rather occurs via activation of specific signaling pathways. In animal behavioral models, LTP signaling deficits can be induced transiently by ADDL administration and reversed by ADDL-neutralizing antibodies. Although memory deficits occur long before frank neurodegeneration, prolonged exposure to ADDLs can lead to degenerative cellular pathology via activation of tau hyperphosphorylation and other transcriptional processes. This presentation summarizes recent structural and functional studies involving ADDLs and stable trimers assembled from a novel Aβ 1-42 peptide analog. The characteristic punctate ADDL binding to dendritic spines occurs at concentrations lower than 50 pM, while the stable trimeric assemblies exhibit no punctate binding, even when applied at 1 μM. The enhanced understanding of ADDL neuronal binding and signaling has led to discovery of small molecule ADDL binding inhibitors, while the design of stable trimeric Aβ peptide assemblies has led to novel vaccine immunogens now in pre-clinical development.

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LRP-1 Regulates Amyloid-Beta-Mediated Cell Death of Cerebrovascular Cells

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Background: Inefficient clearance of amyloid-β (Aβ), caused by impaired blood-brain barrier transport into the circulation seems to be a major cause of Aβ accumulation in the brain of late-onset Alzheimer's disease (AD) patients and hereditary cerebral hemorrhage with amyloidosis Dutch type (HCHWA-D).

The aim of this study was to investigate 1) the association of Aβ-receptors with cerebral amyloid angiopathy (CAA) and senile plaques (SPs) in both AD and HCHWA-D brains, 2) the effect of Aβ on Aβ-receptors expression levels by cultured human brain pericytes (HBPs) and astrocytes, 3) and the role of Aβ-receptors in Aβ internalization and Aβ-mediated cell death of cultured HBPs and astrocytes.

Methods and Results: We observed colocalization of Aβ-receptors such as RAGE, LRP-1, CD36 and LDLR with CAA using immunohistochemistry in both AD and HCHWA-D brain. Expression of both LRP-1 and the LDL receptor by cultured HBPs, leptomeningeal smooth muscle cells and astrocytes increased upon incubation with Aβ. Furthermore, both Aβ internalization and Aβ-mediated cell death by perivascular cells could be antagonized by adding purified receptor-associated protein.

We conclude that: 1) the Aβ-receptors LRP-1 and LDLR are involved in Aβ internalization by cerebral perivascular cells and, 2) perivascular cells may adapt their Aβ clearance capacity to the levels of Aβ present, 3) saturated LRP-1/LDLR-mediated internalization of Aβ results in degeneration of perivascular cells.

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Soluble Amyloid-Beta Leads to Mitochondrial Defects in APP and Tau Transgenic Mice

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Mitochondrial dysfunction has been identified in neurodegenerative disorders including Alzheimer's disease, where accumulation of amyloid beta (A-beta) and oxidative stress seem to play central roles in the pathogenesis, by probably directly leading to mitochondrial dysfunction. In order to study the in vivo effect of A-beta load during aging, we evaluated the mitochondrial function of brain cells from transgenic mice bearing mutant amyloid precursor protein (APP) and non-tg littermate control animals at different ages (1.5, 3, and 6 months). TgAPP mice exhibit onset of A-beta plaques at an age of 6 months, but intracellular soluble A-beta load is already increased at the age of 3 months. In addition, we investigated the effects of different A-beta species on mitochondrial function of brain cells from tau transgenic mice. Basal mitochondrial transmembrane potential was already decreased in tgAPP mice at an age of 1.5 months and decreased further with aging. ATP levels were significantly reduced in tgAPP mice at an age of 3 months. Interestingly, cytochrome c oxidase activity was markedly reduced in tgAPP mice at an age of 1.5 and 3 months. The difference was less pronounced at an age of 6 months. In agreement with our findings in tgAPP, soluble A-beta oligomers induced mitochondrial dysfunction in brain cells from tau transgenic mice. Interestingly, this effect was only present in cortical brain cells, but not in cerebellum. Our results indicate that mitochondrial dysfunction is exacerbated by the presence of soluble A-beta species as a very early event during pathogenesis.

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Structural and Functional MRI in Mild Cognitive Impairment

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We followed 56 MCI subjects for 32 months and used VBM to investigate grey matter atrophy. 13 (23.2 %) subjects progressed to dementia. At baseline, there were no significant differences in age, education or MMSE scores between the stable (sMCI) and progressive (pMCI) MCI subjects. The CDR Sum of Boxes score and delayed verbal recall were significant predictors of conversion to dementia. VBM revealed significant atrophy in the left temporoparietal cortex, the posterior cingulate cortex and precuneus bilaterally in the pMCI subjects compared to the sMCI subjects. There was a trend for atrophy in the right hippocampus. Our results indicate that widespread cortical atrophy is present already two and a half years before a clinical diagnosis of dementia.

Structural and functional magnetic resonance imaging (fMRI) was performed on 21 healthy elderly controls, 14 subjects with MCI and 15 patients with mild AD to investigate changes in fMRI activation in relation to underlying structural atrophy analysed by VBM and hippocampal volumetry. The fMRI paradigm consisted of associative encoding of novel picture-word pairs. Compared to controls, the MCI subjects showed increased fMRI responses in the posterior hippocampal, parahippocampal and fusiform regions, while VBM revealed more atrophy in MCI in the anterior parts of the left hippocampus. Furthermore, the hippocampal volume and parahippocampal activation were negatively correlated in MCI. We suggest that the increased fMRI activation in MCI in the posterior medial temporal and closely connected fusiform regions is compensatory due to the incipient atrophy in the anterior medial temporal lobe.

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Behavioural Abnormalities Associated With Dopaminergic Treatment in Parkinson´S Disease

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Neuropsychiatric treatment-related complications in PD include the so-called dopamine dysregulation syndrome where patients develop a pattern of compulsive dopaminergic drug use, beyond the doses required to treat motor disabilities. This occurs despite the emergence of motor complications, in particular dyskinesias, and despite harmful behavioural consequences.

Predisposing factors include younger age, high dopaminergic drug intake, past drug use, depression, novelty-seeking personality traits, and alcohol intake. The ventral striatum has a central role in natural reward systems and its sensitisation by dopaminergic therapy is believed to underlie dopamine dysregulation.

Punding: This is a behavioural abnormality occasionally associated with high dopaminergic doses. This complex stereotyped behaviour is characterised by an intense fascination with repetitive activities such as manipulation of technical equipment, handling, examining, and sorting of objects, grooming, or using a computer. These activities are carried out during ON-phases, are often associated with dyskinesias, and initially are mainly present at night. They are rarely reported spontaneously. Attempts to interrupt patients typically lead to irritability and dysphoria. It is important to note that punding is distinct from both obsessive-compulsive and manic disorder.

Punding is believed to result from psychomotor stimulation in vulnerable persons, mediated by striatal structures, and an increasing inability to control automatic response mechanisms.

Enhanced reward seeking due to striatal sensitisation is believed to underlie impulse control abnormalities, such as hypersexuality, excessive gambling, or binge eating. While there is evidence of an association with dopamine agonists, no clear associations exist with particular substances and these abnormalities may also occur on L-dopa monotherapy.

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Pittsburgh Compound-B (PIB) Binds to a Range of Neuropathological Amyloid Containing Lesions in Addition to Senile Plaques

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The interaction of a tritium labelled preparation of the molecular imaging probe PIB with neuropathological amyloid lesions associated with Alzheimer's disease was investigated, at low concentrations (0.5 nM), using quantitative autoradiography of fresh frozen human brain sections.

Although, detailed histological and immunochemical analysis of adjacent sections supports previous reports that PIB is able to interact reversibly and with high affinity to senile plaques it extends these findings by demonstrating (i) additional high affinity binding to diffuse plaques, (ii) a variable, non-displaceable binding component associated with cerebrovascular amyloid beta peptide deposits and (iii) a fully displaceable binding to neurofibrillary tangles in entorhinal cortex sections.

These findings strongly suggest that PIB is not specific for senile plaques but that it can in addition bind a range of microscopic lesions containing only minimal amounts of amyloid structure. However, when employed in PET imaging studies the data suggest that the majority of the ligand binding signal is likely to be derived from amyloid beta peptide containing plaques, due to the preponderance of these deposits relative to other amyloid structures.

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Biomarkers for the Early Detection of Parkinson's and Alzheimer's Disease

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In the aging population of many countries in the world both Alzheimer's disease (AD) and Parkinson's disease (PD) are likely to lead to a great public health crisis. Therefore, early therapy and ultimately disease prevention is essential, which is only possible by an early diagnosis. Besides a genetic predisposition epidemiological risk factors seem to play a role for disease development. Based on these, a number of biomakers are being discussed to indicate a vulnerability for these neurodegenerative diseases. For AD they include cerebrospinal fluid, neuroimaging and blood biomarkers as well as subtle neuropsychological deficts and specific aspects of the medical history. For PD, a number of methods have been developed to detect an impairment of the nigrostriatal system already very early in the disease process including hyperechogenicity of the substantia nigra as well as premotor symptoms like olfactory and autonomic dysfunction, depression, REM sleep behaviour disorder, visual and neuropsychological impairment. Moreover, first signs of affection of the substantia nigra like PET and SPECT abnormalities and slight motor signs can be included, as they may be detected before a definite diagnosis can be made.

Although some of these markers are unspecific if singularly evaluated a combination of these features may indeed be valuable to detect a subgroup of the population at risk for AD and PD. However, future studies are necessary to establish the predictive value of these markers singularly and in combination not only to accelerate research on etiology and pathophysiology but also to promote testing for neuroprotective strategies.

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Cognitive Decline and Dementia in PD

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The assertion by James Parkinson that in paralysis agitans "the senses are not affected", is being challenged by observations that a substantial proportion of cases with advanced Parkinson disease (PD) develop cognitive deficits. These are heterogeneous in nature, either having Alzheimer phenomenology or with features reminiscent of "subcortical" dementia.

In PD, autopsy data suggest Alzheimer-like changes, comprising cortical amyloid deposits, temporal lobe atrophy and degenerative changes in the nucleus basalis of Meynert. Therefore it is logical to study the effect of cholinonergic enhancement on cognition in PD.

In recent years another entity has been defined, dementia with Lewy bodies (DLB). In this disorder, cognition is impaired with only minimal extrapyramidal findings (rarely without any). Pathological examination reveals widespread cortical deposition of Lewy bodies, which may also occur in the substantia nigra but only to a light extent. The cortical changes are typically accompanied by Alzheimer changes. Such changes also occur in PD patients with dementia, however these have a more extensive depletion of nigral neurons.

Although typically dementia in PD occurs late into the course of the disease, the latency is quite variable. DLB has been defined when dementia occurred prior to the appearance of parkinsonism, or within a year or two afterwards. However this division is arbitrary and lacks biological explanation or theoretical support.

Clinical manifestations of DLB, like fluctuating cognition, sensitivity to neuroleptics and cognitive improvement by treatment with cholinesterase inhibitors are common to both conditions.

Thus, DLB is a special case of Lewy body disease where widespread cortical deposition of Lewy bodies precedes severe loss of nigral neurons and is manifested by prominent cognitive changes.

Remaining questions include the factors responsible for early cortical deposition of Lewy bodies and the mechanism inducing amyloid deposition in DLB.

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Memantine Effects on Brain Volume, Glucose Metabolism and Cognition in AD Patients - A Randomized Double-Blind, Placebo-Controlled Neuroimaging Study

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Objective: The neuroprotective effect of memantine is well established in preclinical studies. This is the first neuroimaging study evaluating the potential benefits of memantine on brain morphology and metabolism in patients with moderate to severe Alzheimer's disease (AD) over one year.

Methods: Double-blind, placebo-controlled, monocentre pilot study. MRI volumetry (volume loss of whole brain and hippocampus) was done by the automated SIENA method. Metabolic activity (FDG-PET (glucose utilization) and magnetic resonance spectroscopy (N-acetyl-aspartate, NAA, and myoinositol, MI)) was assessed globally and regionally. Clinical outcome measures were ADAS-cog, ADCS-ADL and CDR.

Results: Of 37 randomized patients (mean baseline MMSE 19,3 for placebo and 18,7 for memantine group) , 32 completed the imaging evaluation after six months and 24 after twelve months. Results were non-significant due to the small sample size of this pilot study. After one year, the memantine group showed substantially less hippocampal volume loss on MRI compared with placebo whereas no difference was detected for total brain volume. PET demonstrated less decrease of cerebral glucose metabolism in all investigated brain areas of the memantine group. The intra-individual variability of metabolite levels on MRS was large and concentrations of NAA and MI did not differ between treatments. Memantine treated patients showed a numerical advantage over placebo on clinical outcome variables.

Conclusions: Clinical effects of the study were consistent with previous results. Smaller loss of hippocampal volume in patients treated with memantine is in line with the suggested

neuroprotective effect of memantine. Reduced decrease in glucose metabolism indicates beneficial functional effects.

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The Co-Existence of Two Amyloid Subunits (Adan and A-Beta) in Familial Danish Dementia. Dysregulation of APP by ADAN Precursor.

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Classic arguments sustaining the importance of amyloid in the pathogenesis of cerebral amyloidosis are usually centered in Aβ and its role in neuronal loss in Alzheimer's disease (AD). Two early-onset neurodegenerative conditions, familial British and Danish dementias (FBD and FDD), show extensive pre-amyloid and amyloid deposits, severe congophilic angiopathy and neurofibrillary tangle pathology in limbic areas, closely resembling AD. Genetic defects at or near the stop codon of the 13q14.3 BRI2 gene result in elongated BRI2 precursors containing C-terminal segments not previously seen in the human transcriptome. The newly created amyloid peptides ABri (in FBD) and ADan (in FDD) both differ from Aβ in length and primary structure and yet all share a great propensity to oligomerize and form fibrils in vitro, suggestive of common pathogenic pathways. ABri and ADan emphasize the importance of amyloid as a causative element in the process of neurodegeneration allowing to test the hypothesis that unrelated peptides could adopt similar altered amyloidogenic configurations and trigger comparable downstream detrimental effects in neuronal cells. Moreover, AβX-42 co-localizes with ADan (~1:10 ratio) largely in vascular fibrillar deposits in FDD. Studies in primary FDD fibroblasts show dysregulation of APP processing co-existing with low levels of plasma Aβ40 and Aβ42, a process likely related to the APP-ADan precursor protein interaction in plasma membranes. The recently developed transgenic mice for both BRI2 genetic defects may be ideal models to study the influence of preamyloid lesions and CAA in neuronal cell death and further assess the regulation of APP processing by secretases.

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Physiopathology Linking Stroke and Heart Disease to Alzheimer Disease.

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Background: Heart disease and stroke are two of the major leading causes of death and disability in the world. Mainly affecting the elderly population, heart disease and stroke are important risk factors for Alzheimer's disease (AD). There is mounting evidence linking the development of AD to the presence of chronic brain hypoperfusion (CBH) and its involvement in heart disease and stroke. That evidence indicates a strong association between such risk factors as coronary artery bypass surgery (CABG), atrial fibrillation, aortic/mitral valve damage, hypertension, hypotension, congestive heart failure, cerebrovascular-carotid atherosclerosis, and transient ischemic attacks in promoting CBH.

Methods: In people whose cerebral perfusion is already declining by their advanced age, further cerebral blood flow reductions from heart-brain vascular-related risk factors, increases the probability of AD. A fundamental principle in cell biology involves the use of chemical energy in the form of ATP to assemble, disassemble and alter protein structure.

Results: Our findings and those of others indicate that CBH resulting from stroke or heart disease triggers a neuronal energy crisis in ischemic-sensitive brain regions leading to a cascade of intracellular molecular abnormalities affecting (among other things) protein synthesis, cleavage and folding. These molecular changes eventually result in neurodegenerative lesions such as the deposition of excess amyloid-beta and the formation of neurofibrillary tangles.

Conclusions: Knowledge of how heart disease and stroke can progress to AD may target more precise therapy in preventing, arresting or reversing this dementia.

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Evidence of a Transient Increase in Cerebral Amyloid Angiopathy After Abeta42 Immunization in Human Alzheimer's Disease

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Background and Aim: In Alzheimer's disease (AD) Aβ accumulates as plaques in the cerebral cortex (mainly Aβ42) and in blood vessel walls as cerebral amyloid angiopathy (CAA, mainly Aβ40). Animal and human studies show that the Aβ plaque burden can be reduced by immunotherapy. We hypothesized that plaque Aβ is solubilized following immunization and drains via the perivascular pathway, detectable as an increase in cerebrovascular Aβ.

Methods: We are performing a clinical and neuropathological follow up of patients who were enrolled in the initial Elan Pharmaceuticals trial of Aβ42 immunization in AD. To date we have neuropathology on 9 patients who died between 4 months and 5 years after first immunization dose and show variable degrees of plaque clearance. Immunostaining for Aβ40 and 42 was quantified and compared with unimmunized AD patients.

Results: The cerebrovascular Aβ load was substantially higher in most immunized patients who had evidence of plaque removal. There was an increase in the density of cortical vessels containing Aβ42 and a corresponding increase in Aβ40. However two of the longest survivors, 4-5 years after first immunization, had virtually complete absence of both plaques and CAA, raising the possibility that, given time, Aβ is cleared from the cerebral vasculature.

Conclusion: The findings are consistent with the hypothesis that Aβ immunization results in solubilisation of plaque Aβ42 which, at least in part, exits the brain via the perivascular pathway.

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The BACE-1 Inhibitor GSK188909 Decreases Beta-Cleavage of APP and Abeta Production in Vivo

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The development of drugs with potential for disease modification is pivotal for the effective treatment of Alzheimer's Disease (AD). One of the many strategies being investigated is to eliminate the formation of Abeta that deposits as senile plaques in the brains of AD patients and is thought to play a central role in AD pathogenesis. Abeta is generated physiologically following sequential processing of Amyloid Precursor Protein (APP) by beta-site APP cleaving enzyme (BACE-1) and gamma-secretase. Consequently, inhibition of BACE-1, a rate-limiting enzyme in the production of Abeta, is an attractive therapeutic approach for the treatment of Alzheimer's disease.

Here we describe a selective non-peptidic BACE-1 inhibitor, GSK188909, that potently inhibits beta-cleavage of APP and Abeta production in cells. When administered acutely to TASTPM mice in the presence of an inhibitor of the drug transporter P-glycoprotein to increase the transport of GSK188909 across the blood brain barrier, significant lowering of brain Abeta40 and Abeta42 is observed.

We are now utilising GSK188909 in conjunction with the P-glycoprotein inhibitor to further explore the relationship between brain exposure of GSK188909, BACE-1 inhibition and Abeta lowering in the brain. This should allow us to elucidate the degree of BACE-1 inhibition that is required in the brain to demonstrate efficacy.

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Long-Term Treatment With CHF5074, a Novel Flurbiprofen Analogue, Markedly Reduces Brain Beta-Amyloid in Tg2576 Transgenic Mice

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Background and Aims: A subset of NSAIDs, including flurbiprofen, has been shown to selectively reduce the production of beta-amyloid42 (Abeta42), independently from their cyclooxygenase (COX) inhibiting activity. CHF5074 is a new flurbiprofen analogue devoid of anti-COX activity and with potent in vitro inhibitory activity on Abeta42 secretion. We evaluated the effects of long-term treatment with CHF5074 on brain Abeta pathology in transgenic mice (Tg2576) carrying the double Swedish mutation of APP.

Methods: 10-month old Tg2576 mice were treated with CHF5074 (375 ppm in the diet) for 17 weeks. Control animals received standard diet. Brain Abeta40 and Abeta42 were sequentially extracted with SDS (2%) and formic acid (70%) and measured with a commercial ELISA kit. Brain Abeta plaque load was assayed using standard immunohistochemistry.

Results: Long-term exposure to CHF5074 was well tolerated by Tg2576 mice (no deaths, normal body weight gain and no histopathological findings in peripheral organs). The estimated assumed dose of 62 mg/kg/day produced mean (± SEM) plasma concentrations of 217 ± 10 microM. CHF5074 brain levels were 3.0 ± 0.2% of the corresponding plasma concentrations. Compared to controls, CHF5074 treatment resulted in a marked reduction in both Abeta40 (-50 ± 9%, $p = 0.009$) and Abeta42 (-45 ± 10%, $p = 0.022$) brain levels. Brain histopathological evaluations, including amyloid plaque load, are underway.

Conclusions: Chronic CHF5074 treatment significantly reduced brain Abeta levels in Tg2576 transgenic mice. This novel flurbiprofen analogue has, therefore, the potential to be a safe and promising therapeutic agent for AD treatment.

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Novel Bi-Aromatic Compounds as Anti-Amyloidogenic Agents in Alzheimer's Disease

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Background and Aims: Protein misfolding and aggregation are critical steps in the pathogenesis of a number of neurodegenerative diseases. In many cases, self-assembly of two or more proteins is implicated in these diseases, including Alzheimer's disease, in which aggregation of amyloid-β (Aβ), tau and α-synuclein may all contribute to neurotoxicity. Inhibiting aberrant folding and assembly of one or more of these species is thus of great therapeutic interest.

Methods and Results: We identified a novel class of bi-aromatic compounds that potently inhibit the aggregation of Aβ, tau and α-synuclein in Thioflavin T (ThT) and Thioflavin S (ThS) dye-binding fluorescence assays. The aggregation of both major physiological isoforms of Aβ, i.e. Aβ40 and Aβ42, was inhibited and compounds also caused disassembly of pre-formed aggregates. Further experiments confirmed the anti-amyloidogenic activity of the compounds against Aβ40; circular dichroism studies showed the compounds inhibited the random coil to β-sheet conversion, while Aβ40 binding was studied in proton NMR experiments. Given their activity against three different amyloidogenic proteins, the novel bi-aromatic species are thought to bind to the cross-β-sheet structure common to all, with binding leading to inhibition of aggregate assembly.

Conclusions: The bi-aromatic compounds identified here have the ability to act against three different targets in AD, i.e. Aβ, tau and α-synuclein. Binding to and modulating the aggregation of multiple proteins, each of whose self-assembly contributes to disease pathogenesis, may yield benefits as compared to modulating the proteins individually.

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Effects of Long Term Treatment With Y-Secretase Inhibitor LY-411,575 During Initial Seeding Phases of Amyloid Deposition in Tg2576 Mice

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Background/Aims: y-secretase inhibition (GSI) is a potential therapeutic target of Alzheimer's disease (AD). However, the utility of GSIs could be limited due to inhibition of y-secretase regulated signaling events mediated by other substrates, especially signaling events mediated by Notch. To this end, we asked whether decrease in Ab production during the initial seeding phase of Ab deposition can significantly shift the subsequent temporal accumulation of Ab. Methods: To test this, we administered low tolerable doses of the GSI LY-411, 575 (formulated in mouse chow to deliver ~2.5mg/kg/day) to 2 groups of Tg2576 mice as follows: drug dosing from 4-7 months of age and from 7-10 months of age. Mice were then sacrificed at 12 and 15 months of age and a thorough evaluation Ab deposition and associated pathologies was then conducted. Results: No severe toxic effects were seen using this long term dosing regimen, except for a consistent change in mouse fur coloration and possible skin cell hyperplasia. In the Tg2576 mice dosed from 4-7 months with LY-411, 575 and then sacrificed at 12 months of age, no significant changes in biochemically extracted Abeta levels were seen compared to controls. However, in the GSI treated mice, the numbers of deposited immunostained Abeta plaques were reduced. Interestingly, the deposited plaques in GSI treated mice were relatively larger in size compared to plaques in controls mice. Conclusions: This would indicate that some Abeta plaques continue to accumulate and grow even in the presence of GSI using this dosing regime in Tg2576 mice.

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Intranasal Administration of NAP Results in Functional Enhancement and Reduction of Tau Hyperphosphorylation in Models of Alzheimer Disease

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Background: Activity-dependent neuroprotective protein (ADNP), a protein essential for brain formation, contains a neuroprotective peptide, NAP (NAPVSIPQ), (Bassan et al., J Neurochem. 1999; 72:1283; Gozes et al., CNS Drug Rev. 2005; 11:353; Pinhasov et al., Brain Research 2003; 144: 83). ADNP-like immunoreactivity decorates microtubules (Furman et al., Neuron Glia Biology 2004; 1:193) and NAP stimulates microtubule assembly and reduces tau hyperphosphorylation in vitro (Gozes & Divinski, J Alzheimer's Dis. 2004;6(6 Suppl):S37;Divinski et al., J Biol Chem 2004; 279:28531. J Neurochem 2006; 98:973). Hypothesis/aim: It is hypothesized that NAP acts, to a large extent, through a reduction in tau phosphorylation with resultant effects on microtubule stabilization and functional protection.

Methods/Results and Conclusion: In vivo studies showed that in ADNP-deficient mice there is increased tau phosphorylation that can be decreased by chronic NAP treatment. In a triple transgenic mice model of Alzheimer's disease, NAP reduced tau hyperphosphorylation. In a tauopathy model with double mutant tau (Rosenmann et al., in press) NAP protected against behavioral/cognitive impairments. Based on these preclinical findings, Allon Therapeutics Inc., a Vancouver based company is conducting clinical trials on NAP as a potential neuroprotective drug candidate.

Support: BSF, ISF, Gildor Chair, Elton (Elbaum) Laboratory, NIA, NICHD intramural, Allon Therapeutics Inc. I Gozes serves as the Chief Scientific Officer of Allon Therapeutics Inc.

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Angiotensin Converting Enzyme (ACE) Inhibitors in the Cognitive Performance of People With Mild Cognitive Impairment

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Background: Cross-sectional and clinical trial data suggest reduced incidence of, or slowed cognitive decline in, Alzheimer's disease (AD) amongst people taking Angiotensin Converting Enzyme (ACE) Inhibitors (ACE-Is).(1,2) Supportive evidence has also been produced that ACE-Is are protective against cognitive deterioration after 12month follow-up in individuals who met operational criteria for amnestic Mild Cognitively impairment (MCI).(3)

Aims: In an existing cohort of MCI patients (N=305) collected as part of a longitudinal study for the Development of Screening guidelines and diagnostic Criteria for Predementia Alzheimer's disease (DESCRIPA) we sought to test whether ACE-Is (i) improved cognitive performance and (ii) delayed conversion of MCI to AD after 12 month follow-up.

Results: Users of ACE inhibitors showed 1.94 points less decline on the MMSE at 1-year follow-up after correction for age, gender, education, baseline MMSE score, and systolic and diastolic blood pressure (p=0.053). This effect was not observed for other anti-hypertensive drugs (p>0.14) except for angiotensin antagonists (p=0.007). Neither ACE inhibitors nor any other antihypertensive drug were associated with conversion to AD at follow-up. ACE inhibitors tended to be associated with lower risk for AD or memory decline without dementia (OR=0.57, p=0.13) but not for other antihypertensive drugs (p>0.45). Cross-sectional analysis revealed that ACE users showed less atrophy of the medial temporal lobe (p=0.058). A similar effect was observed for diuretics (p=0.069) but not for other anti-hypertensive drugs.

Conclusions: ACE-Inhibitors and Angiotensin blockers may offer benefit against the rate of cognitive decline in people with Mild Cognitive Impairment

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Disease Modification Trials: Methodology and Regulations

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Background And Aims: Neurodegenerative disorders are characterized by a progressive course that determines increasing levels of disability. Symptomatic treatments which are defined as interventions able to control partially or totally the manifestations of the disease concurrent with their administration are considered insufficient to achieve a meaningful reduction in disability at least in some diseases as Alzheimer disease. Interested parties aim for the development o disease modifying interventions. In this lecture we plan to discuss the clinical trials design features that are paramount to the demonstration of a disease modifying effect taking in account the regulatory requirements worldwide.

Methods: The lecture will be based on a critical analysis of different trials designs that have been proposed in the field including delayed start, randomized withdrawal, classical parallel controlled trials, group sequential trials among others. The mechanistic approach which attempts to demonstrate a physiopathological effect will be confronted with the consequentialistic approach focused on relevant clinical outcomes that reflect actual disability. In addition the role of futility trials will be commented.

Results And Conclusions: In 2007, given the absence of validated surrogate endpoints for any neurodegenerative disorder, clinical trials must be based in clinical endpoints. We will present a parallel, placebo controlled, group sequential design as the ideal model for trial aiming to demonstrate disease modification in a neurodegenerative background.

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Disease Modification Trials: Methodology and Regulations

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The most exciting recent development in the Alzheimer's disease (AD) field concerns the transition from studying symptomatic treatments to the current focus on the development and clinical testing of compounds that target primary pathophysiology and risk factors. These new treatments have the potential, if proven clinically safe and effective, to slow the progression of AD, and ultimately to enable primary prevention. However, while it is gratifying that a growing number of putative disease modifying agents are already being evaluated in clinical trials, standards for designing such trials and measuring outcome are not fully established, and there are no regulatory guidelines to help determine whether there is adequate evidence to warrant drug approval with a disease modification claim. This presentation will review the current state of the art for trial design and measuring outcome in "disease progression" trials, with particular reference to the implementation of these designs and outcome measures in ongoing trials. The discussion will include the potential role of biological surrogates in supporting a disease modification claim. Current "rules of evidence" for interpreting the results of these trials will be examined in relation to potential regulatory guidelines in the U.S. and the E.U.

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Longitudinal MR Imaging and CSF Biomarkers Predict Decline to AD

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Hippocampal formation imaging is useful for recognizing the early features of Alzheimer's disease (AD) at the mild cognitive impairment (MCI) stage. However, conventional MR hippocampal imaging is limited because of costs, available quantitative expertise, and absent specificity for AD. In a 2-year longitudinal study of MCI and control subjects, we examined the hypothesis that CSF markers for tau, A-Beta, and the lipid peroxidation product [8,12-isoiPF2α-VI, isoprostane] (IP) would individually predict the future decline to AD. We also hypothesized that by combining MR imaging with CSF sampling we would improve the early diagnosis of subjects at risk for AD. Moreover, we examined for the first time, whether over three MR and CSF observations there were longitudinal relationships between elevated IP levels (a presumed early feature of neurodegeneration) and MR gray matter loss of the medial temporal lobe. Two complete clinical observations with MR and LP were performed on 22 MCI patients that declined to AD, 43 stable MCI patients, and 21 normal controls. The declining MCI patients were correctly classified by: elevated CSF levels of P-tau231 (83%), total tau (77%), and IP (74%), and reduce A-Beta42 (71%). For 15 MCI patients and controls studied 3 times, the longitudinal elevation in IP was related to and incremented the longitudinal hippocampal gray matter prediction of decline from 88 to 100%. These results demonstrate that MR and CSF biological markers provide diagnostic information for MCI patients at risk for future AD.

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MRI of Alzheimer's and Parkinson's: The Alzheimer's Disease Neuroimaging Initiative (ADNI-Info.Org)

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The goals of ADNI are to develop improved methods and uniform standards for acquiring longitudinal multisite MRI, PET, blood and CSF biomarker data, and to identify and validate biomarkers with high power to distinguish treatment effects. Methods are implemented at 55 sites, and 560 subjects have been entered. AD (n=200), MCI (n=400) and 200 controls will have clinical/cognitive assessments and 1.5 T MRI every 6 months for 2-3 years; 50% will have FDG PET scans, 25% will have 3T MRI. All scans and clinical data are at loni.ucla.edu/ADNI. Blood, urine and CSF samples are stored for later analysis.

In a separate study aimed at predicting cognitive decline, we enrolled 150 non demented elderly subjects with memory complaints or impairments. Atrophy of the entorhinal cortex and reduced cerebral blood flow in the posterior cingulate predicts cognitive decline. Using a 4 Tesla MRI, DTI shows reduced fractional anisotrophy of the cingulum, in MCI and AD. Quantification of hippocampal subfields shows reduced subiculm and entorhinal cortex in AD. Finally, susceptibility-weighted imaging shows signal loss in the substantia nigra in 8 patients with Parkinson's disease and in the striatum in 5 patients with MSA compared to 22 controls. There is also hypoperfusion of premotor regions in PD. The pattern of imaging changes in PD and MSA greatly differs from AD.

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Cell Therapy in Parkinson's Disease

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Clinical studies using transplantation of fetal dopaminergic (DA) cells into brains of Parkinson's disease patients have provided proof of principle that implanted immature, but well-defined post-mitotic neurons can restore function even in a progressive age-dependent neurological disease. A biotechnological and large-scale medical application of this methodology could be achieved by obtaining similar cells derived from human embryonic stem (hES) cells, or perhaps even by stimulating endogenous adult stem cells. However, while several hES cell differentiation protocols have been developed for generating DA neurons, the production of sufficient amounts of the "right" therapeutic DA cell has not yet been accomplished. To achieve this goal, specific criteria have to be fulfilled to produce therapeutically useful DA cells, that include a clinical understanding of what is sufficient cell survival, accurate integration in the brain circuitry to achieve normal function in the absence of tumor formation or immunogenicity, in patients. The biological insights and methods to generate therapeutically relevant cells largely depend on understanding the opportunities arising from normal development, while the medical limitations appear to be defined by the capacity to accomplish physiological integration of the new cells in the patients.

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Adult-Derived Neural Stem Cell Grafting for the Treatment of Parkinson's Disease and Cerebral Ischemia

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Due to the development of stem cell biology, several types of stem cells are expected to be used for the treatment of neurological disorders such as Parkinson's disease and cerebral ischemia. Among them, adult-derived neural stem cells (NSCs) have an advantage that they can be used as autologous donor cells. From the viewpoint of application for regenerative medicine, adult-derived NSCs have similar characteristics as fetus-derived NSCs and the efficacy of intracerebral grafting using adult-derived NSCs have been reported.

We have been using adult-derived NSCs dissected from subventricular zone of rats. We inserted glial cell line-derived neurotrophic factor (GDNF) gene into these cells using adenovirus and obtained GDNF secreting NSCs. When these cells were grafted into the striatum of hemiparkinsonian model rats, protection and repair of nigrostriatal dopaminergic system and behavioral recovery demonstrated by drug-induced rotation were observed. The level of recovery was similar to that observed when fetus-derived NSCs were used as donor cells.

We also used GDNF-secreting adult-derived NSCs for the treatment of cerebral ischemia in rats. Three hours after unilateral middle cerebral artery occlusion, these cells were grafted into ipsilateral penumbra region. The results demonstrated that GDNF-secreting adult-derived NSCs migrated longer distance than conventional adult-derived NSCs and decreased the size of cerebral infarction shown by TTC staining.

In this presentation, merits and issues related to adult-derived NSCs as donor cells for intracerebral grafting will be discussed.

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APP Alters Stem Cell Biology

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We found that human neural stem cells (HNSCs) exposed to high concentrations of amyloid-precursor protein (APP) or transplanted into APP transgenic mice (APP23) primarily differentiated into astrocytes, suggesting that pathological alterations of APP processing in Alzheimer's disease (AD) may prevent neuronal differentiation of HNSCs.

APP induced glial differentiation of neuronal progenitor cells with inductions of CNTF, gp130 and JAK1 gene expressions, and STAT3 phosphorylation, while silencing of these genes by RNA interference suppressed the glial differentiation of the cells, indicating involvement of IL-6/gp130 pathway in the APP function. APP interacted with notch and increased the generation of notch intracellular

domain and gene expression of Hes1, indicating glial differentiation of the neuronal progenitor cells may be also mediated by the physical interaction between APP and notch. Thus, APP may regulate HNSC differentiation through activation of both IL-6/gp130 and notch signaling pathway. Although importance of adult neurogenesis is not clear, premature glial differentiation of HNSCs may harm maintenance of normal brain function and may contribute to the pathophysiology of AD. Also, successful neuroplacement therapy for AD may depend on a management of APP level in the optimal range for neuronal differentiation of HNSCs.

We found treatment with (+)-phenserine, reduced APP protein and active astrocytes in the brain of APP23 mice. HNSCs transplanted into the (+)-phenserine treated APP23 mice migrated and differentiated into neurons. These results indicate that regulation of APP level may prevent astrocytosis under AD pathology and may be essential for the stem cell therapy for this disease.

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Gene Transfer Can Provide Sustained, Targeted Delivery of Neurotrophic Factors to Treat Neurodegenerative Diseases Such as PD and AD

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Background: Neurotrophic factors have the capacity to restore function and prevent further neurodegeneration of dying neurons. They therefore possess the capacity to reduce symptoms while also halting or reversing disease progression. Despite their potential, targeted and sustained delivery of these proteins has proven to be an insurmountable problem. Recently, gene transfer has emerged as an effective and practical solution.

Methods: Ceregene has developed the technology to deliver the genes for neurotrophic factors in a safe and effective manner, including neurturin (NTN) for PD and NGF for AD.

Results: A series of nonclinical studies established that AAV-NTN (CERE-120) can express NTN in a predictable and controlled fashion, can provide trophic support in a variety of animal models of PD and is safe and well tolerated at dose levels hundreds of times higher than those required for efficacy. An open label trial in PD subjects (two dose levels with 6 subjects per dose) indicated that CERE-120 is safe and well tolerated; no serious CERE-120 AEs related were noted. Moreover, a significant reduction in PD symptoms was noted over a period of 12 months, reflected by a 40% reduction in UPDRS-motor off scores, a 2-fold increase in time 'on without dyskinesias', a 50% reduction in 'total off time', and significant improvements in timed motor scores. A multi-center double-blinded trial of is ongoing. Following similarly encouraging results using CERE-110 (AAV-NGF) to support basal forebrain cholinergic neurons in AD subjects, a multi-center controlled study is also planned testing AAV-NGF for AD.

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Moderate and Repetitive Electrical Stimulations of the Sphenopalatine Ganglion Enhance Cerebral Blood Flow and Improve Functional Recovery Following Ischemic Stroke

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Ischemic models generating moderate cerebral hypoperfusion have been instrumental in evaluating the causal relationship between reduced cerebral blood flow (CBF), microvascular pathology and cognitive performance and memory capacity. Reduced CBF has been shown in Alzheimer's disease (AD) patients and cerebral infarcts are the underlying mechanism for vascular dementia. We have hypothesized that a moderate augmentation of cerebral blood circulation may be of potential therapeutic value to enhance perfusion and reduce damage. To examine this hypothesis we took advantage of the ability of the parasympathetic fibers arising from the sphenopalatine ganglion (SPG) to stimulate the cerebral vasculature by mechanisms that possibly involve neurotransmitters release such as NO and VIP. Adult male SPD rats subjected to either permanent or transient middle cerebral artery occlusion (MCAo) and implanted with a designated electrode, were given various electrical pulse regimens at timed intervals to stimulate SPG in order to characterize conditions for a moderate rise of the cerebral blood flow (CBF). Daily trains of electrical stimulations were carried out for at least one week or longer and neurological and neurobehavioral scores and infarct size evaluated. Results indicate that certain trains of electrical stimulation initiated 24h after MCAo significantly improved functional recovery already after one week and over. SPG stimulation in acute stroke is now being tested in a multi-center clinical trial. This therapeutic approach can be of future use for dementia patients through both improvement of CBF and prevention of ischemic events.

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Characterization of Novel Genes Involved in Learning and Memory in Rodent Models

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Aging is often associated with decline of numerous cognitive processes, especially learning and memory. As in humans, individual differences in aged animals can naturally lead to memory impairments. This study is among the first genome wide analysis of genes involved in long-term memory formation that are differentially expressed between aged memory-impaired (AI) and aged memory-unimpaired (AU) animals following stimulation in a spatial memory task. Learning impairments in young adult rats following exposure multiple exposures to scopolamine, a muscarinic receptor blocker, was also studied for comparison. We found that

alterations in hippocampal gene expression of transthyretin (TTR), calcineurin, and NAD(P)H dehydrogenase quinone 2 (NQO2) several hours after training were associated with memory deficits in aged animals. Decrease in protein levels of C/EBP, a transcription factor regulating TTR and NQO2 expression, was observed in AI animals. Memory deficits were also found during aging in mice lacking TTR, a retinol transporter known to prevent amyloid-? peptide aggregation and senile plaque formation as seen in Alzheimer's disease. Treatment with retinoic acid (RA) reversed cognitive deficits in these knock-out mice as well as in aged rats. The expression of various genes was also altered in the scopolamine-induced amnesia model including homer 1a and GABAb receptors. Taken together, our study provides genetic, behavioural and molecular evidence that TTR is involved in the maintenance of normal cognitive processes during aging by acting on the fine regulation of brain RA availability and signalling pathways. Moreover, abnormal balance between gabaergic and glutamatergic neurotransmission is likely involved in learning deficits induced by sustained muscarinic receptor blockade. Supported by a grant from the Canadian Institutes of Health Research.

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Heparan Sulphate Proteoglycans Affect the Biological Activity of Amyloid Beta Protein

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Background. Amyloid beta (Aβ) deposits in Alzheimer's disease brains, i.e. senile plaques and vascular amyloid, also contain heparan sulphate proteoglycans (HSPGs). HSPGs may play a role in the deposition, aggregation or clearance of Aβ. However, it is not known how HSPG / glycosaminoglycans (GAG) expression relates to Aβ expression and how HSPGs/GAGs affect biological activity of Aβ.

Objectives. 1) to study in vitro (in cultured human brain pericytes) the expression of HSPGs in response to Aβ; 2) to study in vitro the role of GAGs in Aβ-mediated cytotoxicity.

Methods. Cultured human brain pericytes and smooth muscle cells were incubated with Aβ and GAGs to study their effects on either HSPG protein (Western blot) and mRNA levels (Taqman assay) or Aβ-induced cytotoxicity.

Results. Agrin and glypican-1 expression by pericytes was upregulated after treatment with Aβ. Furthermore, the degree of glycosylation of these HSPGs was increased after Aβ treatment. These findings were confirmed at the mRNA level by quantitative PCR. Furthermore, several GAGs (heparan sulphate, heparin, dermatan sulphate, keratan sulphate, chondroitin sulphate) dose-dependently inhibited Aβ-mediated cytotoxicity in both pericytes and smooth muscle cells. Finally, we observed that the inhibitory effect was strongly dependent on the degree of sulphation of the GAGs.

Conclusions. GAGs may modulate the biological activity of Aβ and this capacity is directly regulated by the degree of negatively charged sulphate groups present in these GAGs. Furthermore, our data suggest that Aβ induces HSPG expression and glycosylation which may explain the association of HSPGs with Aβ deposits in Alzheimer brains.

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The Cyclin Dependent Kinase 5 Inhibitor (Cip & P5) Reduces a 1-42 and P25/Cdk5-Mediated Tau Hyperphosphorylation and Apoptosis in Neurons

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The extracellular aggregation of amyloid peptides and the intracellular hyperphosphorylation of tau and neurofilament proteins at specific epitopes are pathological hallmarks of neurodegenerative diseases such as Alzheimer's disease (AD) and Amyotrophic Lateral Sclerosis (ALS). Cdk5 is a member of the Cdk family of serine/threonine kinases, most of which are key regulators of the cell cycle. Unlike mitotic Cdks, Cdk5 plays a critical role in brain development, neuronal migration, neurite outgrowth and axon patterning but has no known role in the mitotic cell cycle. Cdk5 activity is regulated through association with its neuron-specific activators, p35 and p39. The deregulation of Cdk5 phosphorylates tau at AD-specific phospho-epitopes when it associates with p25. p25 is a truncated activator, which is produced from the physiological Cdk5 activator, p35, upon exposure to amyloid peptides (A) and calpain activation. We have found that the Cdk5 Inhibitory Peptide (CIP), a 125-residue and a smaller 24 amino acids peptide (P5) derived from p35. have a much higher affinity for Cdk5 than does p25. CIP and P5 effectively and specifically inhibit the activity of Cdk5 in vitro and in siitu. We show that neuronal infections with Cdk5 inhibitory peptide CIP, selectively inhibits p25/Cdk5 activity and suppresses the aberrant tau phosphorylation in cortical neurons. Furthermore, A -induced apoptosis of these cortical neurons is also reduced by co-infection with CIP. Most importantly, our results showed that CIP and P5 inhibition is highly specific: they inhibit the neurotoxic effects produced by p25/Cdk5 complex but did not alter the 'normal' role of p35/Cdk5 complex, which is essential for brain development and survival. Accordingly, we suggest that utilization of the CIP or P5 peptide, or molecules with similar inhibitory properties, could lead to useful agents to ameliorate some of the neuronal pathology produced by p25/Cdk5. This raises the intriguing possibility that such agents might be therapeutic for AD and other neurodegenerative diseases, which exhibit abnormal phosphorylation of neuronal cytoskeletal proteins by p25/Cdk5.

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Accumulation of Reelin in Oligomeric Abeta-Plaques During Aging is Accompanied by Neuronal Loss and Cognitive Impairments: Relevance to Late-Onset AD

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Background: Transgenic animals expressing AD-causing mutations are widely used to investigate the pathophysiological mechanisms of this severe neurodegenerative disorder. However, the majority of AD patients are sporadic late-onset cases with largely unknown aetiology. Here, we tested the hypothesis that alterations in the Reelin signaling cascade are a common phenomenon of aging and involved in neurodegenerative processes of late-onset AD.

Methods: We evaluated brain tissue obtained from behaviorally characterized and naïve wild-type mice, rats, and marmoset monkeys, as well as mice exposed to a prenatal immune challenge and triple-transgenic AD mice using immunohistochemistry and unbiased stereology to assess alterations in Reelin expression during normal and pathological forms of aging.

Results: We report that aging in wild-type rodents and primates is accompanied by accumulations of Reelin-enriched oligomeric proteinous aggregates in the hippocampal formation that are related to the loss of Reelin-expressing neurons. Both phenomena are associated with age-related episodic-like memory impairments in wild-type mice. We provide evidence that normal aging involves loss of Reelin neurons, reduced production and elimination of the extracellular deposits, whereas a prenatal immune challenge or the expression of AD-causing gene products, both result in earlier, higher, and more persistent levels of Reelin-positive plaques.

Conclusions: Our findings suggest that elevated Reelin plaque load represent a major risk factor for late-onset AD and highlight the possibility to investigate mechanisms and signaling pathways underlying age-related neurodegeneration and pathophysiological processes involved in the more common form of AD in wild-type animals.

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Prevention of Tau Aggregation in Vitro and in Cells by Low Mw Compounds

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Tau protein forms anomalous aggregates ("paired helical filaments") in Alzheimer's disease and other brain diseases ("tauopathies"). This is considered to be a possible cause of neurodegeneration. We are therefore searching for compounds which are capable to inhibit and reverse the aggregation of tau protein. The search involves screening of libraries of chemical compounds for their effects on the assembly and disassembly of paired helical filaments (characterized by the assembly-inhibition and disassembly-inducing half-maximal concentrations IC50 and DC50). Previous screens have revealed several active compounds which fall into different chemical groups. From these results we have now developed a new lead compound by defining the core structure of a chemical group and then modifying it. The derivatives were synthesized and tested for their activities of inhibiting PHF assembly or inducing PHF disassembly. About 30% of the tested compounds showed an improvement of the inhibitory potential. The results were used to build up structure-activity relationships and to optimize the reagents with regard to PHF inhibition and cell viability. In addition we generated a cell model of tau pathology which is based on the inducible aggregation of tau protein. This allows one to test whether compounds which inhibit tau polymerisation in vitro are also beneficial for cells. The results show that cellular "tau amyloidosis" can be reduced by inhibitor compounds and that the viability of the neurons can be improved. - Supported by DFG and ISOA.

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Natural Oligomers of the Alzheimer's Amyloid-Beta Protein Induce Reversible Synapse Loss by Activation of An NMDAR Dependent Signaling Pathway

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The onset and progression of Alzheimer's disease (AD) is associated with a decline in synapse density in hippocampus and neocortex, and synaptic loss is the strongest anatomical correlate of the degree of clinical impairment. Although considerable evidence supports a causal role for the amyloid-beta protein (Abeta) in AD, a direct link between a specific form of Abeta and synaptic loss has not been established. Here, we demonstrate that physiological concentrations of naturally secreted Abeta dimers and trimers, but not monomers, induce progressive loss of hippocampal synapses. Pyramidal neurons in organotypic rat hippocampal slices had markedly decreased dendritic spine density and numbers of electrophysiologically active synapses when cultured in the presence of picomolar levels of soluble oligomers of human Abeta. Spine loss was reversible upon washout of the oligomers and was prevented by an Abeta-specific antibody or a small-molecule modulator of Abeta aggregation. Furthermore, Abeta-mediated spine loss required NMDA-type glutamate receptors through a pathway involving cofilin and calcineurin signalling. Our results demonstrate that soluble, low-n oligomers of human Abeta directly trigger hippocampal synapse loss, providing a quantitative cellular model for further elucidating the molecular mechanism of Abeta-mediated neuronal dysfunction.

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Cortical Cytochrome Oxidase Inhibition Decreases Hippocampal Cholinergic Transmission, Increases Neural Plasticity and Impairs Memory in Rats: Prevention by Ladostigil

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Background. A reduction of 25-35% in cerebral cortical cytochrome oxidase (COx) activity is found in Alzheimer's disease (AD) which probably contributes to the neuronal dysfunction. Aims. To replicate this reduction in cortical COx activity in the rat; to determine its effect on memory and hippocampal cholinergic transmission and to see if could be prevented by ladostigil, a cholinesterase inhibitor. Methods. Sodium azide (NaN3) was delivered to male rats via Alzet minipumps for 4 weeks at the rate of 1 mg/kg/hr. Results. Impairment in spatial and episodic memory was seen four weeks after initiation of NaN3 administration, without any signs of cell damage or reduction in cortical or hippocampal neurons. A selective reduction of ChAT immunoreactivity was found in the diagonal band, a major source of cholinergic input to the hippocampus and cingulate cortex, in association with a significant increase in VAChT-immunoreactive varicosities and GAP 43 immunoreactivity in the same layers of the dentate gyrus. Three months after cessation of NaN3, COx and ChAT activity returned to control levels but VAChT and GAP 43 immunoreactivity increased further but the memory deficits remained. Chronic treatment with ladostigil prevented the decrease in ChAT immunoreactivity in the diagonal band, the compensatory increase in cholinergic neural plasticity in the dentate gyrus and the episodic and spatial memory deficits without restoring COx activity. Conclusion. Ladostigil may be of therapeutic benefit in reducing the cognitive deficits in patients with AD associated with a reduction in cortical COx activity and oxidative stress.

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Brain Cortex Muscarinic Transmission is Impaired in Young Adult Transgenic Appswe/Ps1de9 Female Mice

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Mental performance in human naturally declines with age. This decline is at least partially caused by weakening of brain muscarinic cholinergic transmission. In Alzheimer´s disease, progressive deterioration of mental performance is accompanied by a distinct lesion of basal forebrain cholinergic neurons innervating brain cortex that is regularly found in terminal state of the disease at autopsy. However, an important issue is whether dysfunction of cholinergic synapses occurs and may already play a role in the initial stages of Alzheimer´s disease pathogenesis. To approach this question we investigated some presynaptic and postsynaptic markers of cholinergic synapses and efficacy of signal transduction through muscarinic receptors in parietal cortex of young adult (7 months) and aged (17 months) transgenic APPswe/PS1dE9 female mice and compare them with littermate controls. These mice overproduce β-amyloid fragments and develop characteristic Alzheimer´s amyloid pathology. We found age-dependent (choline acetyltransferase, acetylcholinesterase, and butyrylcholinesterase activities) and both age- and transgene-dependent (vesicular acetylcholine transporter and muscarinic receptors densities) decline of investigated cholinergic markers. Most notably, muscarinic receptor-stimulated GTP-γS binding that reflects efficiency of signal transduction revealed both age- and transgene-dependent diminution of potency and efficacy of the muscarinic agonist carbachol in activating receptor/G-protein coupling. Our findings thus demonstrate early impairement of cholinergic synapses at the presynaptic level and muscarinic receptor-mediated signalling in this transgenic model of Alzheimer´s disease. Supported by research project AV0Z 5011922, EU project QLK1-CT-2002-00172, and grants IAA5011206 and LC554.

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A Sapogenin From Zhimu in the Treatment of Dementia

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Zhimu (Rhizoma Anemarrhenae) is a commonly used "Yin Tonic" in traditional Chinese medicine. A compound known as Sarsasapogenin (5b,20a,22a,25S-spirostan- 3b-ol) (ZMS) was purified from Zhimu with high yield (>1%) which could repeat the effect of Zhimu in down-regulating b-adrenergic and/or up-regulating M-cholinergic receptor (MAChR) in several animal models.

In the aged model and two neuro-degeneration models produced by injection of ibotenic acid alone or b-amyloid (Ab1-40) plus small amount of ibotenic acid to nucleus basalis magnocellularis (NBM) of rats, ZMS could significantly improve the learning and memory. It did not inhibit cholinesterase, nor did it occupy the MAChR binding site, but could raised the decreased brain MAChR by elevating the expression of M1 and M2 mRNA.

In the aged animals and the primary culture of cortical neurons intoxicated with Ab, the decreased activity of choline acetyl transferase (ChAT) and decreased ChAT postitve cells was raised by ZMS. This action required the active participation of BDNF. The BDNF content in aged brain or in the culture medium of aged primary cultured cortical neurons was lower than normal and could be raised by ZMS. When the action of BDNF was blocked by either functional anti-BDNF antibody or the TrkB was blocked by k252a, the elevating effect of ZMS on the MAChR density and the ChAT positive cells was abolished.

Therefore, ZMS seems to represent a new approach to the pharmacological regulation of memory in AD and related diseases.

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Alpha-Synuclein and PUFA in Health and Parkinson's Disease

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α-Synuclein (αS) is a protein of unknown function that is strongly implicated in Parkinson's disease (PD). We have reported several lines of evidences suggesting that αS interacts with polyunsaturated fatty acids (PUFAs), among other lipids,

and that this interaction, under adverse cellular conditions, could contribute to αS-related pathogenesis of neuronal dysfunction.

We previously described a correlation between increased cellular levels of PUFA and αS accumulation in higher MW assemblies (soluble oligomers and insoluble aggregates) in vivo. We now report that PUFA-induced αS-oligomerization and aggregation precede the formation of Lewy-like inclusions in cells exposed to specific PUFAs. PUFA-induced inclusions are immunoreactive for αS, ubiquitin and HSP70. As a confirmatory approach, we searched for alterations of αS assembly in brains of mice with certain Peroxisomal Biogenesis Disorders. This group of lipid metabolic disorders is characterized by accumulation of high levels of PUFAs resulting from defective peroxisomes. We found a marked increase in αS accumulation and aggregation in the Pex-/- brains.

Moreover, we reported that αS enrich membranes with PUFAs and thereby increase cellular membrane fluidity. We now show that αS expression plus PUFA exposure in cells induces enhanced membrane trafficking, specifically through receptor-mediated endocytosis. For example, the endocytosis of fluorescently-labeled transferrin by its receptor was enhanced by αS expression plus PUFA treatment, and this could be inhibited by either a dominant negative form of dynamin (K44A) or by siRNA against the clathrin. We are currently examining the mechanisms by which αS interactions with PUFAs affect membrane trafficking parameters.

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Dimebon Improves Cognition, Function and Behavior in Patients With Mild to Moderate Alzheimer's Disease: Results of a Randomized Clinical Trial

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Background and Aims: Dimebon is a novel small molecule therapeutic which inhibits neuronal death, possibly through modulation of mitochondrial-mediated apoptosis, and improves performance in models of AD. This study was conducted to assess the efficacy and safety of Dimebon in the treatment of mild to moderate AD.

Methods: 183 AD patients (MMSE 10-24) in Russia received Dimebon or placebo for 6 months. The primary outcome measure was change in ADAScog score at endpoint (ITT-LOCF). All enrolled subjects were included in the intent-to-treat analysis.

Results: Mean baseline MMSE was 18.5 (SD 3.4) and 84% completed the trial (87.6% Dimebon, 81.9% Placebo). Dimebon treatment resulted in significant improvements on all outcome measures at endpoint: ADAScog (4 points, $p<0.001$), CIBICplus (0.6 units, $p<0.001$), MMSE (2.2 points, $p<0.001$), NPI (3.6 points, $p<0.001$), and ADCS-ADL (3.4 points, $p<0.002$). Additionally, patients were significantly improved over their baseline scores on all 5 outcome measures. Dimebon was well tolerated and fewer Dimebon treated patients experienced serious adverse events versus placebo treated patients (2.2% vs 7.4%). Dry mouth was the most common adverse event (13.5%).

Conclusions: Dimebon is a novel well-tolerated drug that improved cognition, function, and behavior in patients with mild to moderate AD. Importantly, the observed effects resulted from improvement of the treated patients over baseline scores, in addition to greater decline in the placebo treated patients.

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The Insulin Sensitizer Rosiglitazone Reduces Inflammatory Response of Immune Cells and Improves Neuroprotection in Insulin-Resistant Subjects With Alzheimer's Disease

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Background and aims. Glucose metabolism, insulin sensitivity and inflammation have been involved in Alzheimer's disease. In particular, reduced uptake of glucose could be associated with neurodegeneration. We evaluate inflammatory cytokines (TNF-a, IL-6 and IL-8) and of VEGF secreted by circulating peripheral blood mononuclear cells (PBMC) of AD and healthy subjects.

Methods. Cytokines and VEGF were measured during incubation with glucose (1, 5, 10, 20 mM/ml/10e7 PBMC) and with glucose co-incubated with the insulin sensitizer rosiglitazone (0.1, 0.5, 1.0 mg/ ml/10e7 PBMC). Eighty-seven subjects with AD (41 mild to moderate and 46 severe), aged 66-85 yr., and thirty-nine healthy subjects, aged 67-82 yr. were recruited. PBMC were separated by Ficoll-Hypaque and gradient centrifugation; cytokines and VEGF were measured by ELISA (R&D Systems).

Results. HOMA-IR was higher in AD than in healthy subjects (2.7 vs 1.7; $p<0.0001$). The spontaneous and glucose-modulated secretions of cytokines were significantly increased ($p<0.001$) in AD compared to healthy subjects; whereas VEGF was significantly reduced ($p<0.001$) in AD. The co-incubation of PBMC with rosiglitazone significantly blocked the inflammatory response of PBMC of AD subjects during glucose exposure, with a reduction of cytokines from 60% to 85% ($p<0.001$). On the contrary, rosiglitazone significantly restored VEGF production of AD subjects.

Conclusions. These data suggest that in insulin-resistant AD blood glucose exposure significantly increased inflammation, also reducing the secretion of neuroprotective growth factor VEGF; whereas rosiglitazone, antagonise the inflammatory pattern by means of a reduction of insulin resistant condition within immune cells, also improving the neuroprotective effects induced by VEGF.

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Omega-3 Fatty Acid Treatment of 174 Patients With Mild to Moderate Alzheimer´S Disease: A Randomised Double-Blind Trial

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Epidemiological and animal studies have suggested that dietary fish or fish oil rich in omega-3 fatty acids (w3 FA), DHA and EPA may prevent Alzheimer's disease (AD).

To determine effects of dietary w3 FA supplementation to AD patients with mild to moderate disease on cognitive functions.

Methods: Randomized, double-blind, placebo-controlled clinical trial where 204 AD patients with acetylcholine esterase inhibitor treatment and a MMSE >15 points were randomized to daily intake of 1.7 g DHA and 0.6 g EPA or placebo for 6 months. Then, all received the w3 FA for 6 more months.

Cognition measured with Mini Mental State Examination (MMSE) and ADAS-cog scales. Global function (assessed with the Clinical Dementia Rating), safety, tolerability and blood pressure.

174 patients fulfilled the trial. At baseline, mean CDR, MMSE, and ADAS-cog values in all patients were similar. At 6 months the decline in cognitive functions as assessed by the two latter scales did not differ between the groups. However, in a subgroup (n=32) with very mild cognitive dysfunction, MMSE >27 points, a significant ($p<0.05$) reduction in MMSE decline rate was observed in the w3 group compared to the placebo group. Similar arrest in decline rate was observed in this placebo subgroup when receiving w3 FA between 6 and 12 months.

Omega-3 fatty acids given to AD patients to moderate disease did not delay the rate of cognitive decline according to MMSE or ADAS-cog scales. However, positive effects were observed in a small group of patients with very mild AD (MMSE>27

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Immunotherapy Against APP **B**-Secretase Cleavage Site Improves Cognitive Function and Reduces Neuroinflammation in Tg2576 Mice

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Deposition of amyloid β (Aβ) peptides into senile plaques is considered a major event in the pathogenesis of Alzheimer's disease (AD). Active and passive immunization studies have shown that antibodies against Aβ peptides are effective in reducing Aβ levels and plaque pathology, as well as attenuating cognitive deficits in animal models of AD. However, the therapeutic potential of these antibodies in AD patients is limited because of adverse inflammatory reactions and cerebral hemorrhage, which are associated with the treatment.

We developed a novel approach to inhibit Aβ production via antibodies against the β-secretase cleavage site of amyloid precursor protein (APP). This approach limits APP processing by β-secretase, mainly through the endocytic pathway. We have shown that anti β-site antibodies were able to bind human APP expressed by cellular model of AD, internalize into the cells after APP binding at the plasma membrane and partially block Aβ production.

Here, we show that long-term systemic administration of anti-APP β-site antibodies to Tg2576 transgenic mice improved mice cognitive functions associated with a reduction in both brain inflammation and the incidence of microhemorrhage. Furthermore, antibody treatment did not induce any peripheral autoimmune responses. In spite of the beneficial effects observed in antibody-treated mice, brain Aβ levels were not altered as a result of antibody treatment.

Since BACE1 is involved in processing of other non-APP substrates, interference with the specific APP-BACE1 interaction proposed by this approach may overcomes some of the limitations of BACE1 inhibition methodologies.

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Immunization Against Alpha 7 Nicotinic Acetylcholine Receptors Prevents Memory Impairment in the Mouse Model of Sporadic AD

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Alpha 7 nicotinic acetylcholine receptors (alpha7nAChR) are involved in learning and memory, and are implicated in the pathology of Alzheimer's disease (AD). β-amyloid peptides (Aβ) have been shown to form tight complexes with the alpha7nAChR, interfering with transduction of the acetylcholine signal by this nicotinic receptor subtype. The distribution of alpha7nAChR correlates with neuritic plaques in the AD brain. The selective binding of Aβ by this acetylcholine receptor is associated with cytotoxicity. Earlier we revealed that olfactory bulbectomized (BE) animals showed many features which were similar to signs of AD, including the spatial memory impairment, the loss of neurons in specific brain structures, the increase of Aβ level in the brain, as well as dysfunction of cholinergic system in basal structures of the frontal brain. Here we show the possibility of prevention of the memory loss in BE mice by immunizations with two different synthetic fragments of the N-terminal extracellular domain of alpha7nAChR conjugated to KLH (keyhole limpet haemocyanin). The decrease of Aβ level in the brain was revealed 2 months after immunization in BE mice. We suggest that such selective cholinergic interventions would slow the progression of memory impairment and protect neurons from degeneration in patients with AD, as well as

would be perspective for prophylactic of this disease in high-risk group.

This investigation is supported by Program of the Presidium of Russian Academy of Science "Fundamental Science to Medicine" and by research grant from RFBR N 06-04-48710.

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DNA Epitope Vaccine Induced Strong Anti-Ab Antibodies Inhibiting AD Like Pathology in 3xtg-AD Mice and Protecting Them From Cognitive Decline

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Induction of humoral responses against fAb42 peptide is thought to alleviate the onset and progression of AD. Previously, we demonstrated that plasmid encoded Ab42 induced in wildtype mice high and APP/Tg mice low titers of anti-Ab42 antibodies and elicited anti-self Ab42-specific T cell responses implicated in adverse effects such as shown in AD patients from AN1792 trial. Other groups of scientists support these data.

To overcome above mentioned problem we developed chemokine-based DNA vaccine that encodes one or three copies of the self-Ab B cell epitope (Ab1-11/3Ab1-11) and the foreign promiscuous T cell epitope (PADRE) fused in frame with macrophage-derived chemokine (MDC/CCL22). MDC was chosen for its ability to target and deliver antigens to professional antigen-presenting cells, and to elicit Th2-type polarized immune responses. We demonstrated that the pMDC-3Ab1-11-PADRE vaccine induced robust anti-Ab antibody and Th2 type anti-PADRE T cell responses in 3xTg-AD mouse model. These mice were protected from age-related behavioral impairment and significantly inhibited development of Ab plaques, while mice immunized with pMDC-Ab1-11-PADRE vaccine induced only low titers of anti-Ab antibodies and were not protected from cognitive decline. Currently, we are further investigating and will be reporting the quantity of soluble (monomeric/ oligomeric) and insoluble forms of Ab42/40 peptides as well as phosphorylated tau accumulated in the brains of immune and control animals.

Our data along with results demonstrating that soluble forms of Ab amyloid trapped into vasculature of two AD patients vaccinated with AN1792, suggest that anti-Ab vaccine might be effective as prophylactic, but not therapeutic measure.

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Beta-Amyloid-Specific Th1-Type Responses in Young Healthy Individuals Convert to Regulatory Responses in Individuals With Down Syndrome or Alzheimer's Disease

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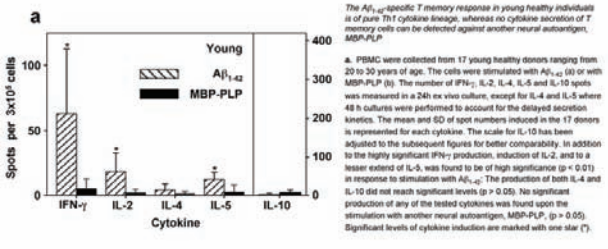
Beta-amyloid 1-42 (Aβ1-42) specific adaptive immunity can protect Alzheimer-prone transgenic mice against the histopathologic and clinical hallmarks of the disease. Vaccination of Alzheimer patients with this antigen showed potentially protective effects, but at the same time evidence of CD4+ Th cell mediated meningoencephalitis as an undesired side effect.

Anti-Aβ1-42 IgG antibodies and T cell proliferation indicate recognition of Aβ1-42 by the adaptive immune system in humans.

We show for the first time that Aβ1-42 –specific Th1-cell memory is present in young healthy humans, with high levels of IFN-γ and IL-2 secretion. With increasing age, the Th-signature is predominated by CD4+ T-cell derived IL-10 production, but Th1-type cytokine prodruction does not totally disappear. However, individuals with Trisomy 21 and patients with Alzheimer's disease show IL-10 production only after Aβ1-42 stimulation. This shift in cytokine signature is specific for the immune response to Aβ1-42, since two control antigens (Mumps antigen and Tetanus toxoid), show similar Th-signatures in healthy controls and in individuals with Trisomy 21 and Alzheimer's disease.



The $A\beta_{1-42}$-specific T memory response in young healthy individuals is of pure Th1 cytokine lineage, whereas no cytokine secretion of T memory cells can be detected against another neural autoantigen, MBP-PLP.

a. PBMC were collected from 17 young healthy donors ranging from 20 to 30 years of age. The cells were stimulated with $A\beta_{1-42}$ (a) or with MBP-PLP (b). The number of IFN-γ, IL-2, IL-4, IL-5 and IL-10 spots was measured in a 24h ex vivo culture, except for IL-4 and IL-5 where 48 h cultures were performed to account for the delayed secretion kinetics. The mean and SD of spot numbers induced in the 17 donors is represented for each cytokine. The scale for IL-10 has been adjusted to the subsequent figures for better comparability. In addition to the highly significant IFN-γ production, induction of IL-2, and to a lesser extend of IL-5, was found to be of high significance ($p < 0.01$) in response to stimulation with $A\beta_{1-42}$. The production of both IL-4 and IL-10 did not reach significant levels ($p > 0.05$). No significant production of any of the tested cytokines was found upon the stimulation with another neural autoantigen, MBP-PLP, ($p > 0.05$). Significant levels of cytokine induction are marked with one star (*).

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Involvement of Both Gaba-Dependent and -Independent Pathways in Tramiprosate Neuroprotective Effects Against Amyloid-Beta Toxicity

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Tramiprosate (3-amino-1-propanesulfonic acid), presently in phase III clinical trials for Alzheimer's disease, acts in part by binding to toxic soluble amyloid-β (Aβ), known to oligomerize, deposit and accumulate in the brain. Tramiprosate is structurally related to the neurotransmitter gamma-amino butyric acid (GABA) and binds with high affinity to the GABAa receptor. Moreover, tramiprosate exerts neuroprotective activity against Aβ-mediated toxicity. In the present study, we assessed the involvement of GABAa receptors in the neuroprotective effect of tramiprosate on Aβ-induced toxicity in primary neurons and organotypic hippocampal slice cultures (OHCs). Primary neurons were treated with Aβ42 in the presence or absence of tramiprosate or muscimol, a classic GABAa receptor agonist. Both drugs were found to decrease basal and Aβ42-induced caspase 3/7 and caspase 9 activities, effects significantly blocked by

pretreating the cells with specific GABAa receptor antagonists. Interestingly, muscimol did not demonstrate neuroprotective activity against Aβ42-induced DNA damage or ERK1/2 activity in primary neurons. The effect of tramiprosate on Aβ-induced DNA damage was unaffected by pretreatment with GABAa receptor antagonists. In OHCs, both tramiprosate and muscimol decreased Aβ42-induced cellular mortality. The effect of tramiprosate was partially reversed by pretreatment with bicucculine. These data suggest that GABAa receptor activation may be involved in the neuroprotective effects of tramiprosate against Aβ-induced caspases in primary neurons and cellular mortality in OHCs. However, tramiprosate neuroprotective effect on Aβ-induced DNA damage seems to be independent of GABAa receptor activation, suggesting that GABA-dependent and -independent activities may be involved in the mechanism of action of tramiprosate.

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Amyloid Beta Peptides Mediate Hypoxic Remodelling of Calcium Channel Expression

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Diseases which deprive the brain of oxygen for sustained periods of time, or acute incidents such as stroke, predispose individuals to development of Alzheimer's disease. We have shown that hypoxia (1-2.5% O2, 6-48h) in vitro causes dramatic remodelling of ion channel functional expression in neurones, as well as remodelling of Ca2+ signalling in astrocytes. In both cell types, such changes are likely to promote Ca2+-mediated degeneration, since they disrupt key aspects of Ca2+ homeostasis. Almost all of these effects are mediated by, and indeed require formation of, amyloid peptides and their direct application mimics many of the effects of exposure to hypoxia. In central neurones, hypoxia causes an amyloid-mediated, selective up-regulation of L-type voltage-gated Ca2+ channels (Neurobiol. Aging (2006) 27, 439-445). This effect was also seen in PC12 cells and, crucially, in a recombinant expression system in which human L-type Ca2+ channels were stably expressed in HEK 293 cells. This latter observation indicated hypoxia acted post-transcriptionally and allowed detailed mechanistic investigation. In brief, hypoxia increased amyloid peptide production which in turn promoted Ca2+ channel insertion in the plasma membrane, an effect due to close interaction of peptides with Ca2+ channel proteins (as indicated by co-immunoprecipitation; FASEB J. (2005) 19, 150-152). This required mitochondrial formation of reactive oxygen species (J. Biol. Chem. (2005) 280, 21706-21712). Our results provide potential mechanisms to account for the increased incidence of dementias in individuals who have previously suffered hypoxic / ischemic episodes.

Supported by the Alzheimer's Research Trust, The Wellcome Trust and the MRC.

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Enhancement of Intracellular Abeta and P53 Degradation: A Novel Therapeutic Strategy for Alzheimer Disease

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[Background and aims] Recent major therapeutic strategies for Alzheimer disease (AD) are to inhibit amyloid beta-protein (Abeta) generation and aggregation, and to promote extracellular Abeta removal. While, Abeta42 appears in the neurons at early stage of AD; and intracellular Abeta42 induces mitochondrial damage, synaptic dysfunction, proteasomal inhibition, and p53-dependent apoptosis. We have recently demonstrated that cytosolic Abeta42 is transferred by Abeta related-Death Inducing Protein (AB-DIP) to the nucleus and activates p53 promoter leading to apoptosis (Ohyagi and Tabira, MRMC, 2006). Since cytosolic Abeta42 and p53 are degraded by proteasome system, activation of proteasome may reduce cytosolic Abeta and p53 levels attenuating neuronal apoptosis.

[Methods] We first established an assay system using cultured cells. To artificially accumulate Abeta in cytosol, human neuroblastoma cells (SH-SY5Y) were incubated with Abeta40 peptide in the hyper-osmotic medium, followed by incubation in hypo-osmotic medium. We then studied degrading process of intracellular Abeta40 from 0 to 30 min by western blot. Next, we treated the cells with a proteasome inhibitor (MG132) to inhibit Abeta degradation and checked some drugs that may counteract such effects. Also, p53 protein levels, proteasome activity, and cell viability were evaluated. Furthermore, we studied protecting effects on mouse primary neuronal cells under oxidative stress.

[Results] We have identified a compound that activates proteasomal activity, enhances cytosolic Abeta/p53 degradation, recovers cell viability, clearly protecting neurons from apoptosis.

[Conclusion] Such a drug may be a new candidate drug to inhibit neurodegeneration in AD.

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Amyloid Precursor Protein Processing But Not Beta Amyloid Neurotoxicity is Regulated by Specific JNK Inhibitor

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The amyloid beta peptides (Abeta 1-40 and 1-42) accumulate in Alzheimer's (AD) brains, they derive from the proteolytic processing of the amyloid precursor protein (APP). It has been demonstrated that the JNK scaffold protein, JIP-1, interacts with the cytoplasmic domain of APP, suggesting that JIP-1 and JNK may play important roles in the metabolism of APP. It has also been established that APP is phosphorylated

at Thr 668 by JNK. This phosphorylation in the cytoplasmic domain of APP may result in a regulation of the APP processing. We examined the role of JNK in AD pathogenesis using a specific cell-permeable JNK inhibitor peptide, D-JNKI-1, and two different in vitro models: primary cortical neurons and H4-15x cells, stably transfected with human APP695 carrying Swedish mutation. In cortical neurons, D-JNKI-1 (6µM) reduced of about 60% the level of APPs in both lysates and media, and this also correlated with a decrease in the media of Abeta fragments. Similar results were obtained in H4-15x cells. In both cellular models D-JNKI-1 prevented phosphorylation of APP at Thr 668 in a dose-dependent way. We also evaluated the effect of D-JNK-1 against Abeta 1-42 neurotoxicity in cortical cultures in comparison with SP600125, a synthetic JNK inhibitor with a JBD-independent mechanism. SP600125 antagonized the Abeta toxicity while D-JNK-1 was unaffected. These data indicate JNK is implicated in both the APP processing and Abeta toxicity with a different mechanism, the effect of APP processing is a JBD-dependent JNK mechanism, while the Abeta_toxicity is a JBD independent JNK mechanism.

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Neuroprotective Agents From Chinese Medicine

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Neuroprotection, methods to protect or preserve healthy neuron, is rapidly become an areas of major importance in medicine. As global population is aging, the health problems in age-related neurodegenerative diseases such as Alzheimer's disease are rapidly expanding. One useful approach to protect the neurons against damage from harmful toxin is to use extracts from neuroprotective Chinese Medicine. In this presentation, the isolation and characterization of novel polysaccharides from neuroprotective Chinese Medicine will be discussed. Their neuroprotective effects using neuronal cell culture will also be presented. The data suggested that these polysaccharide extracts could significantly protect neurotoxicity against many toxins including human β-amyloid peptide 1-42. As β-amyloid peptide has long been considered to be one of the toxins triggering neuronal apoptosis, the study of neuroprotective polysaccharides from Chinese Medicine may open a new therapeutic window for the prevention of Alzheimer's disease.

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Clinical Features and Frequency of Vascular Dementia

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Background : Cerebrovascular insult is a frequent cause of dementia. Both are major health problems in our country.

Purpose: To analyze patients with ischemic cerebrovascular insult who had developed vascular dementia and to assess its frequency, associated risk factors and clinical features.

Methods: We made a retrospective analysis of 450 patients aged >65 years, who were hospitalized at the Department for Urgent Neurology, with acute ischemic stroke for a period of five years. During admission, demographic data, vascular risk factors, stroke features and neurological status information were assessed. All eligible patients were subjected to a battery of neuropsychological tests during admission and three months after the cerebrovascular insult.

Results: Clinical features of vascular dementia were found in 63 (14%) patients. Statistical analysis showed that vascular dementia was associated with involvement of middle cerebral artery circulation, leucoaraiosis, multiple ischemic lesions, cortical lesions; diabetes mellitus, atrial fibrillation, dysphasia and gait impairment. Older age and low educational level were significant, independent correlates of vascular dementia ($p<0, 01$).

Conclusion: In our study population, vascular dementia was present in about one-sixth of the patients. The clinician should be aware of the potential risk factors and take preventive measures in order to decrease the frequency of vascular dementia.

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The Relationship Between Pain and Depression in Parkinson's Disease

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Background: Depression is common in Parkinson's disease (PD). An association between pain and depression is common, but little is known about this relationship in PD. Such information may improve the management of patients with PD. Therefore, we examined the relationship between pain and depression in patients with PD.

Methods: 227 patients with PD were drawn from a community-based prevalence study. Pain was assessed by employing the pain section of the Nottingham Health Profile (NHP) and depression by using the Montgomery- sberg Depression Rating Scale (MADRS) and the Beck Depression Inventory (BDI).

General linear models and regression analyses were used to control for confounding factors on depression as both continous and categorical variables.

Results: 67% of 227 patients suffered from pain. They had more severe depressive symptoms, indicated by higher mean scores on both depression scales, and were more likely to have major depression. In multivariate analyses, the

presence of pain was significantly associated with BDI but not with MADRS. Both MADRS and BDI sum scores correlated significantly with pain, and in the multiple linear regression analyses.The effect of pain severity was statistically significant in the model with MADRS and remained as a trend in the model with BDI.

Conclusion: Our results indicate a relationship between pain and depression. Pain issues should be integrated in the management of depression in PD.

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Stroke-Related Dementia

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Background and aim: To analyze psychological changes and quality of life in patients with stroke-related dementia. Methods: 354 patients with stroke were evaluated. 241 (68, 1%) had cerebral thrombosis, 78 (22%) intracerebral haemorrhage and 35 (9, 9%) subarachnoidal haemorrhage.Results: Of the analyzed risk factors, hypertension dominated in 263 (74, 3%) and stress condition in 153 (43, 2%) of the cases. 102 of the patients (74, 3%) had lethal outcome. After the treatment and recovery of the patients had been completed, it was noted that part of them have shown signs of dementia. More distinct psychological changes were registered in patients with left sided neurological deficits. Patients underwent psychological testing (scale for dementia evaluation; cognitive behavior, noncognitive behavior, etc), in order to evaluate the degree of forgetfulness, distinct dementia or other psychological changes. It was shown that 82 (23, 1%) of the patients were completely dependent on another person's care and nursing: hey were completely unable to take care of themselves; 98 (27, 7%) of the patients needed another person's care, with slowing of the psychological functions, but they could (under supervision) partially take care of themselves. The remaining 72 patients (20, 3%) were quite alright and could perform all psychological functions on their own, and did not register any psychological changes. Conclusion: About 2/3 of the patients with stroke-related dementia had impaired quality of life. Preventive measures should be taken towards the potential risk factors for stroke and subsequent development of stroke-related dementia.

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Dementology - Solution for the Future

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Sometimes certain medical problems outgrow even themselves and start to live their own life.In 1996 van Gooi and van Grevel highlighted the fact that 1400 papers had been written on diagnosis and treatment of AD over the 5 years.However,there appeared to be a discrepancy between the enthusiasm for the potential of this new knowledge and the conservation of traditional methods in everyday practice.

Fortunately the situation has begun to change recently. Promising developments from the field of molecular biology , neurogenetics,neuroimaging and markers are helping to eliminate the word "Probable" from dementia definition and the right of pathologist to stamp the final diagnosis.

Models of care for AD patients consist of a series of pharmacologic and nonpharmacologic interventions in order to delay progression.Strategies to prevent AD need to be applied in early midlife.This rapid growth in clinical and research aspects of dementia encompasses many speres of medicine and deserve recognition as indipendent specialty on its own. DEMENTOLOGY should be the science of dementias,its prevention,diagnosis,treatment and follow up.The term Dementologist might be used for a neurologist,psychiatrist and/or geriatrician who through practice and interest develops expertise in this field without having formal training or board certification.In the second context Dementologist refers to a physician whose practice and/or research efforts are concentrated on dementia care having pregraduate,postgraduate training and certification in Dementology.This is what is called for.

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A Romanian Descripa Project Presentation

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Background: Patients recruited in the study: patients admitted to the hospital between 1st of July 2004-31st of June 2005, the total of 448 patients of whom 55 were patients with MCI included in the study. Objective(s): To asses the prevalence of various risk factors and co-morbidities in all new patients with mild cognitive impairment admitted in the memory unit. To ascertain the validity of the international model for the distribution of co-morbidities and risk factors for MCI in Romania. Methods: There were inclusion and exclusion criteria for these patients. There were charts revealing the distribution of patients regarding gender and age, education, place of residence, prevalence of alcoholism, myocardial infarction, depression, relationship between prevalence of depression and the loss of memory, prevalence of osteoporosis. Results: During that period has been ascertained the validity of the international model for the distribution of co-morbidities and risk factors of MCI in Romania. Conclusions: The study actively screened for MCI patients admitted in The Day Hospital of Memory Impairments, using international criteria for diagnostic, this study promoted correct evaluation and treatment of the patients, and is the first descriptive study in East-European countries on MCI. Within the study group, prevalence of co-morbidities such as cardiovascular, psychiatric or metabolic diseases was evaluated and a model of distribution, adjusted for Romania was made. The study showed that The Day Hospital of Memory Impairments founded by "Ana Aslan" International Academy of Aging in Romania is able to initiate and manage a larger group study.

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Predictors of 1-Year Outcome in Subjects With Mild Cognitive Impairment. Results From the Descripa Study

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Background: Only a subgroup of subjects with MCI have AD but it is difficult to identify these subjects. The DESCRIPA study aims to develop criteria for AD in the predementia stage. It is a multicentre study of the European Alzheimer's disease consortium. Markers of AD investigated include age, MMSE score, functional impairment, cognitive test performance, APOE genotype, medial temporal lobe atrophy (MTA), and beta amyloid 1-42 and p-tau 181 levels in CSF. We also investigated the predictive accuracy of the Predementia Alzheimer's disease Scale (PAS) which combines 6 markers of AD.

Objective: To investigate predictors of conversion to AD after 1 year.

Subjects: Inclusion criteria were age >55 years and new referral to a memory clinic. Exclusion criteria were dementia and disorders causing cognitive impairment. The present analysis selected subjects with data on the 1-year follow-up (n=549). Subjects were on average 70 years old, scored 27.3 on the MMSE, and had 10.6 years of education. Data on APOE genotype, MTA, and CSF values were collected in a subgroup only. Main outcome measure was the area under the curve (AUC).

Results: Predictors of AD were age (AUC=0.61), MMSE score (AUC=0.66), CDR-sum of boxes (AUC=0.63), delayed recall (AUC=0.66), MTA score (AUC=0.61), p-tau181 level (AUC=0.67), and the PAS score (AUC=0.73).

Conclusions: A combination of variables can best predict AD after 1 year. Predictive accuracy was generally low as subjects are likely to convert to AD at longer follow-up intervals as well.

Acknowledgement: The European Commission funded the study within the 5th framework programme.

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The Relation Between Agitation in Residents With Dementia and Job Stress, Demand and Physical Health in Care Staff

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Background: Within the nursing profession stress and burnout are considered to be widely present and problematic, to have an impact on job satisfaction and to affect the retention of nurses. Behavioral disturbances in residents with dementia are generally associated with stress and burn out in caregiver. However, few studies have measured the actual correlation between behavior in patient and stress in staff caring for the patient.

Aim: We have measured frequency of agitation in patients, and the care staff perception of frequency and disturbance of agitation in the same group of patients, and explored their relationship with job stress, job demand and physical health in care staff

Methods: The subjects were 211 patients and 197 care staff in Norwegian nursing homes.

Results: We found a relatively low level of perceived stress and subjective health complaints. Job stress in care staff is not related to patient agitation in itself, but it is related to the care staff perception of patient agitation. Discussion To prevent job stress, sick leave and turnover in care staff, we need to consider care staff perception and experience of patient agitation, as well as consider patient agitation. These findings suggest that we need to combine management of agitation in patient, with an opportunity for staff to relieve own stress, reflect on work situation and patient relation, to improve work environment for care staff and thus improve the quality of care towards patients

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The Relationships of GDS and MMSEAssessments to Domains of Cognitive Function Assessed by the CDR System in Alzheimer's Disease

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Background: The Cognitive Drug Research (CDR) computerised cognitive assessment system has been widely applied in dementia research for 20 years. A cohort of 744 Alzheimer's patients was studied over a 26 week period. CDR testing, the Global Deterioration Scale (GDS) and MMSE were conducted at baseline. The objective is first to evaluate the relationship between the GDS, MMSE and core domain scores from the CDR system, and second to identify whether the rate of decline over 6 months is related to initial scores on the GDS and MMSE.

Methods: Composite scores for the major CDR domains have been established by factor analysis. These involve important aspects of attention, working and episodic memory. The nature of the relationship between the baseline scores on the GDS, MMSE and CDR domains was evaluated as was the relationship of decline on the CDR domains over 26 weeks to initial GDS and MMSE scores.

Results: Clear differences existed on all CDR domains according to the level of the GDS. For the MMSE, a clear linear relationship was identified with the core CDR domains. The relationship between initial GDS & MMSE scores and subsequent decline was less clear.

Conclusion: Objective evaluations of cognitive function in Alzheimer's patients show a clear relationship to independently conducted assessments made by clinicians. While this may be expected for domains of memory, it is less obvious that this should occur for aspects of attention, and this shows that attention deficits form a central part of the symptomatology of Alzheimer's disease.

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Screening for FTLD-U in Alzheimer's Disease and Hippocampal Sclerosis With Tdp-43 Immunohistochemistry

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Frontotemporal lobar degenerations (FTLD) are clinically and pathologically heterogeneous. Pathologically, the hallmark histopathologic findings are dystrophic neurites as well as neuronal cytoplasmic and sometimes intranuclear inclusions that are immunoreactive for ubiquitin, FTLD-U. Recently, the TAR DNA binding protein (TDP-43) was found to be another marker for the neuronal lesions in FTLD-U. TDP-43 has been reported to be specific to the lesions in FTLD-U and not found in other degenerative disorders, such as Alzheimer's disease (AD). This offers the possibility of detecting FTLD-U in the setting of other neurodegenerative disease processes. With this in mind, we screened a series of AD cases with TDP-43 immunohistochemistry. Given that about 80% of FTLD-U cases have neuronal loss and gliosis in the hippocampus, consistent with hippocampal sclerosis (HpScl), we also screened cases with HpScl. Of the 21 cases of HpScl screened, 13 (62%) had TDP-43 immunoreactivity. Of 75 AD cases screened, 44 (58%) had TDP-43 immunoreactivity, including 35 of 46 (76%) cases of AD with HpScl and 9 of 30 AD without HpScl (30%). Double labeling immunohistochemistry for tau protein and TDP-43 at the light and electron microscopic level showed that in most cases the two types of lesions were separate, but some inclusions had co-localization of tau and TDP-43, either as separate structures within the same neuron or as double stained inclusions. The frequency of tau-TDP43 co-localization was 21%. If specificity of TDP-43 for FTLD-U is true, the results suggest that occult FTLD-U in the setting of HpScl and AD is not uncommon.

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Lexical-Semantic Processing in Amnesic Mild Cognitive Impairment and Parkinson's Disease

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In the last years many researches reported a semantic memory disorder in Parkinson's disease without dementia (PD). The aim of the present study was to compare the semantic dysfunction pattern of PD patients with that one found in Amnesic mild cognitive impairment (aMCI), as this condition may be characterized by a light semantic disorder too.

Materials and Methods: Three groups of subjects have been selected. The first one was composed by 13 PD patients, the second one was of 11 aMCI patients according to Petersen's criteria and we selected a control group of 10 normal subjects (NC) too.

All the groups underwent a screening neuropsychological battery for excluding dementia and for detecting a normal cognitive condition in NC subjects.

Semantic memory was furthermore investigated with naming of line drawing test and semantic feature question task. The items of the tests were divided in living and no living stimuli.

The ANOVA univariated and the Tukey test were used to compare the values of the three groups.

Results: PD patients showed significantly worse performances than NC subjects on naming and semantic feature question task, both in living and no living items.

AMCI group performed significantly worse than NC group in semantic feature question task, only for living items.

Although PD patients showed the lowest performances, they did not significantly differ in comparison with aMCI group.

These results give support to previous researches reporting early semantic dysfunction in Parkinson's disease.

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Analysis of Chlamydophila (Chlamydia) Pneumoniae in the Alzheimer Brain

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Background and Aims: Our studies have focused on pathogen involvement as a risk or causative factor in sporadic late-onset Alzheimer's disease (LOAD). Chlamydophila (Chlamydia) pneumoniae has been shown to be localized in human AD brain tissues (Balin et al., 1998, Arking et al., 1999, Gerard et al., 2006). We are using immunolabeling techniques to develop greater consistency between laboratories in detecting infection in AD brains.

Methods: The current investigation involves immunolabeling of AD brain sections and tissue homogenates for C. pneumoniae. We used a battery of anti-chlamydial antibodies and observed differential immunolabeling patterns for C. pneumoniae from AD brains.

Results: On AD brain sections, immunodetection revealed intracellular labeling of neuronal, glial, and endothelial cells, as well as diffuse extracellular labeling in regions of neuropathology. The labeling profiles were determinant on antibody specificity. This included whether the antibodies were generated against organisms in the acute vs chronic/persistent stage of infection, whether the organism was in the elementary (EB) or reticulate body (RB) stage of development, and whether the antibody was generated against a specific antigen (eg, outer membrane protein vs lipopolysaccharide). From AD brain homogenates, immunoblotting demonstrated unique patterns of labeling, again dependent on the anti-chlamydial antibodies used.

Conclusions: Our data suggest that C. pneumoniae was present in areas of neuropathology in AD brain tissues, that C. pneumoniae, at times, may colocalize or interact with eukaryotic proteins, and that a battery of antibodies must be used to accurately detect infection with C. pneumoniae in the AD brain.

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Extracellular Matrix in the Human Substantia Nigra and Subthalamic Nucleus

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The substantia nigra (SN) and the subthalamic nucleus are critically involved in the pathology of Parkinson€™s disease. These areas are dominated by dopaminergic neurons in the SN pars compacta, GABAergic neurons in the SN pars reticulata (SNpr) and glutamatergic neurons in the subthalamic nucleus. Virtually nothing is known about the characteristics of ECM in human neurodegenerative diseases.

Remarkable structures in the adult brain that are enriched in ECM molecules are the perineuronal nets (PNs) consisting of aggregating chondroitin sulphate proteoglycans (CSPG) connected to hyaluronan and tenascin-R. Probable functions of PNs are the stabilization of synaptic contacts and a support of permanently fast neuronal activity of net-associated neurons via the special hydrodynamic properties of PNs. Further, PNs show potential neuroprotective effects due to their polyanionic charge by possibly reducing the local oxidative potential in the neuronal microenvironment. Here we firstly present detailed investigations on the distribution, morphology and composition of PNs in the human midbrain. Using the immunocytochemical detection of CSPG combined with antibodies to cytoskeletal, synaptic and transmitter-specific markers as well as calcium binding proteins, we could show a region specific appearance of PNs in the investigated nuclei. Whereas pigmented, tyrosine hydroxylase-positive, neurons were devoid of PNs, the SNpr and the subthalamic nucleus displayed region-specific phenotypes of the perineuronal ECM. These data indicate that the ECM is composed according to physiological functions in the different neuronal systems. It is conceivable that the organization pattern of the ECM is related to the differential vulnerability of neurons integrated in the nigral complex.

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The Clock Drawing Test: An Opportunity to Identify Patients at High Risk for Progression to Subcortical Vascular Dementia

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Mild cognitive impairment (MCI) is a heterogeneous condition with possible progression to different forms of dementia. 3 MCI subgroups have been described: amnestic MCI, multiple-domain MCI (md-MCI) and single-non-memory-domain MCI. Md-MCI is characterized by impairment in several cognitive domains without dementia and it is likely to evolve towards vascular dementia (VD).

Objective of this study was to examine contribution of cognitive, neuropsychological and functional parameters in identifying subjects at risk of developing VD.

83 elderly with md-MCI have been evaluated in a 3-year-follow-up in order to identify possible progression to dementia.

23 subjects converted to dementia in a mean time of 2.5 ± 1.1 years: 19 converted to subcortical VD and 4 to AD. Given similar scores at the MMSE, at baseline the average score in the Clock Drawing Test (CDT) resulted significantly lower in subjects evolving to VD ($p<0.05$). The Trail Making Test (TMT) part B ($p<0.05$) and the Cancellation Test ($p<0.001$) were found to be the most predictive tests for progression to VD.

Analysis of the sub-items of the Instrumental Activities of Daily Living (Lawton's IADL) showed that md-MCI subjects evolving to dementia had greater impairment in some complex activities, such as shopping ($p<0.05$).

Conclusions: impairment in selective items of the IADL and in the CDT seems to correlate to the risk of evolution to VD. If these data will be confirmed in larger studies, CDT could be applied within the md-MCI group as a screening test to identify subjects at high risk of dementia.

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Hereditary Cerebral Hemorrhage With Amyloidosis-Dutch Type (HCHWA-D): A Case With An Unusual Presentation

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Background: The primary clinical presentation in patients with the amyloid β (Aβ) E693Q mutation of HCHWA-D is hemorrhagic stroke due to severe cerebral amyloid angiopathy (CAA).

Case Presentation: We report a 50-year-old, previously healthy, female member of an HCHWA-D family, who presented with headache, nausea, and vomiting and showed a rapid cognitive deterioration within weeks with progressive aphasia. CT and MR imaging (MRI) revealed prominent leukoaraiosis but no evidence of microbleeds. MRI also showed high signal intensity of the gray matter of the temporal gyri. Diffusion-weighted images evidenced hemorrhage in the center of this region and areas of high signal intensity in the right frontal and parietal cortices indicative of vasogenic and cytotoxic edema. Cerebrospinal fluid showed marked elevation of protein and aseptic lymphocytic pleiocytosis. On suspicion of vasculitis or acute disseminating encephalomyelitis the patient was treated with corticosteroids. After initial improvement she deteriorated despite the therapy. A subsequently performed biopsy of the right frontal cortex disclosed Aβ CAA associated with granulomatous angiitis inflammation and multinucleated giant cells. DNA analysis in the patient demonstrated the HCHWA-D mutation.

Comment: CAA-associated granulomatous inflammation with giant cells is a rare finding in HCHWA-D brain. The unusual clinical presentation and pathologic findings in this case add to the evidence that inflammatory CAA-related perivascular inflammation may represent a subset of CAA with clinically distinct symptoms. Imaging may reveal

abnormalities of the cortical gray matter in addition to white matter changes.

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Focal Dystonia - Diagnosis and Treatment

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DYSTONIA refers to rigidity and athetosis with abnormally heightened or lessened muscle tone. Voluntary movements are pre-programmed in the brain. Abnormal sensory input to the motor cortex might contribute to excessive cocontraction of antagonistic muscles. Levodopa intensified Akinesia and freezing claimed to be caused by a noradrenaline (NA) deficiency. Locus ceruleus stimulation and intracerebral NA infusion results in dystonia. The frequent "bizarre" presentation of patients with dystonia presumed psychogenic, misdiagnosed in over 40% were often subjected to futile psychiatric treatment.

Von Monakow and Mourge (1927), influenced by Freudian theories speculated, involuntary movements are psychoneurotic manifestations, stating "the psychopathology had an Oedipal configuration with castration anxiety". Others referred to "exhibitionistic quality", or "hypermotility as uncontrollable motor expression of hostile and aggressive impulses". Dystonias can be symptomatic or inherited as in torsion dystonia. Focal TASK SPECIFIC DYSTONIA (TSD) occurs with writing, typing, playing instruments and sports-related activities. The CUMULATIVE TRAUMA DISORDER (CTD), is different from TSD, claimed a "disturbance of central motor units and fatigue" resulting in tendonitis and entrapment neuropathy.

In RSD sympathetic blocks of the affected limb or spinal anesthesia alleviate tonic dystonia. AKINESIA lasting for hours and TARDIVE DYSKINESIA following neuroleptic treatment, resolved immediately with Stellate Ganglion Blocks (SGB). In five cases of focal TSD in writing (3), typing (1), and playing piano (1) unsuccessfully treated with prolonged psychotherapy were immediately relieved with SGBs. Spastic torticollis and blepharospasm were only temporarily alleviated with SGBs.

Considering the guarded prognosis of TSD treatment with SGBs is advocated.

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The Characteristics of Cognitive Impairment in Parkinson Disease and Recognition of Cognitive Symptom by Caregiver

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The purpose of this study is to evaluate the frequency of cognitive impairment and the characteristics of cognitive deficits in neuropsychiatric test in PD patients, and to know that the early detection of cognitive deficit may be possible in outpatient clinics with the questionnaire for patients and caregivers. Eighty-five patients performed neuropsychiatric testing and questionnaires. All patients had motor symptoms with Hoehn and Yahr stage 0.5 to 3 (mean: 1.98±0.617), and evaluated with cognition by MMSE, 7-MS test, and demographic features including onset age, disease duration, L-dopa dosage. We compared the characteristics of cognitive deficits in neuropsychiatric testing and symptoms detected by questionnaire between the cognitive impairment and non-impairment group. The frequency of cognitive impairment of PD patients was 47% (40/85), among them 75% (30/40) patients complained the symptom of memory decline. The characteristics of neuropsychiatric testing were decreased performance in free recall and improvement with enhanced cued recall, which means the retrieval defect in memory function. The risk factors of cognitive impairment in PD patients were old age, women, older age of onset of PD motor signs, severity of motor symptoms, and lower educational level. With the questionnaire, the symptom of memory decline and difficulties in activity of daily living were important points of cognitive deficit in PD patients. Complaints of cognitive deficits were influenced by old age and depressive symptoms. The short questionnaire for caregiver about memory and ADL might help the early detection of cognitive impairment in PD patients.

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Neuropsychological Clusters Differentiate Subgroups of Vascular Dementia From Alzheimer's Disease and MCI

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Background and aims: The neuropsychological differentiation of Alzheimer's disease (AD) and vascular dementia (VaD) constitutes a major challenge in dementia diagnostics. To determine whether this challenging differentiation is due to the heterogeneity of the VaD, we examined whether the discrimination improves after splitting the VaD group into more homogeneous subgroups. Methods: 99 patients who underwent a comprehensive clinical, neuropsychological and MRI examination met the NINCDS-ADRDA and NINDS-AIREN criteria for AD (n = 53) and VaD (n = 46). To examine the suitability of neuropsychological variables to discriminate between diagnostic groups (e.g. MCI, AD and FTD), logistic regression analyses were performed, adjusting for age, gender and education. Results: Whereas the neuropsychological variables discriminated well between most diagnostic groups (classification rates: 90,5% -100%), the differentiation of AD vs. VaD was inaccurate (correct classifications: 76%, sensitivity: 93,5%, specificity: 47,4%). To examine whether this could partly be explained by the heterogeneity of the VaD, hierarchical cluster analyses were performed to split the VaD group into homogeneous subgroups. The cluster analyses yielded 4 clusters which, in a second step, were individually differentiated from AD, again using logistic regression. The resulting classification rates were remarkably high (95,5% - 100%) and could not be accounted for by the small group sizes of some VaD clusters. Conclusions: The neuropsychological differential diagnosis of AD vs. VaD can be improved by partitioning the VaD into more homogeneous subgroups. These subgroups need to be related to neuroimaging measures in further analyses to investigate their pathophysiological relevance.

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Long-Term Outcome of Depressive Pseudodementia in the Elderly

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Background: The term depressive pseudodementia has proved to be a popular clinical concept. Little is known about the long-term outcome of this syndrome.

Aims: To compare depressed elderly patients with reversible cognitive impairment and cognitively intact depressed elderly patients.

Methods: All patients suffering from moderate or severe depression admitted to St Margaret's Hospital, UK as inpatients or day hospital outpatients between January 1 1997 and December 31 1999 (n=182) were screened for entry into the study. Eligible patients were divided into those presenting with pseudodementia and those who were cognitively intact and followed up for 5 to 7 years.

Results: Seventy one percent point four percent of those suffering from pseudodementia had converted into dementia at follow up compared to only 18.2% in the cognitively intact group. The relative risk was 3.929 (95% CI: 1.985 to 7.775) and the 'number needed to harm' 1.88.

Conclusions: Reversible cognitive impairment in late-life moderate to severe depression appears to be a strong predictor of dementia. Patients with pseudodementia should probably have a full dementia screening, comprehensive cognitive testing and ongoing monitoring of their cognitive function.

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Neuropsychological Aspects of Cognitive Impairments Associated With the Body Imbalance

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Purpose: Possibility that the dementia in elderly people may accompany body imbalance was examined. Methods: 96 subjects with the mean age of 81.7±0.5(SEM) years old first underwent two kinds of neuropsychological assessment to classify the cognitive impairments into three grades, i.e. A, B+D, and C, having a mean test score of 24.8±0.3, 20.9±0.6, and 14±0.9, respectively. The procedure had been reported more in detail (AD/PD/2005). To study the body sway, each subject was asked to keep upright posture on a posturographic apparatus during the eyes open and closed. The length of body sway or LNG in cm, and the power spectrum of low(0.02~0.2Hz), medium(0.2~2.0Hz), and high(2 ~20Hz) frequency component of the sway in log scale were studied. Results: An inverse relationship between LNG and the mean test scores was obtained. In other words, a greater body unbalance equivalent to a longer LNG occurred at low test scores, and vice visa. In each subject, the relative power of three frequency components of a LNG was also plotted against the three grades or a scale of the test scores. Our records at grade A showed that the power of low frequency component during eyes open decreased by 6.8 % immediately after eyes closed. Instead, the power of medium frequency increased by 6.2 % as a compensation of body unbalance seen only at highest test scores. Conclusions: The body imbalance caused by deprivation of visual signals differed in magnitude according to the grade of cognitive impairments expressed in terms of neuropsychological test scores.

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Role of Connections of Degenerating Hippocampus for Symptom Induction in Alzheimer's Disease

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Background: Alzheimer's Disease (AD) is most common form of dementia. As hippocampus is part of brain which undergo neurodegeneration in initial stages of disease with disease symptoms, so its afferent and efferent connections have a prominent role for induction of neurological and psychiatric symptoms of AD. Objectives: Pathophysiological effects of neurodegeneration in hippocampus would be covered with relative therapeutic methods. Methods: It is a review to study pathophysiological effects of hippocampal neurodegenration on parts of brain, which receive efferent and give afferent to hippocampus. Results: Afferent connections of hippocampus are with entorhinal cortex, indusium griseum, cingulate gyrus, contralateral hippocampus, septal neclei, gyrus rectus, nucleus accumbens, mammilary body and ventromedial hypothelemic nucleus. Efferent connections are to cingulate gyrus, internal capsule, anterior nucleus of thalamus, medial thalamus, septal nuclei, amygdaloid nucleus, tegmentum of mid-brain, lateral peroptic area, anterior part of hypothalamus and habenular nuclei. As hippocampal neurons degenerate so parts of brain receiving afferent and efferent connections from hippocampus would have an alteration in physiological function. With respect to afferent, it is difficult to restore normal function of afferent sending areas to degenerated hippocampus in AD because afferent receiving structure is mostly degenerated. But efferent-receiving areas could have a role to decrease disease symptoms, if efferent neurotransmitter from hippocampus to respective area restored. Conclusion: In this review, methods to restore function of parts receiving efferent from degenerated hippocampus are discussed. These methods are either supply of relevant neurotransmitter or managing receptor response to area receiving efferent from degenerated hippocampus in AD.

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Restless Legs Syndrome After Electricity-Induced Spinal Cord Injury

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Background: The pathophysiology of restless legs syndrome (RLS) is complex and has not been completely elucidated. Dopaminergic dysfunction and iron metabolism

have been suggested as the underlying mechanisms. There have also been reports of an association between RLS and structural spinal cord lesions. Injury due to high-voltage electricity can cause delayed spinal cord injury.

Methods: From 1995 to 2005, we prospectively recruited patients who developed RLS after electricity-induced delayed myelopathy. All patients had symptoms and signs of myelopathy, and a central conduction defect was observed on studying the posterior tibial somatosensory-evoked potential.

Results: A total of 1,306 patients were admitted to our hospital due to electrical burns. Of them, 19 were diagnosed with electricity-induced delayed myelopathy. After 4 months (1–6 months), 4 patients (21%) developed RLS. In all patients, nerve conduction studies of the low extremities yielded normal results; the haemoglobin level was above 10.0 g/dl, and the creatinine level was below 1.0 mg/dl. The patients responded well to L-dopa or dopa agonists.

Conclusions: RLS is a relatively common complication of spinal cord injury, especially that induced by electricity. Because RLS patients respond well to medication, careful examination of their medical history can alleviate their symptoms

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Parkinson's Disease: Role of PINK1 in Mitochondria Dysfunction

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The pathological hallmark of Parkinson's Disease (PD), a progressive neurodegenerative disorder, is characterized by loss of dopaminergic neurons in the substantia nigra and presence of cytoplasmic inclusions termed Lewy bodies in affected brain areas. The etiology of PD remains unknown, although clinical and experimental evidence implicate the involvement of mitochondrial dysfunction and oxidative stress. The case for mitochondrial dysfunction is most persuasive in the PD field. This mitochondrial connection started when it was demonstrated that the exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine caused parkinsonism in humans and laboratory animals.

Rare hereditary forms of PD have provided insights into the molecular pathways of this disorder. Mutations in at least five genes have been linked to PD including alpha-synuclein, parkin, DJ-1, LRRK2 and PINK1. The mutations found in PINK1 (PTEN-induced putative kinase 1) are associated with recessive forms of early onset PD. The PINK1 gene encodes a 581 aminoacid protein with an N-terminal mitochondria targeting peptide and a putative serine/tyrosine kinase domain. The potential targeting of PINK1 to the mitochondria suggests that this protein may play a role in protecting cells from loss of mitochondrial membrane potential and possibly oxidative stress.

In our studies we focused on understanding the role of PINK1 in mitochondria dynamics and function. For this we determined the localization of PINK1 in the mitochondria through confocal immunostaining and fractionation studies. Additionally, we analyzed the effect that down-regulation of PINK1 caused on apoptosis, mitochondrial membrane potential and mitochondria morphology. Furthermore, these findings lead to a mechanistic pathway between mitochondrial dysfunction and PD.

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Signs of Apoptosis of Immunocompetent Cells in Patients With

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Parkinsonism is widespread disease of central nervous system,which affects mainly extrapyramidal system.Main pathologic feature of parkinsonism is degeneration of dopaminergic neurons of substantia nigra.It results in disbalance of other neurotransmitters. Immunopathologic mechanism may involve in this process.

Aim: Aim of this study was to learn apoptosis of immunocompetent cells of peripheral blood in patients suffering from parkinsonism.

Materials and methods: 66 patients aged from 44 to 82 years(29 male and 37 females) were investigated.Mean age was 65 years . Besides anamestic and neurological investigation immunoassay tests were made. These tests consisted of determination of Fas-receptors CD-95,which are markers of apoptosis. Obtained data were compared with values of control group(66 persons) and processed statistically.

Results: All patients showed clinical signs of parkinsonism-hypomimi expression,hypodynemia,muscle rigidity,tremors,vegetative and emotional disorders,monotonous speech,postural instability.Immunoassay examination showed reliable($p<0,001$) increase of CD-95 in patients(22,606+/-0,02) as compared to control group(7,0+/-0,02). All patients were divided in to 3 groups regarding disease duration-33 pts with disease duration less than 5 years,25 pts-from 5 to 10 years,8 patients-more than 10 years. Direct correlation was found. In first group CD-95 level was 19,485%, in second group – 25,24%, in third group – 27,25%.

Conclusion: Performed study showed increase of Apo-Fas-receptors level in parkinsonism.This level correlates with disease duration and severity. This fact confirms participation of apoptosis in pathogenesis of parkinsonism.

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Theory of Parkinson's Disease: Mechanisms of Clinical Phenomena

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This research is a further development of the theory originally described a decade ago (Baev, 1995, Neurol. Res. 17, 38-48; Baev, 1997, Progr. Neurobiol. 51, 129-166). According to it, skeletomotor cortico – basal ganglia – thalamocortical loop, the highest motor control level, possesses an internal model of object behavior (feedforward model). This model is a predictive mechanism that can function properly only when it receives error signals, i.e., signals that are generated when there are prediction errors. Dopaminergic neurons of the substantia nigra pars compacta are an important part of the error distribution system that

conveys each signal about prediction error to a specific basal ganglia subcircuitry that made the prediction error. Within this theoretical framework, Parkinson's disease is conceptualized as the disease of error distribution system. Death of dopaminergic neurons leads to incorrect predictions and, hence, to erroneous controlling commands sent to lower motor control levels. Parkinsonian clinical motor symptoms find good explanation within this theoretical framework. Skeletomotor loop is a hierarchical system, and motor symptoms depend on the hierarchical level that makes prediction errors. Mechanisms of functional neurosurgical procedures like partial lesion of basal ganglia structures or their deep brain stimulation also find good explanation based on the proposed theoretical ideas.

Supported by: St. Joseph's Hospital and Medical Center

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Phospholipid Methabolism and Free Radical Peroxidation Disorders in White Rats Erythrocytes Membranes in Corazolum-Induced Epileptoid Seizures

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The attention neurologysts is focused on the molecular mechanisms of pathogenesis of acute epileptoid seizures. In this study we investigate qualitative and quantitative disturbances of phospholipids (PL) in erythrocyte membranes (EM) white rats with experimental epileptoid seizures induced by corazolum and to search possible approaches for the most efficient antioxidant defense conditioning by vitamin E and sodium thiosulfate (STS). Studies were performed with 50 unbred male albino rats weighing 180-200 g, which received single intramuscular injections of corazolum (dose, 8-9 mg per animal), STS (1 mg per animal), and vitamin E (0.4 mg per animal). According to the data obtained acute epileptoid seizures are characterized by the considerable interfractional shifts of PL. A marked decrease in the content of phosphatidylcholines (PC) was accompanied with an adequate increase of the level of lysophosphatidylcholines (LPC), which can be regarded as activation of phospholipase A2. Next, we attempted to determine the actual role of ultralow concentrations of vitamin E and STS as factors known for their pronounced antioxidant properties and have a mobilizing effect on endogenous antiradical defense in cells. A characteristic feature observed in the case of corazolum-induced epileptoid seizures was permanent prevalence of LPC over of their content in control. The observed statistically significant prevalence of the content of LPC is the subject of a special discussion in the light of role of LPC in the induction of interferon, maintenance of immunological status of the organism, and, therefore, functioning of regulatory mechanisms of cell activity in general.

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Tissue Plasminogen Activator (TPA) Induces Microglia Activation Through a Molecular Mechanism Involving Erk1/2 Activation

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Tissue plasminogen activator (tPA) participates in relevant physiological roles in brain such as memory and learning but it has also been involved in a number of pathological situations where it can be either neuroprotective or mediator of cell death. In a recent report (Medina et al, 2005) we have described a relationship between tPA and Alzheimer's disease (AD): i) tPA is over expressed in amyloid rich areas and senile plaques of AD; ii) tPA induces activation of Erk1/2 intracellular signalling, tau phosphorylation and neuronal death in hippocampal neurons; iii) tPA mediates amyloid toxicity in vitro.

Neuroinflammation around senile plaques is a persistent pathological hallmark in AD and it has been proposed that high levels of Aβ induce microglial activation and the release of several pro-inflammatory cytokines that can cause neuronal cell death. Previous reports have described that microglia cells produce tPA and that this protease mediates microglial activation via a non-proteolytic mechanism. However the molecular mechanism leading to these events have not been elucidated. Here, we have analyzed the molecular pathways induced by tPA in mixed and purified microglial primary cultures. We show that tPA induces a dramatic change in the morphology of both cell cultures and also an increase in the production of reactive oxygen radicals, consistent with the role of tPA in glial activation. We also report an activation of Erk1/2, AKT and JNK pathways in response to tPA both in mixed and microglial cells, suggesting that these pathways could be responsible of tPA-mediated activation in microglial cells.

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There is a Dysmetabolic Guam Syndrome?

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The authors studied 3 cases (2 women and 1 man), mean age 40 years old, with Guam syndrome through severe hypoglycemia(23mg/dl),recurrent, determined by a pancreatic insulinoma.

The patients presented : resting tremor with asymetric onset, bradykinesia, rigidity - without any response at levodopa drugs,early dystonia at legs with rapid course in the last 6 month. It were also associated variable cognitive

disorders and psychomotor seizures preceded by confusive episodes,especially in the morning.

At addmission in our clinic, in all 3 cases we find parkinsonian syndrome with elements of limbic dementia, partial amiotrophy at shoulder girdle and fasciculations.

Electrophisiological exams confirm the signs of denervation atrophy.

Cerebral and spinal MRI were normal.

EEG: diffuse alteration type theta, delta, without focalisation.

In the hospital, the patients presented severe hypoglycemia episodes (23-29 mg/dl)and agravation of the pshychomotor seizures.

Paraclinical investigation revealed in all 3 cases the presence of a pancreatic tumor type secretant insulinoma.

Excision of the tumor goes to disappearance of hypoglycemia and all of neurological manifestation.

The authors disscuss the physiopathological mechanisms which interfere in the neurological picture of hypoglycemia - Guam syndrome.They insist on the implication of hypoglycemia in the sufference oh the hipocampus, ventro-lateral talamus,basal ganglia,cerebellum, brainstem and anteror horn cells in the spinal cord.

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Phonological Processing in Parkinson's Disease and Normal Ageing: Evidence From Nonword Reading and Repetition

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We explore how Parkinson's disease (PD) affects metalinguistic abilities - specifically phonological processing. As our previous research has shown that individuals with PD have impaired phonological abilities, we predict that tasks which place heavy demands on phonological processing will be particularly problematic for individuals with PD. We first examined errors in a word and nonword reading task. We found an interaction between experimental group and word type, whereby individuals with PD made more pronunciation errors when reading nonwords, compared to controls and their own performance on matched words (e.g. "sleep"-"sleeb"). In a second task, participants were asked to repeat high phonotactic probability (highly word-like) nonwords and low phonotactic probability (low word-like) nonwords. The 'wordlikeness' of novel stimuli provides a very good predictor of their repetition accuracy. Repetition accuracy of highly wordlike stimuli is greater as both phonological knowledge and phonological working memory contribute to the maintenance of the novel phonological form. For less word-like stimuli, little lexical support is available, thus individuals are more dependent on representations in phonological working memory to retain the novel stimuli for repetition. As expected, individuals with PD made more pronunciation errors overall and more when repeating low vs. high phonotactic probability nonwords than controls. Taken together, results are consistent with the hypothesis that individuals with PD have impaired phonological ability and have difficulty successfully completing tasks that place demands on phonological processes. We argue that damage to frontal-systems leads to a general metalinguistic deficit, including a general phonological deficit.

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Dementia With Distinctive Brain Histopathology in Spinal and Bulbar Muscular Atrophy (Kennedy´S Disease)

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Background: Kennedy's disease has so far not been connected with dementia.

Case report: We describe here clinico-pathologic results obtained from a patient (age 51) with a 20 y history of a typical Kennedy's disease (48 CAG repeats in exon 1 of the androgen receptor gene) who developed, in addition, a dementia at age 48. The patient deceased as a result of the dementia after 3 years. Clinically the dementia was classified as a frontal lobe variant of FTLD. Both brothers (age 45 and 56) of the patient were also affected with Kennedy's disease but had no dementia. Histopathology on brain and spinal cord showed neurons with cytoplasmic Pick-like inclusion bodies in neocortex, striatum and entorhinal cortex. These were partially immunoreactive with anti-polyglutamine antibody 1C2. The inclusions were negative for: Ubiquitin, Tau, beta-amyloid, prion protein, alpha-synuclein and silver impregnation. CAG repeat length was identical in brain and peripheral blood lymphocytes. Since CAG repeat mutations usually result in nuclear protein aggregation yet the patient showed cytoplasmic protein aggregates, we sequenced exons 2 to 8 of the androgen receptor gene for mutations of the relevant nuclear localization signals with no results. Mutations for Huntington's disease or myotonic dystrophy 1 (DM1) were not present.

Conclusion: The presented case strongly suggests a causal association between Kennedy's disease and dementia, mostly because the present dementia was clearly distinct from known dementias such as AD, DLB, FTLD, and including tauopathies, ubiquitinopathies, TSE, HD and DM1.

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Relationships Among the Number of Remaining Teeth, Cognitive Function,and Adl in Elderly People

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Background and aims: The objective of this study is to investigate and measure the relationships among the number of remaining teeth, cognitive function, and ADL in a group of elderly people.

Methods: A sample population of 96 community-dwellers aged 60 years and over was recruited on an island in Shimane,

Japan. The subjects were divided into two groups: those with 10 or more teeth and those with 0-9 teeth. The Revised version of Hasegawa Dementia Scale (HDSR) and the timed up and go test were used to assess cognition and ADL.

Results: The mean number of remaining teeth was 15.3. The HDSR in those with 10 or more teeth were significantly higher than in those with 0-9 teeth. Those with 10 or more teeth also showed significantly better results in the timed up and go test than those with 0-9 teeth. There were significant correlations between the number of remaining teeth and cognitive function, and the number of remaining teeth was related to the timed up and go test in all subjects.

Conclusions: Our results suggest that elderly people with fewer remaining teeth have a higher risk for cognitive and ADL decline.

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Amnesic Syndrome in a Unilateral Mammillothalamic Tract Infarction

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Background And Aims: It is controversial whether isolated lesions of mammillothalamic tract (MTT) produce significant amnesia. Since the MTT is small and adjacent to several important structures for memory, amnesia associated with isolated MTT infarction has been rarely reported. We report a patient who developed amnesia following an infarction of the left MTT that spared adjacent memory-related structures including the anterior thalamic nucleus.

Methods: Personal observation.

Results: The patient's memory deficit was characterized by a severe anterograde encoding deficit and retrograde amnesia with a temporal gradient. In contrast, he showed no frontal executive dysfunction and personality change that are frequently recognized in the anterior and medial thalamic lesion.

Conclusions: We postulate that pure amnesic syndrome can develop following discrete lesions of the MTT.

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Verbal Fluency in Patients With Occipital-Mesial Temporal Stroke

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Background & aim: In Alzheimer's disease (AD), language disturbance usually presents with initial word finding difficulty progressing to anomia and impaired comprehension. There are also an inability to retrieve words with circumlocution and poor word-list generation, particularly for words in a given semantic category in early stage of AD. Mesial temporal area such as hippocampus and parahippocampal region is involved earlier in AD. We investigate the feature of verbal fluency in patients with occipital-mesial temporal stroke distributed by posterior cerebral artery (PCA). Methods: Eight patients (6 men and 2 women with ranged from 53 to 73 years) with acute PCA infarction were selected. Controlled oral word association test was performed. Results: The stroke lesion was confined to mesial and basal temporal area, and occipital lobe in all patients. The lesion was located in only the cuneus in one and the remaining seven patients had the lesion involving the lingual gyrus and the occipital part of fusiform gyrus. Four of the former seven patients also had the parahippocampal lesion. Comparing with phonemic word fluency, semantic word fluency was significantly impaired in all stroke patients with the lingual and occipital part of fusiform gyrus. In contrast, no difference of performance in both word fluency tests was observed in the patient with cuneus lesion. Conclusions: The decline of semantic verbal fluency observed in the early stage of AD might be more related with the dysfunction of the lingual and the fusiform gyrus than parahippocampal gyrus.

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Peripheral Arterial Occlusive Disease in Dementia With Ischemic Cerebral Vascular Accident

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Background and aims: The toe-brachial index(TBI) has been receiving attention for non-invasive method for measurement of atherosclerosis which is generally regarded as a risk factor for vascular factor in dementia. In this study, we investigated the relation between a low TBI and vascular factor in dementia.

Methods: We reviewed Ilsan Hospital stroke database of recent 3 years. Total 50 vascular dementia patients and TBI study was included.

Results: According to degree of TBI, peripheral arterial occlusive disease(PAOD) was classified as normal (TBI>0.8), borderline (0.6<TBI<0.8) and abnormal (TBI<0.6). Abnormal TBI(PAOD) was observed in 9(18%) patients. PAOD was more common in large artery atherosclerosis patients than small vessel occlusion and cardioembolism patients between subgroups by TOAST classification.

Conclusions: PAOD was observed in 18% and more common in large artery atherosclerosis patients among vascular dementia patients. Toe brachial index can be a useful marker which reflects the degree of peripheral atherosclerosis for vascular factor in dementia.

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Neuropsychological Assessment of Progression and Conversion to Alzheimer's Disease in MCI

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Background and Aims: Alzheimer's disease is characterised by longitudinal cognitive decline. Accordingly,

this study employed a longitudinal design to identify optimal diagnostic criteria and accurate predictors of progressive cognitive decline leading to AD. Our aim was to examine the profile of impairment in patients with mild cognitive impairment (MCI), the proposed amnestic prodrome of AD, who progressed to frank dementia according to NINCDS-ADRDA criteria (McKhann et al. 1984).

Methods: Twenty-three patients with MCI, were administered a detailed neuropsychological test battery with emphasis on different memory components, incorporating semantic, episodic, and associative learning tasks. Additionally, a novel associative learning battery was developed. The novel battery incorporated four stimuli: famous buildings and famous faces which are familiar stimuli requiring semantic processing; and patterns and unfamiliar faces which are novel with no semantic content. Patients were tested at baseline and after 12 months.

Results: Neuropsychological performance of MCI patients who progressed to Alzheimer's disease after 12 months compared to those who did not progress, was best characterised by impairment in episodic delayed recall, associative learning of novel stimuli and semantic recall.

Conclusions: The results suggest that associative learning is an important aspect of episodic memory in early assessment, but also specific semantic deficits are of clinical relevance in defining those at increased risk of developing Alzheimer's disease.

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Spatial Associative Learning in Mild Cognitive Impairment and Behavioural Variant Frontotemporal Dementia

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Background: Patients with behavioural variant frontotemporal dementia (bvFTD) are widely held to have preserved episodic memory compared to Alzheimer's disease inclusive of preserved spatial associative memory, yet recent studies have cast some doubts upon this assumption.

Aim: The present study investigated episodic memory in bvFTD and amnestic mild cognitive impairment (MCI).

Method: 11 bvFTD and 23 MCI patients were administered a series of novel spatial associative learning tasks (SAL) and a battery of neuropsychological tests covering language, visuospatial functioning, executive functioning, attention, working memory, episodic and semantic memory and global cognition.

Results: bvFTD patients performed significantly worse than controls and were indistinguishable in performance from MCI patients on a novel SAL task. Pertaining to task demand, we hypothesised that bvFTD performance may be due to a genuine memory deficit, working memory or executive deficit. SAL scores significantly correlated with visuospatial function, executive function, episodic and semantic memory. There was no significant correlation with language, working memory, intelligence or attention.

Conclusions: These findings suggest that there is a subtle memory deficit in bvFTD, particularly sensitive to spatial associative learning tasks. Executive impairment may be contributing to this memory impairment by influencing ability to implement an active strategy for effective learning and retrieval of information. The finding of episodic memory problems in bvFTD makes differential diagnosis difficult, suggesting emphasis should be placed on personality and behavioural symptoms in bvFTD to discriminate it from MCI.

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Predicting the Preservation of Object and Action Knowledge in Alzheimer Disease and Focal Brain Injury Patients

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Previous studies have yielded inconsistent evidence concerning the relative preservation of action and object knowledge in Alzheimer´s patients.

We investigated the confrontation naming of three groups of Spanish-speaking individuals, extending research on action and object naming to a morphologically rich language.

We were interested in the possibility that variation in the type of brain injury could impact on the relative preservation of object and action knowlege. Thus we compared naming in elderly controls with naming in patients with focal (aphasic patients) or distributed (patients diagnosed as probable Alzheimer's) brain injury.

Participants were asked to name pictured 50 objects and 50 actions matched on frequency, Age-of-Acquistion (AoA), imageability, length, visual complexity and name agreement.

We found no significant difference between the mean accuracy of object compared to action naming in any group. However, the comparison of group means hides important features in the character of naming performance.

Regression analyses indicated that the predictors of object and action naming accuracy differed in each group. In Alzheimer's patients, naming accuracy was unaffected by grammatical class but was affected by name agreement. In contrast, aphasic naming was predicted by a main effect of AoA and the interaction of AoA and class. Control performance was at ceiling. In both groups, actions tended to elicit visual confusion and unrelated errors, whereas objects elicited more coordinate semantic errors and null responses.

Our data indicate significant differences between patient types, and between object and action performance, not in overall accuracy but in the significance of performance predictors.

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Clinicopathological Correlates of Alzheimer Disease in a General Autopsy Series From Brazil

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Background and Aims: The current used neuropathological staging models of

AD have been developed in the last 20 years. Nevertheless, they were mostly tested in Caucasians of northern European ancestry or Asians .A comparison of those criteria had never been done in a Brazilian series. Our aim was to examine which neuropathological criteria best discriminated AD from normal aging in a clinicopathological series of 76 subjects belonging to the Brazilian Brain Bank of the Aging Brain Study Group. Methods: The cases were clinically and neuropathologically fully evaluated. Immunohistochemistry methods were used in every case. Clinical status was assessed through an interview with a reliable informant. CDR score was compared to Braak staging, CERAD score and Ronald Reagan–NIA score (RR). Subjects with other neuropathological diagnosis such as vascular changes and PD were excluded. Results: The CDR distribution among the 57 selected subjects is: CDR0=28; CDR0.5=12; CDR1=4; CDR2=5; CDR3=8. CERAD score showed the best correlation (table 1). In nine subjects, classification according to RR was not possible due to discrepancies between the number of neuritic plaques and neurofibrilary tangles. Conclusion: In this series, the CERAD criteria better discriminated between the CDR groups, however closely followed by Braak Staging

Table 1. Clinicopathological correlation, by score. The "r" and "p" values are depicted on first and second lines, respectively

	CDR	CERAD (N=57)	BRAAK (N=57)	RR (N=48)
CDR	1.0000			
CERAD	0.5303* 0.0000	1.0000		
BRAAK	0.5294* 0.0000	0.7563* 0.0000	1.0000	
RR	0.5076* 0.0002	0.9189* 0.0000	0.9102* 0.0000	1.0000

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Research of Aspiration Pneumonia in Patients With Cerebral Infarction

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Background and Aims: Aspiration pneumonia is often a complication in patients with cerebral infarction. Pneumonia is the fourth cause of senior citizen death in Japan and its prevention is important in the extension of senior citizen longevity.

Methods: We investigated factors related to the attacks of pneumonia in our recovery stage ward. The subjects were 91 stroke patients discharged from our recovery stage ward between January 1, 2003 and December 31, 2005. Difference in the rates of pneumonia were analyzed with regard to age, sex, number of comorbidities, regions of the cerebral infarction, cilostazol treatment, smoking, the presence of diabetes mellitus, length of hospitalization and housing after discharge. Details were investigated using logistic regression analysis.

Results: The incidence of pneumonia in three year follow up was 6.6%.

Conclusions: A strong correlation in the apperance of pneumonia was observed for sex, number of comorbidities, region of cerebral infarction, cilostazoke treatment, length of hospitalization and housing after discharge.

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Down Syndrome With AD: Case With Extensive Subcortical NFT, CAA and Infarct

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Background: Down's syndrome patients (DS) usually develop Alzheimer's disease (AD) with an increased risk of cerebral amyloid angiopathy (CAA) resulting in strokes. As in AD without DS, the amyloid cascade hypothesis suggests that excess amyloid beta production and deposition due to overexpression and proteolysis of APP (amyloid precursor protein) leads to neurofibrillary pathology in the form of neuritic plaques (NP) and neurofibrillary tangles (NFT).

Methods: The brain of a 48 year old man with DS, dementia, seizures, spasticity and falls was studied for the presence of NP, NFT and neuropil threads (NT) using an antibody to tau (Tau5). Congo red was used to visualize fibrillar amyloid in NP and vessels (CAA).

Results: In addition to an infarct, there was extensive AD pathology in both cortical and sub cortical sites, particularly in hypothalamic and thalamic nuclei. CAA affected both the cerebral and cerebellar cortices. Of 19 brain regions containing NFT (selected for their relevance to AD pathology

and associated projection areas) 14 also had congophilic plaques. The remaining 5 regions lacked fibrillar amyloid.

Conclusions: Enhanced amyloidogenesis in DS enables testing of the amyloid hypothesis of NFT formation. Lack of such topographic association in as much as 26 % of sites with extensive NFT argues against an obligatory relationship between amyloid deposition and neurofibrillary pathology. The prominence of subcortical NFT in this case may correlate with the propensity for falls. CAA affecting cerebellar cortex without amyloid deposits in the underlying parenchyma also questions a mandatory relation between parenchymal amyloid deposition and CAA.

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Stance Adaptation to Unilateral Achilles Tendon Vibration in Parkinson Disease and Vascular Dementia Patients

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In Parkinson Disease (PD) and Vascular Dementia (VD) patients, postural instability represents one of characteristic clinical signs of advanced disease stage. The pathophysiological mechanisms of posture instability are not fully explained. Aim of our study was to examine patient ability of upright posture adaptation to unilateral Achilles tendon vibration.

Vibration stimulation during stance was unilaterally applied on right and left Achilles tendon. Vibration (fr. 100 Hz, ampl. 1mm, duration 20s.) was started 10 seconds after beginning of measurement. DC motor with eccentric weight was used as vibrator. Changes in the center of foot pressure (COP) were measured in anterior-posterior and lateral direction. The measurement lasted 50 s. The mean positions of COP were determined for the following measurements periods 0-10 s., 20-30 s. and 40-50 s. Amplitude and direction of mean COP displacements were calculated.

Unilateral vibration of Achilles tendon in control subjects induce body shift backwards and to unstimulated side until subjects reached new mean position of COP. The same standing behavior was observed in PD patients. However, the amplitude of COP displacement has tendency to be smaller than in control subjects. In VD patients, the vibration evoked large COP displacement, which resulted in the falling reaction. VD patients have much more problems to keep upright position during vibration compared to the PD patients. Our results indicate that VD patients may have a problem with re-weighting proprioceptive signal.

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Lipid Peroxidation in Alzheimer Disease: Mechanisms of Toxicity

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Substantial evidence exists for a role of increased lipid peroxidation in the pathogenesis of AD. The hallmark lesions of AD are marked by adduct formation with reactive lipoxidation-derived aldehydes such as 4-hydroxy-2-nonenal (HNE). In addition to our finding that HNE-protein adducts are seen diffusely distributed also in apparently normal neurons in AD, suggesting that oxidative stress plays a contributory role in the demise of neurons in the disease, numerous studies show that HNE is toxic to neurons in culture. At micromolar concentrations, HNE has been shown to trigger apoptosis, but at lower concentration, HNE appears to behave as a signaling molecule, possibly by interacting with transcription elements. Recent studies have shown that the lipid peroxidation cousin of HNE, 4-oxo-2-nonenal (ONE), is more reactive than HNE as a protein modification and cross-linking agent, and more toxic to neurons in culture. The higher reactivity of ONE suggests that it may be a more potent contributor to AD pathogenesis than HNE. The work reported will present comparative data on the toxicity of HNE and related lipid oxidation products to neurons in culture, and on the mechanisms responsible for cytotoxicity. These data will be correlated with the relative levels of lipoxidation-derived aldehyde protein modification in AD tissue.

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Is There a Morphological Substrate of Parkinsonian Rest Tremor? A Voxel-Based Morphometry Study

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Objective: To investigate morphological substrates of rest tremor in patients with Parkinson's disease (PD).

Background: Rest tremor is a hallmark of PD and its pathogenesis remains incompletely understood. Nigrostriatal dopaminergic deficiency correlates with bradykinesia, but not with rest tremor. A central oscillator originating in an interaction of basal ganglia- and cerebello-thalamo-cortical ciruits is postulated to cause tremor.

Methods: We investigated 24 men (median, range [years], age 62, 55-70, at onset 55, 42- 67, disease duration 7, 3-20) with mild to moderate PD (Hoehn Yahr [HY] 2 and 3). Exclusion criteria were advanced PD (HY 4-5), dementia and other significant brain pathology. Patients with (n=14) and without rest tremor (akinetic-rigid patients; n=10) were compared. Demographical and clinical profile were similar, except for higher prevalence of motor fluctuations (90% vs 14%, $p<0.01$) and impaired balance (100% vs 50%, $p<0.01$) in akinetic-rigid patients. Voxel-based morphometry of 3 T T1-weighted MRI image, pre-processed with optimized protocol using SPM2, was performed. The resulting normalized grey matter volume distributions of the two groups were tested for local differences by a voxel-wise ANCOVA.

Results: Grey matter density is decreased in the right > left posterior quadrangular lobe and declive of the cerebellum in PD with tremor compared to akinetic-rigid patients ($P_{FDR} < 0.05$).

Conclusion: These results underscore the involvement of the cerebellum and cerebello-thalamo-cortical circuit in the pathogenesis of Parkinsonian rest tremor.

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Intracellular Iron: Quantitative Element Microscopy of Perineuronal Net-Ensheathed Neuron

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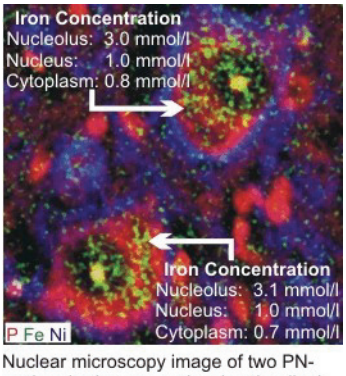
A subpopulation of neurons with less vulnerability against neurodegenerative diseases possesses a specialised extracellular matrix in form of a perineuronal net (PN). Due to its negatively charged chondroitin sulphate proteoglycans, the PN is able to bind iron specifically. Thus, the PNs possibly have a neuroprotective effect against iron induced radical generation by reducing the local oxidative stress in the neuronal microenvironment.

We have quantitatively analysed the elemental concentrations (especially iron) of perineuronal net-ensheathed neurons by using a nuclear microscopy technique (Particle Induced X-Ray emission). We prepared rat brain sections of substantia nigra, Subiculum and parietal cortex. The neurons with PNs were identified by lectinhistochemical staining with Wisteria floribunda agglutinin intensified by DAB-nickel enabling the identification of the PNs for nuclear microscopy analysis.

We could show a significantly higher concentration of cytoplasmic iron in PN-ensheathed neurons compared to neurons without a PN.

The difference in intracellular iron concentrations could be influenced by the PNs while acting as spatial buffer for iron ions. We hypothesise that there is an altered activity of iron specific proteins (transporters, reductases) in PN-associated neurons, which transform the iron into the less toxic Fe3+ with subsequent storage inside the cell. This could probably delay functional changes as well as metabolic imbalances in the course of Parkinson's and Alzheimer's disease.



Nuclear microscopy image of two PN-ensheathed neurons showing the distribution of P, Fe, Ni (100 µm × 100 µm).

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Standardization and Validation Study of Korean Behavioral Dyscontrol Scale

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Objective: The Behavioral Dyscontrol Scale(BDS) is a brief cognitive screening tool for detecting executive dysfunction. The authors examined the validity and reliability of the Korean version of the BDS (BDS-K) in elderly patients and normal controls.

Methods: The BDS-K, a Korean version of the Mini-Mental State Examination (MMSE), and neuropsychological batteries were administered to 104 elderly persons in an elderly outpatient clinic (mild Alzheimer's disease (AD)=25, Normal controls (NC)=79). Concurrent validity was evaluated using correlations with the MMSE. Receiver operating characteristics analysis was performed to assess the discriminatory ability of the BDS-K and to define cut-off scores for the detection of AD. Internal consistency and test-retest reliability were evaluated.

Results: BDS-K scores were highly correlated with those of MMSE. Using a cutoff score of 10/11, the BDS-K had an excellent sensitivity of 78% and a good specificity of 78% for the detection of AD. Internal consistency and test-retest reliability were good.

Conclusions: The results obtained show that the BDS-K is brief, reliable, and suitable for use as a screening tool for the detection of AD in primary care and elderly outpatient clinic settings. It may be used in vascular dementia screening.

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A Prospective Analysis of Parkinson's Disease as a Consequence of Head Injury

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Background: The etiology of Parkinson's disease (PD) remains uncertain. Head injury is an inconsistently reported risk factor for PD. A review of epidemiological studies of head injury and PD carried out by Goldman et al. (2006) showed relative risks between 0.6 and 6.2. Most reported studies were based on retrospective analyses, which may have a systematic bias, since patients seek an explanation for their illness and remember head injury as a possible cause. Prospective studies circumvent this problem. The aim of this study was to carry out a prospective analysis of the association between head injury and PD in a national population.

Methods: All people in Denmark aged 21-59 years 1 January 1996 (N = 2,905,713) were followed for hospital contacts due to head trauma (ICD-10: S00 - S09) during 1995 and for hospital contacts due to PD (ICD-10: G20) 1996-2004. Only principal diagnoses were regarded. An age and gender standardised morbidity ratio (SMR) was calculated to compare incidence rates of PD among those who received hospital treatment for a head injury during 1995 (N = 50,238) with the rates in the total population.

Results: We observed a total number of 9 cases of subsequent PD among the people with head injury in 1995. The expected number was 12.2, which yielded the SMR 0.738 (95% CI: 0.337 – 1.40).

Conclusion: At least in the short term perspective (9 years) there is no association between head injury and subsequent PD. Thus, head injury does not seem to trigger PD.

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Executive Dysfunction in Early Subcortical Ischemic Vascular Dementia and Early Alzheimer's Disease

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Introduction: Clinico-anatomical studies suggest that the site most commonly affected in subcortical ischemic vascular dementia (SIVD) is the frontal-subcortical region. Disruption to the frontal subcortical circuits is likely to result in executive deficits.

Objective: To compare the executive function of patients with early subcortical vascular dementia and early AD.

Methods: Retrospective analysis of patients with early SIVD and early AD who presented to the Singapore General Hospital memory clinic between January 2004 and June 2006.The clinical dementia rating scale (CDR) was used to identify patients with early dementia. SIVD was diagnosed by the NINDS-AIREN criteria and AD was diagnosed by the NINCDS-ADRDA criteria. The Frontal Assessment Battery (FAB) was used to evaluate the executive profile.

Results: We identified twenty two SIVD and forty two AD patients with a CDR score of 1.There were 35 male and 29 female patients. The mean age was 72.6 years and the mean MMSE score was 20.1.

Patients with SIVD showed statistically poorer performance in the FAB, digit cancellation task, symbol digit modalities test, WAIS-R Block design and auditory detection tests compared to AD patients. Performance on the digit span backward and visual span backward was also poorer among patients with SIVD but did not reach statistical significance.

Conclusion: Our findings suggest that patients with early SIVD have poorer performance in tests of attention, visospatial function and executive function compared to patients with early AD. The FAB which is simple and brief was effective in detecting executive dysfunction among patients with SIVD.

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Modification of Illness Perceptions: An Avenue for Treatment of Depression in MCI?

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Background & Aim: The high prevalence of depression in patients MCI has been well-documented. However, little is known about the psychological factors underlying adjustment to MCI. Illness perceptions have been found to predict variance in depression in a number of physical health complaints and interventions aimed at altering illness perceptions have been shown to improve outcomes. The aim of the present study was to investigate the relationship between beliefs about Mild Cognitive Impairment (MCI) and severity of depression.

Methods: This was a longitudinal study involving 98 participants with MCI. Illness perceptions and depression were measured at baseline and six months using a modified version of the Illness Perception Questionnaire (Revised) and the Geriatric Depression Scale.

Results: Illness perceptions were found to explain 33.6% of variance in depression, in addition to that explained by demographics, and duration and severity of cognitive impairment. Specifically: perceptions of serious consequences, strong personal blame and attribution of memory problems to mental attitude predicted higher depression in the cross-sectional analysis. At six month follow-up, baseline illness perceptions explained 23.4% of variance in depression scores with perceptions of serious consequences of memory problems and high personal blame for memory problems predicting greater depression.

Conclusion: Beliefs regarding MCI explained significant variance in depression scores. The findings suggest that interventions aimed at altering illness perceptions may be a promising new avenue for research into treatments to alleviate depression in people with MCI.

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Prevalence of Metabolic Syndrome as Defined by Different Criteria in Japanese Outpatients With Parkinson's Disease

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Objective: To estimate the prevalence of obesity and metabolic syndrome (MetS) in Japanese outpatients with Parkinson's disease (PD).

Patients and methods: We diagnosed MetS in 94 patients with PD (40 men, 54 women) and 102 patients with cerebrovascular diseases (CVD) (66 men, 36 women) according to criteria proposed by The Japanese Society of Internal Medicine in 2005, The International Diabetes Federation (IDF), and Adult Treatment Panel III (ATPIII). Age was 50-80 years in both groups.

Results: Mean BMI was about 23 kg/m2 in both PD and CVD. The prevalence of MetS in PD (men, 22.5%; women, 9.3%) was similar to that of general Japanese adults in the National Health and Nutrition Examination Survey Japan. According to the IDF criteria, 27.5% of men and 13.0% of women had MetS. When the ATPIII criteria were used, the incidence of MetS was 12.5% in men and 22.2% in women. The incidence of MetS in men and women with CVD was respectively 47.0% and 25.0% according to the Japanese criteria, 53.0% and 33.3% according to the IDF criteria, and 59.1% and 66.7% according to the ATPIII criteria. The

prevalence of MetS according to the APTIII criteria in Japanese patients with CVD was similar to that in general adults in the US.

Conclusion: The risk of atherosclerotic diseases as assessed by ethic-specific diagnostic criteria for MetS is similar in Japanese outpatients with PD and the general population. Strategies designed to reduce risk factors for MetS are urgently required for patients with PD.

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Preliminary Study on the Convergent Validity of a Telephone Version of the Mini-Mental State Examination

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Objectives: To determine the convergent validity of a telephone version of the Mini-Mental State Examination (tMMSE) for patients with Alzheimer's disease (AD).

Patients and methods: Longitudinal and observational study of a clinical sample consisting of patients with AD. The tMMSE was translated and retrotranslated into Spanish. Consecutive probabilistic sampling of participants was used and the patients were randomly assigned to two different study groups depending on the administration of the in-person/telephone test (P-T) or telephone/in-person (T-P) test. Convergent validity was determined by calculating Pearson's Correlation Coefficient (PCC). Multivariate linear regression analyses were used to determine the effect of hypoacusia and education on test administration and performance.

Results: The sample consisted of 35 patients per group (n=70). There were no significant clinical differences between both groups. Mean time between administration of the two versions was 10.65 days (SD=3.6) with no differences between the two groups (p=0.895). The overall PCC for the MMSE and the tMMSE scores was 0.767. PCC was 0.851 for the P-T group and 0.686 for the T-P group. The effect of education or schooling years and the auditive state did not affect patient performance on the tMMSE, accounting for only 2.75 of the score variability.

Conclusions: The tMMSE provides good concurrent validity and can be a useful tool to estimate the MMSE score in AD patients that are unable to attend healthcare centres on an out-patient basis.

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Validity and Reliability of Quantitative Gait Measures in Geriatric Patients With and Without Dementia

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Background and Aims: Gait and mobility problems are prevalent in elderly people and associated with falls and loss of independence, even more in dementia. Early detection of gait and mobility problems can prevent adverse outcomes. We investigated reliability and validity (sensitivity to change) of quantitative gait measures in geriatric inpatients with and without dementia.

Methods: Cohort study of consecutively admitted geriatric inpatients. Dementia was diagnosed with DSM-IV-TR. Gait velocity (Gaitrite), Timed Up and Go-test (TUG) and other mobility tests were measured on admission and two weeks later. Direct and two-week test-retest reliability in stable patients was expressed in Intraclass Correlation Coefficients (ICCs). Three experts decided from video recordings about the clinical relevance of changes in gait, defined as change in the expected risk of falling. Sensitivity to change was expressed as responsiveness index (RI).

Results: 85 patients (mean age 75.8, 46 female) participated. Demented patients (N=39, mean MMSE 19.1±5.2) were older and had more comorbidity. Mean gait velocity was similar (0.61 vs 0.65 m/s). Demented patients had slower TUG-times (29.8 vs 19.3s), lower Berg Balance Scores (39.6 vs 45.1) and higher stride variability (all $P<0.05$); ICCs were comparable for both groups and ranged from 0.74 to 0.95. Gait velocity and TUG were most sensitive to change; RIs were highest in demented patients: RI gait velocity 7.2 vs 3.8 and RI TUG 4.8 vs 1.8.

Conclusion: Quantitative gait analysis with an electronic walkway and mobility tests was reliable and sensitive to relevant changes in geriatric patients, even in case of dementia.

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Progression of Episodic Memory and Executive Dysfunction in Minimal and Mild Alzheimer's Disease

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Episodic memory and executive dysfunction are among the first symptoms of Alzheimer's Disease (AD). The aim of this study was to determine the progression of these deficits early in the course of AD. Three groups of participants matched on age, education, physical health and depressive symptoms (all $p > .10$), namely 15 patients with minimal AD (10F-4M; MMS : 24-30), 12 patients with mild AD (9F-3M; MMS : 18-23), and 10 normal controls (9F-1M; all MMS $\geq$ 27), were studied. Episodic memory was assessed with Buschke's Free and Cued Selective Reminding Test (FCSRT, Buschke, 1984), and executive functions with the Frontal Assessment Battery (FAB, Dubois, 1997). Minimal AD patients had lower performance than controls on FCSRT measures of free recall ($p = .0001$, delayed recall ($p = .005$), and total score ($p = .0001$), but did not differ from controls on cued recall and recognition (both $p > .10$). Minimal AD patients also differed from controls on the total FAB score ($p = .0001$), and on FAB subtests of mental flexibility (p.=.005), motor programming (p.=.007), sensitivity to interference (p.=.050) and inhibitory control (p.=.022). Minimal AD patients were comparable to controls on FAB subtests of conceptualization and environmental autonomy (both $p > .10$). Performance of minimal and mild AD patients did not differ on any measure (all $p > .10$). Results suggest that selective aspects of episodic memory and executive function are

affected in minimal AD relative to controls, but further impairment does not occur from minimal to mild AD.

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Visual Perception and Mental Imagery in Mild Alzheimer's Disease

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The aim of this study was to determine whether visual perceptual and mental imagery abilities are affected in patients with mild Alzheimer's Disease (AD). The Battery of Mental Imagery (BMI) is a new set of tasks designed to assess a wide and comparable range of visual perceptual and mental imagery abilities relying on the dorsal spatial-properties system or on the ventral object-properties system. Selected tasks from the BMI, namely mental rotation, image scanning, image maintenance and motor imagery (dorsal system tasks), as well as object and color imagery (ventral system tasks) were used. Thirteen patients with mild AD (MMS : 17-23) and 13 normal controls (MMS $\geq$ 27) were studied. The two groups were matched on sex, age and education. Mild AD patients made overall more errors than controls and/or were slower on all perceptual and imagery tasks. However, mild AD patients did not make disproportionately more errors and were not disproportionately slowed in the difficult relative to the easy condition for each task relative to controls. These results suggest that although mild AD patients make overall more errors and/or are slower than controls on perceptual and imagery tasks, the visual perceptual and mental imagery processes themselves may be relatively preserved. Because similar patterns of results were obtained for perceptual and imagery tasks relying on the dorsal and ventral visual pathways, the processes mediated by these pathways do not appear to be differentially affected in mild AD.

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Neurocognitive and Olfactory Traits in Alzheimer's Disease: Free_Radical Insights

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Background and Aim: Cognitive and olfactory deficits are characteristic domains of AD. These traits should have common nature and specific free-radical mechanisms underlying AD etiology and pathogenesis.

Methods: Suggested novel insights into radicals dual nature and functions in CNS are based on original concepts of radicals charge transfer and physiological ambivalence and complementarity, and dynamic free-radical homeostasis.

Results: Cognitive and olfactory deficits in AD in generalized sense of neural information processing reflect changes in molecular pathways ranging from perturbations in radicals redox homeodynamics, including alteration of NO-superoxide complementarity, responsive redox signaling networks, concomitant alterations in genes expression, transcription and apoptosis, redox control of neurotransmission pattern, synaptic circuitry and plasticity to changes in neurogenesis and functional hemispheric asymmetry.

Conclusions: Based on novel model, neurocognitive and olfactory deficits plausibly manifest predisposition to neurodegeneration and AD, and represent characteristic markers/vestiges. This analysis is also important for deeper understanding molecular mechanisms of neurodegeneration and AD etiology under gene-environment interaction, radiation and chemical exposure, and intervention.

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The Differences of Clinical Features Between Alzheimer's Disease and Vascular Dementia According to Progression

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Background: The differences of clinical features are important in differentiating the Alzheimer's disease and vascular dementia. There have been many studies which compared the severity of progression. They assessed individual symptoms but could not explain the differences and global change of progression comprehensively. We have evaluated the cognitive and non-cognitive functions at the same time and evaluated the differences between AD and VD.

Methods: One hundred and thirty eight dementia patients from outpatient clinics which belonged to Busan•Gyeongnam Dementia Association were analyzed. All of the patients underwent the Korean version of Mini-Mental State Examination, the expanded version of Korean Clinical Dementia Rating Scale, the Korean version of Neuropsychiatric Inventory, Korean version of Neuropsychiatric Inventory, scales for activity of daily living, and the Short form of Samsung Dementia Questionnaire.

Results: There were 93 patients with AD and 45 with VD. VD patients revealed more severe Barthel Index of Activity of Daily Living deficits. AD patients had more severe memory and orientation deficiency in CDR 1 and CDR 2. VD patients revealed much faster decline of K-MMSE score between CDR 2 and CDR 3.

Conclusions: These results suggest that VD patients have more severe B-ADL difficulty, while AD patients more severe memory difficulty and disorientation. B-ADL progresses in the earlier stages in VD and in the later stages in AD. Global cognitive dysfunction progression is the opposite: in the earlier stages in AD and in the later stages in VD.

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Bone Metabolism in Huntington's Disease

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Introduction: Huntington´s disease (HD) is an autosomal dominantly inherited disorder characterized by neurodegenerative symptoms (psychiatric manifestations, movement disorders, progressive dementia)due to an increase of CAG repeats in chromosome 4p13. The CAG repeat expansion is present in all cells and so non-brainrelated changes outside the CNS should be possible. The aim of the present study was to investigate correlation between bone mineral density (BMD), body weight and antipsychotic burden in HD.

Methods: 40 patients (18 male, 22 female) with HD were investigated in this study. Blood samples were drawn (total serum calcium, phosphate, alkaline phosphatase, thyroid hormones, free thyroxine, prolactine, 24(OH)vit-D3, CTX-crosslaps) and BMD was measured at the lumbar spine and the hip by dual-energy X-ray absorptiometry.

Results: CAG repeats had a significant influence on age-adjusted total hip BMD and on body weight. Age-adjusted BMD in HD patients was significant lower at the hip compared to controls (p = 0.031). When comparing CAG repeats with antropomorphic data we obtained significant relations with total hip BMD (p=0.001), lumbar spine BMD (p=0.001) and weight (p=0.019). 25(OH)vit-D3 levels were significantly lower (p=0.007) and serum cross laps were significantly higher in HD patients (p= 0.015). The influence of antipsychotics on BMD is neglectable.

Conclusions: We found that body weight and BMD is CAG dependent in HD patients. BMD in HD patients is lower compared to weight-adjusted controls. It now appears that the Huntingtin mutation produces similar disturbances in other parts of the body, yet the affected cells somehow cope.

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Urodynamic Dysfunction in Huntington's Disease

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Introduction: Dysfunction in urinary bladder is common in patients with Huntington's disease (HD), which is an autosomal dominantly inherited neurodegenerative disorder due to an increase of CAG repeats in chromosome 4p13. HD is characterised by neurodegenerative symptoms, psychiatric manifestations and progressive dementia.

We tried to investigate if there is a characteristic urodynamic pattern affecting the vesico-urethral function in HD patients, in correlation to genetic imprinting (CAG-Repeats) and the state of disease.

Methods: 31 HD patients (18 male, 13 female) underwent urological measurements (nativflow, urinary-bladder detrusorpressure, maximum capacity of bladder, changes in pressure during filling and depleting, occurence of detrusorcontraction, loss of urine) and general evaluations of urological complaints and were compared to 31 sex- and age-matched healthy controls.

Results: HD patients had significant variances (p=0.014) in urodynamic parameters concerning the dysfunction of the urinary bladder (in particular in detrusorcontraction and -pressure) in all stages of disease. HD patients had a significantly increased detrusorpressure (44%) as compared to controls (p=0,014). 29% of HD patients featuring bladder-contractions (7% of healthy controls). The frequency of nocturnal miction in HD patients is 105,5% comparing to controls.

Conclusions: HD patients had significant noticeable problems in urodynamic compared to controls. We found no correlation between the urodynamic parameters and the quantity of CAG-repeats or the stage of disease. None the less our findings indicate the question to which extent disturbances in the function of urinary bladder influence the quality of life.

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Aggression in Huntington's Disease

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Introduction: Aggression is one of the most frequent psychiatric symptoms in Huntington's disease. The clinical observations showed an increased aggressive behavior which comes with the progression of the illness. The increased aggression presents a big problem for the patients as well as for their social environment. In this study we wanted to figure out the occurrence of aggression in patients with Huntington's disease.

Methods And Patients: 41 patients (18 female and 23 male) in different stages (1 to 4) with genetically diagnosed HD were examined for this study at the Dept. of Psychiatry at the Medical University Graz. For the assessment of aggression, 4 standardized tests (FAF, BDF, STAXI and AF SE) which describe a total of 25 qualities of aggression were used.

The period of illness was operationalized by the illness stage, illness time, CAG-Repeats and the UHDRS.

Results: High correlations between the aggression questionnaires and the UHDRS subscale "cognitive function" were found. Especially for the subscale "reactive aggression" (p=0.002), "debt" (p = 0.001), "aggression" (p=0,004), "anger state" (p=0.004) and the subscale "sum of aggression" (p=0.019). Furthermore the aggression properties correlate significantly with the subscale "functional capacity", "independence" and "psychosocial function" of the UHDRS, with age, gender, illness stage, illness duration and the CAG-Repeats.

Discussion: Our results prove the clinical observation that increased aggressive behavior depends on the period of illness. We can say that UHDRS and its subscales show a good predictor for the development of aggressive behavior in HD.

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A Voxel-Based Morphometry Study of Brain Volumetry and Diffusivity in Als Patients With Mild Disability

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Background and Aims: The pathological process associated to amyotrophic lateral sclerosis (ALS), albeit more pronounced in the motor/premotor cortices and along the corticospinal tracts (CST), does not spare extra-motor central nervous system (CNS) structures. The aim of this study was to obtain additional insights on the timing of the different CNS structures involvement and its impact on the disease-related neurological manifestations.

Methods: We used a voxel-based morphometry analysis to investigate the regional gray matter (GM) density changes and to quantify GM and white matter (WM) diffusivity alterations from 25 mildly disabled ALS patients and 18 matched healthy controls.

Results: Compared with controls, ALS patients had significant clusters of locally reduced GM density ($p<0.001$) in the right premotor cortex, left inferior frontal gyrus (IFG), and superior temporal gyrus (STG), bilaterally. In ALS patients contrasted to controls, we found significant clusters of locally increased MD ($p<0.001$) in the splenium of the corpus callosum (CC) and in the WM adjacent to the IFG, STG and middle temporal gyrus (MTG) of the right hemisphere, and in the WM adjacent to the MTG and lingual gyrus in the left hemisphere. Compared with controls, ALS patients had significant clusters of locally decreased FA values ($p<0.001$) in the CST in the midbrain and CC, bilaterally.

Conclusions: This study supports the notion that ALS is a multisystem disorder and suggests that extra-motor involvement may be an early feature of the disease and an useful predictor of subsequent development of cognitive impairment, known to occur in ALS.

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Which Neuropsychological Investigation in Amnesic Mild Cognitive Impairment?

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Background and Aims: the aims of the present study were to explore lexical semantic system, executive function domain and episodic memory in amnesic Mild Cognitive Impairment (aMCI) and to find neuropsychological predictors of conversion to Alzheimer's disease (AD).

Methods: a sample of 16 patients affected by aMCI according to R.C. Petersen's criteria and a matched group of 16 control normal subjects (NS) have been selected. All groups underwent a neuropsychological battery composed by Rey Auditory Verbal Learning Test (RAVLT), Free and Cued Selective Reminding Test (FCSRT), Trail Making A and B (TMT), Naming to Verbal Description Test, Picture Naming Test, Semantic Feature Question Test. A 2 yr follow up evaluation was carried out for 12 patients.

Results: the results show significant differences between the two groups in overall domains explored (aMCI<NS). The follow up showed that 5 patients converted to AD according to DSM IV criteria, the other 7 did not present any clinical modification. A statistical analysis of baseline evaluation of the two groups (converters/non converters) found worse performances for the converters on episodic memory domain (Delayed recall of RAVLT; many indexes of FCSRT), and semantic memory (Living Picture Naming Test, Semantic Feature Question Test).

Conclusion: the authors suggest the use of a deep evaluation of episodic and semantic memory as prognostic tool of aMCI.

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The Relationship of Anosognosia in AD and Caregiver Stress

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Background and Aims: Clinicians and researchers commonly observe deficits in awareness in Alzheimer's disease (AD) patients. This abnormality is referred to as anosognosia. Researchers have noted the importance of understanding anosognosia and its relationship to a caregivers quality of life (Regnier and Pynoos, 1992; Barco, 1991), however there has been limited research in this area. This study explores the possible relationship between anosognosia and caregiver stress.

Objective: To determine if a relationship exists between degree of anosognosia and caregiver stress. To determine if AD patients with anosognosia have caregivers with significantly higher stress when compared to those without anosognosia.

Methods: 73 Mild to Moderate AD patients and their caregivers participated. The patient and caregiver were asked to complete the Cole Anosognosia Scale for Alzheimer's disease (CAS-AD). The caregivers were also asked to complete the Zarit Caregiver Burden Interview (ZCBI). The CAS-AD is a 10-item scale measuring the degree of anosognosia in a patient. ZCBI is a 22-item scale measuring the level of caregiver stress.

Results: Using pearson-r correlation coefficient a moderate level of significance was found between the CAS-AD and Zarit ($r=.274$). The participants were also divided into patients with anosognosia ($n=31$) and those without ($n=42$). Using a t-test a significantly higher level of stress was found in caregivers of patients with anosognosia then those without (t-test = 4.07, $p<0.001$).

Conclusion: The study shows the potential importance of identifying and expressing to caregivers the effects of anosognosia in an attempt to alleviate caregiver stress.

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Diffusion Tensor Mr Imaging for Evaluation of Fronto-Subcortical Neural Pathway Changes in Parkinson's Disease With Dementia

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Background and Aims: The prevalence of the Parkinson's disease with dementia (PDD) is more than 40% in Parkinson's disease patients. The purpose of this study was to evaluate the fronto-subcortical neural pathway changes in PDD patients by using diffusion tensor magnetic resonance imaging (DTI).

Methods: Six PDD patients, six PD patients and six normal control group were enrolled, and the fractional anisotrophy (FA) of eight regions of interest (ROIs; globus pallidus, white matter of frontal, parietal and temporal lobe, the genu and splenium of corpus callosum, pericallosal area, and caudate nucleus) were evaluated.

Results: Significant differences of FA in the pericallosal area (F1) and frontal white matter (F2) and globus pallidus were observed among the three groups ($p<0.05$).

Conclusions: Reduced FA in the frontal (F1, F2) white matter and globus pallidus suggests the neural pathway changes from the basal ganglia to frontal subcortical areas in PDD patients. And this also suggests dementia in PD which is characterized by frontal lobe dysfunction may be attributable to nigro-striato-cortical pathway abnormality

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Association of Il-10 Promoter Polymorphism in Italian Alzheimer's Disease.

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Background and aims:Alzheimer's disease (AD) is a complex disease which involves several genetic and environmental mechanisms. Recent studies have reported a genetic association between the single nucleotide polymorphisms (SNP) in the promoter region of IL10 and AD with conflicting results. IL10 is the most important anti-inflammatory cytochine, its gene maps to chromosome 1 and it is polymorphic and its expression is correlated to the allelic variant of two SNP's in the promoter region (-1082G/A, -819T/C). In the present study, we have analyzed genotypes, allele distributions and haplotypes of IL10 promoter polymorphisms in an Italian sample of 222 AD sporadic patients and 179 controls. Patients were enrolled at the Dept. of Neurology of the University of Florence, clinical assessments were done according to published guidelines and the AD diagnosis fulfilled the Diagnostic and Statistical Manual of Mental Disorders criteria. The local ethical committee approved the study protocol

Methods The polymorphisms of IL10 and ApoE genotype were determined in 401 subjects using PCR RFLP methods.

Results We reported a positive association between the -819T/C polymorphism and AD. For the -1082A genotype and allele distribution, no overexpression was found in AD patients, although an association within the AT haplotype was detected. Moreover our data show a significant difference in the ApoE ε4 non-carrier AD patients compared with the ApoE ε4 non-carrier control group.

Conclusions Our results indicate that IL10 polymorphisms may be involved in the risk of developing AD.

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PET Amyloid Ligand [11C]PIB Uptake in Mild Cognitive Impairment and Early AD

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Background and Aim: In this study our aim was to employ voxel-based analysis method to identify brain regions with statistically significant increases in [11C]PIB uptake in early Alzheimer's disease (AD) and mild cognitive impairment (MCI) as compared to healthy control subjects, and to investigate whether patients with amnestic MCI would show increased [11C]PIB uptake indicating early AD process.

Methods: 17 13 patients with amnestic MCI and 14 control subjects were studied with PET using [11C] PIB as tracer. Parametric images were computed by calculating the region-to-cerebellum ratio in each voxel over 60 to 90 minutes. Group differences in [11C]PIB uptake were analysed with statistical parametric mapping (SPM) and automated region-of-interest (ROI) analysis..

Results: In AD patients SPM showed significantly increased [11C]PIB uptake ($p < 0.001$) in the frontal, parietal and lateral temporal cortices, in the posterior cingulate and the striatum. The patients with MCI had significantly higher [11C]PIB uptake ($p<0.01$) as compared to controls in the frontal, parietal and lateral temporal cortices and in the posterior cingulated. These results both in AD and MCI were supported by the automated ROI analysis. Individually, in the frontal cortex and posterior cingulate, 8 / 13 MCI patients had [11C]PIB uptake values above 2 SD from the control mean.

Conclusions: At group level the elevated [11C]PIB uptake in MCI patients resembled that seen in AD. At individual level about half of the MCI patients had [11C]PIB uptake in the AD range, suggestive of early AD process.

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1h Mrs Study of Cerebral Metabolism in Patients With Alzheimer's Disease (AD)

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Background and Aims:The aim of our comparative study is to characterize the metabolic changes in the brain of patients with AD, mild cognitive impairment (MCI), vascular dementia (VD), and normal elderly subjects (N) on the basis of analysis of 1H MRS data.

Methods:Three groups of patients are studied by MRI and 1H MRS with 1.5T Magnetom Vision (SIEMENS). The 1st group includes 85 patients (56-84y) with AD and MCI, the 2nd: 40 patients with VD (52-73y), the 3rd:(N) consists of 50 healthy volunteers (60-75y). 1H spectra are recorded in hippocampus (H), amygdala (A), frontal lobes (FL) and in entothinal cortex (EC).

Results:From the spectra the peak areas of cerebral metabolites: NAA, Cr, Cho and mIns are obtained. In the 1st verse the 3rd group the significant decrease of NAA and Cr and the increase of Cho peak areas and also decrease of NAA/Cr, and Cho/Cr in H and FL are observed. In the 1st group:NAA/Cr=1.01; mIns/Cr=0.82; Cho/NAA=0.96, in the 2nd:NAA/Cr=1.14; mIns/Cr=0.58; Cho/NAA=0.86, in the 3rd: NAA/Cr=1.25; mIns/Cr=0.62; Cho/NAA=0.53. For all patients a significant reduction of NAA/Cr in the left hemisphere and non-significant -in the right hemisphere are found.

Conclusions:1H MRS data are useful for identification of cognitively normal subjects at risk for developing AD.

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Increased Amyloid Load in Parkinson's Disease Dementia (PDD) and Lewy Body Dementia (LBD) Measured With 11c-Pib PET

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Background and Aims: Neuropathological studies have shown that varying amount of amyloid pathology is seen in PDD and LBD. 11C-PIB is a PET tracer which binds to fibrillar amyloid. The aim of this study was to quantify the amyloid deposition in PDD and LBD, and to compare this with healthy control subjects and Parkinson's disease patients without dementia (PD).

Methods: We investigated 22 control subjects, 10 PD, 13 PDD and 13 LBD patients (ages of the subjects ranging between 50-80 yrs). Each subject underwent detailed clinical evaluation, neuropsychological assessment, brain MRI and a 90 minute 11C-PIB PET scan. Amyloid load was quantified as 60-90' target-to-cerebellum uptake ratios. Cortical 11C-PIB uptake was assessed by ROI (region of interest) analysis.

Results: Nine of the 10 PDD patients showed no significant increase in amyloid uptake, while one patient showed a mild significant increase (1.5x) in cortical areas. As a group the LBD patients showed a significant increase in mean 11C-PIB uptake in cortical association regions. Individually 5 of the eight LBD patients showed significant increases (>1.5x) in cortical amyloid load. None of the PD patients or healthy controls showed any significant 11C-PIB uptake.

Conclusion: This study suggests that amyloid plaque load is low or absent in PDD but is significant in over 60% of LBD cases. This finding might help to explain why dementia is a late feature of PDD which has a slower progression compared to LBD. Anti amyloid strategies may also be relevant for some LBD cases.

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The Clinical Evaluation of Automated Diagnosis System of Alzheimer's Disease

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The clinical evaluation of automated diagnosis system of Alzheimer's disease

Objective: The purpose of this study is to estimate the clinical applicability of easy Z-score imaging system specific VOI analysis (eZIS) of ECD-SPECT combined with the voxel-based specific region analysis for AD (VSRAD) of MRI.

Methods: 75 patients with probable AD, which was based on NINCDS-ADRDA and 18 with probable amnestic mild cognitive impairment (MCI), which showed selective impairment of delayed recall with no apparent loss in general cognitive (MMSE>24, WMS-R logical1 < 13 and logical 2 < 8) were studied with both eZIS and VSRAD. The specific area Z-score map for ECD-SPECT was posterior cingulated cortex and lateral parietal-temporal cortex by comparison with age matched normal database and that for MRI was medial temporal areas including entorhinal cortex.

Results: Average of severity, extent and ratio was respectively 2.12+/-0.62 (1.87+/-0.55), 29.02+/-12.32(20.56+/-8.54) and 3.76+/-1.67 (2.56+/-1.23) for AD (MCI). In VSRAD, average of Z-score severity was 1.23+/-0.89 for MCI and the score for AD was 2.89+/-0.65. Although severity of eZIS was liner corresponded to that of VSRAD for AD, discordant findings were observed for MCI.

Conclusions: eZIS of ECD-SPECT were more sensitive to diagnose MCI in comparison with morphological study of MRI. The clinical application of these automated diagnosis system was very useful for the urgency and accuracy of diagnosing AD at an early stage.

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Multiple Cerebral Cavernous Malformations Presenting With Slowly Progressive Subcortical Ischemic Vascular Dementia

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Background: Cerebral cavernous malformations (CCMs) are angiographically occult vascular anomalies that can cause seizures, intracranial hemorrhages, focal neurological deficits, and migraine-like episodes. They can occur in either a sporadic or an autosomal dominant condition, the latter being suggested by the presence of multiple CCM lesions.

Case Presentation: A 78-year-old right-handed woman developed progressive cognitive and behavioral deterioration over a 12-month period. Her daughter first noticed personality changes, awkwardness and subtle dysarthria.. Ever since she got back surgery due to multiple compression fracture on her vertebrae, she became more agitated, anxious and forgetful. Neurological examination revealed minimal dysarthria, normal finding on cranial nerves. Language skills were only slightly impaired with subfluent speech. Copying of geometric designs was very poor. Most salient neuropsychological deficits were noted on frontal executive function tests, i.e., marked impairments in motor programming, mental set maintenance and shifting, generative naming. Routine hematological and biochemical tests, electrocardiogram, chest radiograph were normal. T2*-weighted gradient-echo sequence revealed multiple hypointense void resulting from hemosiderin deposition.

Conclusion: Although common symptoms of CCM are seizures, intracranial hemorrhages, focal neurological deficits, our patient disclose progressive cognitive and behavioral deficits which are suggestive of subcortical ischemic vascular dementia (SIVD). In sum, the neurobehavioral deficits manifested by this case demonstrates that multiple CCMs are additional cause of SIVD and can be added to the growing list of disorders responsible for this syndrome. ACKNOWLEDGEMENTS "This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea.(A050079)"

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Cognitive Function and Dementia Risk in Hypertensive Patients

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Detect Cognitive impairment in population of hypertensive (> 65y) Estipulate others risk factors besides hypertension and detect the Mild cognitive impairment or Dementia. Data was extracted from a sample of hypertensive patients (270) diagnosed in our Clinic during 3 years (2003-2005). They were submitted to: Clinical history, Physical, Neurological examination, Neuropsychological battery; Laboratory, Carotid ecography and Neuroimage. Information was used for; age, sex, education , race,cardiopathy, diabetes,previos stroke, depression, familiar history ,dyslipidemia.We use: Minimental, CDR,Hachinsky,Blessed,Wais,Clock, Delayed recall and Depression Scale's.We find 190 Cognitive impairment patients : MCI AD=16,MCI VASCULAR=82,MCI PD=7, MCI NORMAL AGE=12, MCI /DEPRESSION=20; OTHERS=11; ALZHEIMER= 23; Vascular Dementia=10;Mixta=9. Systolic hypertension and Alzheimer=19. Relation of SAH (especially high Systolic) suggests that it may be a risk factor for Alzheimer. Highly prevalent subtype of vascular cognitive impairment is significant . Incidence of cognitive impairment and depression =45 % is important; these represents a high risk group for Dementia, . Cognitive impairment in hypertensive diabetics was: = 82%.SAH and cardiopathy is high = 89%). There was a very high incidence of cognitive impairment in patients with both pathologies (Diabetes and Cardiopathy) = 93,7%. . We use RMI /CT to identifies strokes and leucoaraiosis(.Conclusion: Relationship between SAH and cognitive impairment was made and association to others risk factors and its prevalence. Treatment and screening, tries to prevent potentially dementing pathologies, with the high grade of dependence and morbidity and immense costs for the health public system.

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Which is Correlated With Clinical Features, Sevierity or Extent of Lesion in Alzheimer's Disease in Spect?

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Objectives: We have reported perfusion decline in the posterior cingulate gyrus, the precuneus and the hippocampus in the very early stage of Alzheimer's disease (AD) as a predictive marker for progression. This study is to evaluate whether clinical severity is correlated with extent and severity of these lesions in SPECT in AD.

Methods: Forty-one patients (13 males, age 71 +/- 7.8 yrs, mean +/- SD) with AD according to NINCDS-ADRDA underwent 123I-IMP SPECT and neuropsychological examinations including Mini Mental State Examination (MMSE), Alzheimer's Disease Assessment Scale Japanese version (ADAS-J cog), Wechsler Adult Intelligence Scale-Revised (WAIS-R) at initial visit. Image sets were transformed to the bicommissural stereotactic coordinate system, and regional anatomical activities were examined by three-dimensional stereotactic surface projections (3D-SSP). An abnormal Z-score was defined as >1.64 (corresponding to $p<0.05$). The extent of lesions was defined as a total number of the coordinates with abnormal Z-score in the cerebral hemisphere, and severity as an average Z-value in the same region. Spearman correlation coefficients were used to evaluate whether clinical severity was correlated with extent and severity of these lesions on 3D-SSP.

Results: All patients underwent MMSE (mean score 16.28, range 3-25) and WAIS-R (mean score 79.3, range 43-110), and 21 patients underwent ADAS-J cog (mean score 21.0, range 8.3-40.7). The extent was significantly correlated with ADAS-J cog score ($r = 0.62$, $p = 0.02$), however, the severity showed no correlation ($r = 0.31$, $p = 0.24$).

Conclusions: Diffuse rather than focal perfusion abnormality reflects progression of clinical symptom in AD.

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Differences in Brain [18f]FDG-PET Findings Between Alzheimer's Disease and Parkinson's Disease Associated With Dementia

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Background & Objectives: Early and reliable antemortem detection of Parkinson's disease associated with Alzheimer's disease (PDAD) as well as distinguishing PDAD from Alzheimer's disease are critically important in clinical practise. We conducted a study to see what the difference would be

between patients with PDAD and AD in terms of [18F]-2-fluoro-deoxy-D-glucose(FDG) PET findings and to look at whether FDG-PET would provide a valuable diagnostic aid to differentiate PDAD from AD. Method : FDG-PET images from 38 patients with AD and 18 patients with PDAD were obtained and analyzed by the statistical parametric mapping (SPM) method. AD was defined by clinically probable AD according to NINCDS-ADRDA criteria. PDAD was defined by clinically probable dementia with Lewy bodies (DLB) according to the consortium on DLB criteria. Parkinson's disease dementia (PDD) was included in the category of PDAD. Results : The SPM analysis demonstrated a more widespread metabolic reduction in the PDAD group as compared to the AD group. The reduction was particularly pronounced in the occipital cortex, the cerebellum, the temporal pole, and the midfrontal area in the PDAD group compared to the AD group. Also was noted a hemispheric asymmetry in the PDAD group - a hypometabolic left hemisphere. Conclusions : These findings suggest that functional neuroimaging, such as FDG-PET, will serve as a valuable diagnostic tool to differentiate PDAD from AD. Further study is warranted to test the value of FDG-PET for the early detection of PDAD.

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Diffusion-Tensor Magnetic Resonance Imaging: Possible Applications in the Early Diagnosis of Alzheimer's Disease

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Alzheimer's disease (AD) is a neurodegenerative disorder associated with progressive destruction of neuronal function.

Most studies have focused on identifying lesions at the level of the grey matter, whereas pathological studies have shown alterations to affect the white matter as well. The hypothesis of white matter alterations points to the possible existence of other mechanisms associated with the deposition of beta-amyloid and tau, mechanisms which lead to impaired axonal transport, myelin changes or functional inflammatory alterations that precede damage to the grey matter .

The aim of this study was to evaluate fractional anisotropy (FA) on DTI MRI of the subcortical white matter of the frontal, parietal, temporal and occipital lobes and corpus callosum rostrum and splenium in a group of subjects with amnestic Mild Cognitive Impairment according to Petersen's criteria, a group of subjects with mild Alzheimer's disease according to the DSM-IV criteria and a control group. The number of fibres at the level of the corpus callosum was also measured.

The study population was composed of 47 subjects, 15 with a diagnosis of amnestic Mild Cognitive Impairment, 14 with mild Alzheimer's disease and 18 controls.

In conclusion, the corpus callosum is compromised in both MCI and AD, demonstrating a significant reduction in the number of fibres in its posterior portion even in the preclinical phases of disease.

In subjects with AD there is also anterior functional damage that is demonstrated by the reduction in fractional anisotropy.

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A Disturbed Brain Network for Target Detection in Patients With MCI

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To learn more about the neurobiological changes in MCI a target detection task could reveal brain activity abnormalities Functional MRI was used to test the hypothesis that patients with MCI would show diffuse impairment in brain activity associated with target detection relative to matched control subjects.

12 patients with MCI and 13 elderly healthy control subjects participated. A standard three stimulus auditory oddball paradigm with two runs of 240 stimuli (12.5 % targets) was used. Functional magnetic resonance imaging (fMRI) with GE-EPI sequences as well as a high resolution T1 weighted structural scan was performed and data analysis was done by SPM2.

Target stimuli compared to standard stimuli elicited strong and widespread hemodynamic responses mainly in frontal, parietal and temporal brain areas in the control goup. MCI-patients showed activations comparable areas, but the activated regions were weaker and less extended. A direct group comparison of the effect of target stimuli showed reduced activation for the MCI patients in a number of brain regions, a region in the left anterior superior temporal cortex showed the most reliable reduction in the MCI patients.

A widespread network of brain areas showed higher activation during processing of salient (target) stimuli in our elderly healthy control subjects. These cerebral activations were reduced in MCI-patients demonstrating an disturbance of brain networks in this neurodegenerative disease.

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Role of DAT/SPET Scan in Diagnosis of Parkinsonian Disorders

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It is a retrospective study of 39 patients who were seen by one consultant with 25 years experience in Parkinson disorder. This eliminated clinician bias and inconsistency in diagnosing the disorder. Although the diagnosis was based on UK PDRS and H&Y template, scoring as such was not done in accordance with UK Parkinson Disease rating scale or the Modified Hoen & Yahr scale were used for diagnosis and disability assessment.

Patient characteristics were 26 males and 13 females. Mean age was 73.9 years. Median was 77years and the youngest was 56 years old and the oldest was 91 years old. The approximate average duration of symptoms was 4 years. This figure is an approximation as it is difficult to interpret the actual duration of symptoms from the clinic letters available and also these were mixture of cases with wide variation in duration of symptoms. 37 patients were already on Dopamine replacement therapy prior to the DAT scan

5 Groups were identified.

- initial Dx of PD definite
- Initial Dx of PD Probable
- initial Dx of PD with Basal Ganglion Infarct
- MSA
- DLB/PDD/Miscellaneous

Conclusion: We feel that DAT scanning is useful to support, confirm and to exclude the diagnosis of Parkinson's disease at District General Hospital level. This allows us to avoid starting potentially toxic medication and stop any unnecessary medication. It also allows us to be able to give an explanation of the patient's symptoms and relevant prognosis to the patient themselves and their family.

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^{1}H-MRS Experiences After Bilateral Dbs of the Stn in Parkinson's Disease

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The objective of this study were to evaluate the changes of the brain metabolites concentrations (NAA – N acethyl aspartate, Cho - choline, Crea – creatinine) in patients with Parkinson disease (PD) before and after bilateral deep brain stimulation (DBS) of the subthalamic nucleus (STN). Material and methods: 13 patients were evaluated at baseline and repeatedly 3 months after surgery. The NAA/Chol, NAA/Crea, Chol/Crea ratios were determined by single voxel ^{1}H-MRS studies on 1.0 T unit using stimulated echo acquisition mode (STEAM) sequence (TR/TM/TE:2000/10/30 ms). Spectra were obtained from right and left globus pallidus (Gp), and fronto-basal cortex (FBC). The patients were also assessed according to the UPDRS part III, in the "medication-on" and "medication-off' conditions. Results: in all patients motor scores (UPDRS III) improved significantly (Student's T test: $p< 0.01$). In all patients, decreased NAA/Cho ratios were observed from the selected voxels in left and right Gp, however significant increase of NAA/Cho, NAA/Crea, and decrease of Chol/crea ratio was observed in FBC after surgery in patients with clinical improvement. Conclusions: significant improvements were observed in the motor scores (UPDRS III) and in cortex NAA/Cho, NAA/Crea ratio increased significantly following bilateral DBS of the STN. These improvements were strongly correlated with improvements in motor function, primarily with regard to bradykinesia. Our results suggest that NAA/Cho and NAA/Crea ratio may be a valuable criterion for evaluation of Parkinson's disease patients with the clinical improvement following DBS of the STN.

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Content and T2 Relaxation Times of Cerebral Metabolites in Patients With Parkinson's Disease (PD): in Vivo 1h Mrs Study

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Background and aims:We propose the quantitative indicators for the characteristics of the brain state in patients with PD.

Methods:Two groups of patients are studied by 1H MRS with 1.5T Magnetom Vision (SIEMENS). The 1st group (PG) includes 120 patients with PD (48-74y), the 2nd:(VG)-20 healthy volunteers (50-73y). Spectra are recorded in the temporoparietal cortex (TC), lentiform nucleus (LN), putamen (P) and substantia nigra (SN).

Results:We introduce two indicators: the metabolite content AM as the peak area and the concentration CM= AM/S. We describe the metabolic state by the triad T*={ACho, ACr, ANAA}, where ACho, ACr, and ANAA are the peak areas of the signals of metabolites. We assign three values:1,2,3, to obtain six symbolic spectral configurations:1*={1,2,3}, 2*={2,1,3}, 3*={1,3,2}, 4*={3,2,1}, 5*={3,1,2}, 6*={2,3,1}. In the PG the most frequent configurations in TC are 2* and 5*, in LN, P, SN - 5*,6*. In the PG a decrease of CNAA and similar values of CCr and CCho in LN and in TC are obtained. T2M values in P, LN and SN in the both groups the shortening of T2M in the PG are obtained. In the TC the T2M values are similar in PG and VG.

Conclusions:Our approach gives a new insight into brain biochemistry in patients with PD.

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Hemichorea in Hyperglycemia With Bilateral Basal Ganglia Lesions on Brain MRI

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Hemichorea is usually caused by lesion in the unilateral and contralateral subthalamus or basal ganglia. Ipsilateral or bilateral lesions have rarely been reported to be responsible for the abnormal hemichoreic movement. An 85-year-old woman with unnoticed diabetes mellitus showed acute onset of hemichorea with hyperglycemia. A brain MRI showed nearly symmetric high and low sinal intensites in bilateral basal ganglia on T1 and T2-weighted images, respectively. Her hemichoreic movement was persisted with lowering of blood glucose level to normoglycemia, but gradually disappeared after drug therapy with tetrabenazine over 2 weeks. It is unknown why the involuntary movement presents unilaterally in spite of the bilateral lesions on brain image.

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Loss of Dopamine Transporter Binding Correlates With Disease Severity and Motor Handicap in Patients With Parkinson's Disease

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This study investigated the correlation between striatal dopamine transporter (DAT) binding, measured with [123I]FP-CIT SPECT, and motor handicap in a group of 35 patients diagnosed with Parkinson's disease. Patients were examined using the motor subscale of the Unified Parkinson's Disease Rating Scale (UPDRS) and the Modified Hoehn and Yahr (H&Y) scale.

Results demonstrated a statistically significant negative correlation between H&Y and UPDRS motor score (i.e., with disease stage and motor handicap) and the binding ratio for right and left caudate and putamen regions. Thus, the loss of DAT binding correlates with disease severity and motor handicap in patients with Parkinson's disease.

[123I]FP-CIT SPECT showed good sensitivity, specificity, negative and positive predictive values. In conjunction with the clinical data this method is considered as of significant clinical importance, not only for the objective confirmation of presynaptic nigrostriatal degeneration, but also and in particular for the early differential diagnosis from non-degenerative movement disorders.

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Transferrin Appeneded Nanoparticles Bearing Temozolamide for Brain Tumor Targeting

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Conventional nanoparticles were rapidly removed after IV administration from the blood stream by the macrophages of the mono nuclear phagocyte system. This limits their application in the field of controlled drug delivery and drug targeting to the tissues. Surface engineering may leads the nanoparticles evading to some extent and exhibiting their prolonged residence time in blood. The potential of targeting to a specific site in the body would be great benefit in the therapy of several diseases. Therefore the application of nanoparticles for drug targeting invivo has attracted a lot of interest in order to achieve the objective. The presence of the hydrophilic coating on the surface of the nanoparticles is thought to sterically stabilize them against opsonization and phagocytes between the hydrophilic polymers. PEG found to be a particularly effective steric stabilizer, probably due to its high hydrophilicity, chain formation, electrical neutrality and absence of functional group, which prevents interactions with biological components invivo. The stability of the PEG surface layer to desorption/displacement invivo is essential for the long circulation effect. PEG has been shown to be more effective when covalently bound on nanoparticles surface when adsorbed in reducing complement activation and interaction of the nanoparticles with macrophages invitro, as well as the uptake of nanoparticles by MPS invivo.

PLGA-mPEG Nanoparticles were prepared by using precipitation solvent evaporation technique. Transferin coupled nanoparticles prepared by coupling of transferin to PEG-nanoparticles using periodate oxidation method. Then the nanoparticles were characterized for various attributes. Invivo studies were carried on albino rats

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The Gerstmann Syndrome : An Atypical Onset of Degenerative Dementia

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Background and purpose: There is evidence to suggest that certain insidious progressive focal parietal syndromes are actually an atypical onset of Pre-senile Alzheimer´s disease.

We report the case of a 45 year old man who's process of dementia began with a left focal parietal degeneration similar to the Gerstmann Syndrome.

Methods: A full neurological and neuropsychological assessment was completed. The following cognitive areas were explored: orientation, attention, memory, language, gnosis, visual-spatial and visual-constructive functions, calculation and frontal functions. A MR and a cerebral SPECT were performed.

Results: The neuropsychological evaluation showed a left focal parietal affectation with digital agnosia, left-right disorientation, agrafia y acalculia. In addition to the typical symptonms of Gerstmann´s symdrome the neuropsychological tests revealed an important attention deficit, alexia, and imitation apraxia. Verbal memory was slightly altered, improving with recognition, and no language deficits were observed. The family reported that the onset was sudden and his condition degenerated quickly over the first year.

MRI results showed a subtle meninge enlargement in the left hemisphere without other significant discoveries.

Conclusions: Atypical onsets have been documented in some degenerative dementias. Some display a focal parietal syndrome cases with bilateral affection. However, the patient only showed signs of left progressive parietal degeneration. We observed a probable case of progressive Gerstmann´s syndrome as a rare onset to dementia.

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Gender Difference and Aging Effect of Corpus Callosum in Korean Adult and Its Study of Functional Significance

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The sexual dimorphism and change with aging of corpus callosum(CC) is a matter or ongoing dispute.Because there are no conclusive data about these, we investigate that neurologically normal Korean adult's CC using MRI shows changes in aspect of gender difference and aging. To study the sexual and aging effect of CC, we analyzed the midsagittal MRI morphometry in 239 adults. Total area, subarea, linear parameter including height, length, and five specific angle of the CC were analyzed. The subjects were composed of 108 young group (3rd decade; male:51, female:57) and 131 old group (7-8th decade; male:60, female: 71). The selected images were analyzed with NIH Image. The area, linear

parameter were measured with V-Works program. In young group, there is gender difference were observed in the area of splenium and length. The young male CC have larger splenium ,longer length than female. Angle B was wider in female compared to male in young group. Angle A, C, D showed gender difference in old group. In aspect of aging effect, the shrinkage of CC area was significantly observed in male group only. The height and length of CC became longer with aging in male and female group. Anterior width of CC became shorter with aging. Spatial rearrangement was observed with aging (Angle A: increase, Angle C: decrease, angle D: increase) This study reports Korean standard CC data based healthy normal adults. These results can be useful to various fields and helpful to diagnose the neurological disease which bring with CC change.

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Comparison of Reduced Cardiac Uptake and Enhanced Washout of 123i-MIBG in IPD, PSP, MSA and CBD. Our Experience.

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Background and aims: Reduced cardiac uptake of (123)I-metaiodobenzylguanidine (MIBG) is considered to show the dysfunction of the postganglionic neurons of the cardiac sympathetic nervous system. Many reports indicate that cardiac uptake of (123)I-MIBG is decreased in patients with idiopathic Parkinson's disease (IPD), dementia with Lewy bodies (DLB) and pure autonomic failure (PAF), and not decreased in patients with progressive supranuclear palsy (PSP), multi system atrophy (MSA) and corticobasal degeneration (CBD). We reviewed our cases, and examined how useful it is in patients with extrapyramidal symptoms to differentiate IPD from PSP, MSA and CBD.

Methods: We reviewed 31 patients (5 IPD, 11 PSP, 6 MSA-C, 5 MSA-P, 4 CBD) admitted to our hospital from April 2004 to November 2006, and evaluated decreasing severity of heart-mediastinum (H/M) ratios.

Results: In IPD and MSA-P patients, the severe reduction of H/M ratio was found. It is difficult to differentiate IPD from MSA-P by myocardial (123)I-MIBG scintigraphy. In IPD, MSA-P and CBD patients, the reductive rate was larger in delay images than in early images, and no tendency in MSA-C and PSP.

Conclusions: Although myocardial (123)I-MIBG scintigraphy is useful to differentiate IPD from other disease with extrapyramidal symptoms, it is difficult to differentiate IPD from MSA-P or CBD in some cases. There are some reports that the H/M ratio is reduced in CBD, MSA or PSP patients, and some that is not. Therefore careful evaluation is needed in suspicious cases of MSA-P or CBD.

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Depressive Symptoms in Corticobasal Syndrome Correlate With Decreased Perfusion in the Ventral Anterior Cingulate: A Brain Spect Study

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Background: Corticobasal Syndrome (CBS) is a neurodegenerative disorder characterized by an asymmetric akinetic-rigid syndrome, apraxia, cognitive deficits and often depression. Resting state PET metabolic studies of depression have shown hypometabolism in the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate. Purpose: The current study investigated the relationship between perfusion in the limbic-prefrontal cortical circuit with depressive symptoms in CBS. Methods: 21 consecutive patients with CBS were recruited from a longitudinal dementia study. Depressive symptoms were assessed using the Cornell Scale for Depression in Dementia (CSDD). All participants underwent standardized brain SPECT scanning using 99mTc-ECD. Perfusion ratios were calculated referenced to the cerebellum using an MRI-guided region of interest template. Regression methods were used to investigate brain-behaviour relationships. Results: In our CBS cohort, 50% had scores falling within the range of clinical depression (i.e., CSDD > 25%; mean CSDD±SD% =24.9±14.6%). Pearson correlations were used to explore the relationship between perfusion ratios in the anterior cingulate and DLPFC bilaterally with CSDD scores. Perfusion in the left and right ventral anterior cingulate was significantly correlated with CSDD. These were entered into a multiple linear regression model along with disease duration and MMSE. Perfusion in the ventral anterior cingulate bilaterally and also disease duration were significantly correlated with CSDD [$F(2, 17)=15.9$; $p<0.0005$; $r2=63.7\%$]. Decreased perfusion in the ventral anterior cingulate predicted severity of depression ($r2 =41\%$ of variance). Conclusion: Clinically-significant depression is highly prevalent in CBS. Brain SPECT helps to elucidate the neural substrates of depressive symptoms seen in CBS.

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Inhibition of the Da Transporter for Parkinson's Disease: in Vivo Evaluation by PET

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In Parkinson's Disease, synaptic dopamine (DA) levels are depressed. The use of an inhibitor of the dopamine transporter (DAT) has been proposed as a way to maintain higher synaptic DA concentrations. We investigated the compensatory mechanisms that occur in response to acute and chronic DAT inhibition by brasofensine (NS-2214). PET

studies with the DA D2 receptor ligand [11C]-raclopride were used to evaluate variations in endogenous concentrations of synaptic DA, with binding potential (BP) as the parameter of interest. [11C]-methylphenidate, [18F]L-dopa, and dihydrotetrabenazine were used as markers of DAT, dopa decarboxylase, and VMAT activity respectively. As expected, DAT binding was decreased, while VMAT binding , an index of DA terminals integrity, did not change in either acute or chronic conditions. Acutely, there was a 23% decrease in raclopride BP due to a decreased receptor affinity to raclopride, accompanied by a decrease in DA synthesis likely due to initial overstimulation of DA D2 autoreceptors. After 4 weeks of treatment, the 21% decrease in raclopride BP reflected a decrease in D2 receptor density, suggesting prolonged increased in intrasynaptic DA, while DA synthesis had returned to baseline, presumably due to downregulation of D2 autoreceptors. Additionally, it was observed that MPTP-lesioned animals treated with brasofensine exhibited motor improvements. These data suggest that DAT inhibitors may be useful adjuncts to L-dopa therapy, increasing the synaptic concentrations of DA without adversely affecting DA synthesis.

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T1 Relaxation Enhancement in the Substantia Nigra of Patients With Parkinson Disease

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Background and aims: Parkinson's disease (PD) is characterised by dopaminergic cell loss in the substantia nigra (SN) for which there is no diagnostic hallmark in MRI. There are controversial findings on T2 relaxation enhancement reflecting increased iron levels. Concomitant T1 relaxation enhancement has not been systematically studied, but there are qualitative data suggesting T1 signal changes in idiopathic PD. Aim of this study was to quantify T1 relaxation in the substantia nigra of PD brains.

Methods: 7 patients with idiopathic PD (mean 62.8, 52-77 years, 1-7 years of disease duration) and 5 age matched controls were studied at 3T (Philips Achieva, Netherlands) using a 3D inversion recovery technique with eleven inversion times. T1 maps were estimated using in-house software and regional analysis for the SN was done using MRICro.

Results: Two subjects had to be discarded for motion artefacts. SN T1 values were significantly reduced in patients (983 (44) ms vs 1046 (7.5) ms, $p<0.05$, t-test). In the 5 patients with asymmetric symptoms, the difference was more marked for the more affected SN with a non significant reduction contralaterally. There was a trend for reduced contralateral to ipsilateral SN T1 (965 vs 997ms, $p<0.1$).

Conclusions: This pilot study at 3T shows reduced T1 relaxation times in the substantia nigra of PD patients in the early phase of the disease and furthermore an asymmetry reflecting the clinical progression. Further studies are warranted to assess the diagnostic value of T1 relaxometry for diagnosis and disease monitoring in PD.

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Damage to Parietal White Matter Tracts Occurs in Dementia With Lewy Bodies

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Background and Aims: Diffusion tensor imaging is a novel MRI technique which allows visualisation of the integrity of white matter tracts by measuring diffusion of water. This is the first study to investigate such anatomical connectivity in subjects with Dementia with Lewy bodies (DLB) compared to subjects with Alzheimer's disease (AD). We hypothesized that diffusion tensor imaging would reveal temporal lobe abnormalities in the AD group and more prominent parietal changes in the DLB group.

Methods: We recruited 15 people with AD, 16 with DLB and 15 healthy control subjects of similar age. They were scanned on a 1.5T MRI system with diffusion tensor FLAIR imaging. Apparent diffusion coefficient (ADC) and fractional anisotropy (FA) maps were calculated and data were analysed using predefined regions of interest and SPM.

Results: We found a significant decrease in the FA map in a region of interest in the parietal lobe (precuneus) of the DLB group compared to the AD and control groups. Using SPM we found raised ADC in the left temporal lobe of AD subjects compared to controls. There were no other significant differences between groups.

Conclusions: We conclude that there are subtle, disease specific alterations in the integrity of the white matter tracts visible with diffusion imaging in DLB and AD which help explain key clinical differences between the two disorders. Such changes may also underlie the previously unexplained differing patterns seen on perfusion imaging in DLB (parieto-occipital) and AD (temporo-parietal). Our findings may aid diagnosis and treatment of these conditions.

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Magnetic Resonance Spectroscopy of the Hippocampus to Detect Metabolite Alterations in Alzheimer Disease

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Conventional methods of diagnosing and tracking Alzheimer Disease (AD) involve neuropsychological assessments of cognitive performance including the Mini-

Mental Status Exam (MMSE). The hippocampus is a brain structure affected early in AD. It is postulated that metabolite changes in this region may serve as surrogate markers for disease progression and may be more sensitive than clinical assessments. We hypothesized that cognitive decline in Mild Cognitive Impairment (MCI) and AD is associated with a decrease in N-acetylaspartate (NAA) and glutamate (Glu).

Data were gathered from 15 control subjects [mean±standard deviation, age = 78.3±5.9 years, MMSE = 29.2±0.9], 7 MCI patients [age = 69.6±13.0 years, MMSE = 28.0±1.3] and 16 mild to moderate AD patients [age = 73.7±7.6 years, MMSE = 22.1±3.2] taking donepezil or galantamine. A 4 Tesla Varian/Siemens MRI scanner was used to acquire short echo time [TE=46ms, TR=3.2s] proton Magnetic Resonance spectra from a localized tissue volume [3.8 ± 0.7 mL] mainly within the right hippocampus. Absolute metabolite levels were calculated using semi-automated software that incorporated prior knowledge of metabolite lineshapes.

A trend toward a decrease in Glu ($p<0.1$, Mann-Whitney U-test) was noted between MCI and control groups. A significant decrease was found in NAA levels ($p<0.05$, 2-tailed t-test) and Glu ($p<0.01$, 2-tailed t-test) between AD and controls. No significant correlations were found between metabolite levels and MMSE scores. In conclusion, although differences between MCI and controls did not reach significance with this small sample size, decreased NAA and Glu were detected in AD patients.

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White Matter Changes of the Corpus Callosum in Patients With MCI and Early AD: A Diffusion Tensor Image Study

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Background & Aims: MCI and AD mainly affect grey matter, but recent studies demonstrate that white matter (WM) damages also occur. This study is to investigate the WM damage of corpus callosum (CC)in patients with MCI and AD with diffusion tensor magnetic resonance imaging (DTI) to identify regional specificity for the development of dementia. Method : We examined ten patients with AD, twelve patients with MCI, and eleven age-matched controls. The WM changes were accessed by DTI indexes, fractional anisotrophy (FA) and mean diffusivity (MD), in seven regions in the CC, containing fibers projecting into the corresponding cerebral regions which were identified with DTI-based tractography. Results: FA values are lower in MCI and AD patients in the regions connect to the orbitofrontal, dorsolateral prefrontal and parietal areas than the normal controls. MD values are higher in the same three regions in MCI and AD patients. An MD value of the CC connects to the dorsolateral prefrontal area is the only index that is different between MCI and AD patients. Conclusion: White matter changes in the specific areas of CC, associated with the emotional, executive and perceptual function could be identified in MCI and AD patients. Of the above mentioned areas, the region interconnecting dorsolateral prefrontal area is the most important for the development of dementia in MCI patients. This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A050079)

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Evaluation of Amyloid Burden in Ageing Subjects With and Without Cognitive Decline

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Background and aims: Up to 30% of ageing healthy controls (HC) show Aβ deposition at autopsy. We have evaluated the relation between Aβ burden as assessed by PIB PET and cognitive decline.

Methods: PIB PET studies were performed on 33 elderly participants (age 73 ± 6) from the Melbourne Healthy Aging Study (MHAS), a 10-year longitudinal study of the cognitive consequences of ageing. Aβ burden was quantified using Standardized Uptake Value normalized to cerebellar cortex (SUVR). An independent behavioural neurologist reviewed all the neuropsychological and medical historical data for all subjects, and classified each subject as being cognitively "stable? or "declining.?

Results: Nine subjects were classified as declining. Three had MCI, one had Alzheimer's disease (AD), and six showed decline on word list recall but remained on the normal range for age. Seven of the 9 declining subjects and 3 of the 24 stable subjects, had cortical PIB binding. Aβ burden correlated with memory impairment in the declining group (SUVRvs CVLT II: $r = -0.77$, $p < 0.02$), and was higher in subjects with an ApoE-ε4 allele.

Conclusions: Declining subjects were more likely to show cortical PIB retention -with a similar distribution as in AD patients- than stable participants. Non-invasive longitudinal studies are warranted to better understand the role of Aβ deposition in the course of neurodegeneration and to determine if Aβ deposition in cognitively declining though non-demented subjects represents preclinical AD.

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Cortical Thickness in Single Versus Multiple Domain Amnestic Mild Cognitive Impairment

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Background and aims: Amnestic mild cognitive impairment (aMCI) can be classified into single domain and multiple domain. However, there have been no studies that specifically investigate the structural differences that support this classification. In an attempt to compare regional cortical thickness in two subtypes of amnestic MCI, we aimed to map the distribution of cortical thinning using surface based cortical analysis of magnetic resonance imaging (MRI). Methods: Nine patients with single domain amnestic MCI (S-aMCI), 22 patients with multiple domain amnestic MCI (M-aMCI), and 61 normal healthy groups were measured with cortical thickness across the entire brain. In order to learn the different pattern of cortical thinning in S-aMCI and M-aMCI, ANCOVA was performed based on a vertex-by-vertex procedure, and a statistical map of differences in cortical thickness between groups was constructed on a surface model. Results: Compared to controls, S-aMCI patients showed cortical thinning in left medial temporal lobe. Compared to controls, M-aMCI patients showed cortical thinning in left medial temporal lobe, precuneus, anterior and inferior basal temporal, insula, temporal association cortex. When the two MCI groups were directly compared, M-aMCI patients showed cortical thinning in left precuneus. Conclusions: Our studies suggest that M-aMCI may be the transitional state between S-aMCI and AD and the cortical thinning of posterior medial parietal region may be responsible for multiple cognitive impairment of M-aMCI.

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Voxel-Based Hypointesity Load in T2-Weighted MRI Images of Alzheimer's Disease Patients

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The Alzheimer's disease (AD) hallmarks, such as, beta-amyloid plaques are correlated with the hippocampus size reduction and brain overload of iron. This overload is characteristic of brain neurodegeneration and it is involved in the generation of beta-amyloid plaques were it is accumulated. A related property is the iron overload in the Basal Ganglia, in nuclei that include the Putamen, Globus Pallidus, and the Nucleus Caudate. We describe a method for the computation of voxel-based hypointensity load in T2-weighted MRI images of nuclei in the Basal Ganglia. This method consists of the steps of: (i) automatically selecting structures in the Basal Ganglia by determining a volume-of-interest given by MRI brain volume slices and a region-of-interest that contains these structures in each slice, (ii) normalizing the MRI images, (iii) determining a threshold for which voxels with image brightness larger then this threshold are considered as hypointense in the normalized images. This threshold is determined based on the analysis of receiver operating characteristic (ROC) curves obtained by comparing, voxel wise, the result of our method with that of a manually annotated golden standard for T2-weighted MRI images especially developed by clinical experts for this method. We processed, among others, a data set made up of MRI brain volumes of patients with AD and we verified the occurrence of regions in the Basal Ganglia with an abnormal T2-weighted hypointensity profile that is associated with iron overload in this region. These results indicate the possibility of using T2-weighted hypointensity load as a marker for AD.

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Functional MRI Activation and Deactivation in the Diagnosis and Treatment of Alzheimer Disease

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Functional MRI (FMRI) in AD reveals an abnormal deactivation network during cognition involving the posterior cingulate cortex (PCC). Clinical and imaging trials in AD confirm that frontal lobe behavior improvements and activity in the working memory (WM) network relate to treatment response. We examined the use of FMRI in these networks for confirming AD diagnosis and predicting treatment response.

12 mild/untreated AD patients and 4 aged-matched controls performed normally on a 1-Back WM task. FMRI of WM was done in an 8-cycle block-design at 1.5T. AD patients started on galantamine and were stratified as AD-Responders or AD-NonResponders based on caregiver feedback on a Frontal Lobe Behavior scale at least 8 weeks after treatment. Control and AD FMRI data were analyzed by regression modeling of the task to generate z-maps of significant activation and deactivation. Z-maps were also derived for AD-Responders and AD-NonResponders. These maps were combined to make masks for voxelwise statistical comparisons between groups within the two networks.

Mann-Whitney tests revealed significantly more deactivated voxels in the PCC of controls compared to the AD group and subgroups. In the WM network, significantly more activation occurred in all network regions in the controls and AD-Responders compared to AD-NonResponders. Controls had more activation in ventrolateral frontal regions than AD-Responders.

Consistent with other studies, pretreatment FMRI confirms that AD patients are unable to deactivate the PCC. Frontal behavioral improvement with galantamine is associated relatively intact pretreatment WM network. This suggests that FMRI may be a biomarker for AD diagnosis and treatment response.

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Development of a New Class of Compounds With Very High Affinity for the Amyloid Plaques

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Objective: To develop SPECT imaging agents to quantify amyloid plaque burden in Alzheimer Disease patients.

Background: Recent interest in Alzheimer (AD) therapy with drugs targeting reduction of β-amyloid burden has underscored the need for non-invasive scintigraphic methods

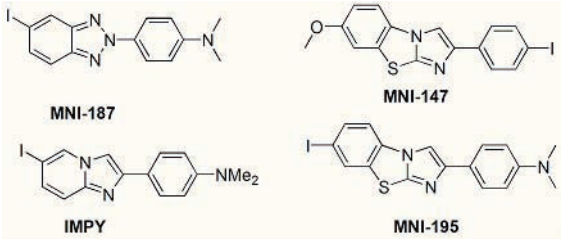
for interrogating plaque deposition for both new drug development and elucidating pathophysiological changes in AD patients. There are only limited radiotracers available for imaging amyloid plaques deposition, all in early stages. We have synthesized and radiolabeled a series of new ligands with iodine-123 and iodine-125

Results: Compounds of the novel series, represented by MNI-147, MNI-187 and MNI-195 (figure 1), were synthesized, their affinities for the β-amyloid protein were evaluated using human AD brain tissue and compared to that of IMPY, an established amyloid ligand. Their binding affinities are 2 to 10-fold better than that of IMPY.

Figure 1

The radiolabeling of the novel compounds was carried out under commonly-used conditions (Na123/125I, oxidizer, acidic medium) at ambient temperature. Radiochemical yields averaged 35-90%. The radiolabeled compounds were readily purified by reverse-phase HPLC, and their radiochemical purity exceeded 95%. Lipophilicity and protein binding of the novel SPECT probes are comparable to those commonly used in human (e.g., for MNI-187, LogD at pH 7.4 is 2.7, and the free ligand fraction in plasma is 4%).

Conclusion: These preliminary data suggest that these new compounds warrant their evaluation in human using SPECT imaging.



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Butyrylcholinesterase K Variant and Cognitive Dysfunctions in Alzheimer's Disease, Mild Cognitive Impairment and Normal Elderly in Korea

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Background and aims: Previous studies reported that butyrylcholinesterase (BCHE) K variant increased the risk for Alzheimer's disease. However, there have been no previous studies for mild cognitive impairment.

Methods: We included sixty-three patients with AD diagnosed with the NINCDS-ADRDA criteria, nineteen patients with mild cognitive impairment (MCI) with Petersen's criteria, and twelve normal comparisons. Venous blood samples were genotyped blind to clinical data for the BCHE K variant.

Results: The frequencies of K variant were 33.9% in AD, 21.1% in MCI, and 8.3% in normal comparisons with significant difference ($\chi 2 = 4.2$, $p = 0.04$). Two cases in AD have K/K allele, but no cases in both MCI and normal comparisons. Among AD patients, the MMSE scores were 17.2 (S.D. 7.4) with K variant and 14.1 (S.D. 7.1) without K variant, but they did not show statistically significant difference.

Conclusions: Butyrylcholinesterase K variant is found more frequently in AD than in MCI or normal elderly in Korea.

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Neurocognitive and Cerebral Blood Flow Pattern in Non-Demented Patients With Parkinson's Disease

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Background: Even in the early stages of Parkinson's disease (PD), cognitive alterations can be demonstrated by careful neuropsychological test. The aim of this study is to clarify the cognitive and perfusion patterns of the non-demented PD patients according to clinical characteristics.

Methods: Total 141 PD patients (65 men, age 40-76 years, Hohen and Yahr (HY) stage 1-4) were included in this study. They were divided into two groups according to the age of onset; <60 & >60) We also divided the patients into two types of PD; tremor- and akinetic-rigid type. They were all matched for the duration of symptoms and treatment, equivalent levodopa dose, motor scores and HY stage. Patients with PD who had dementia clinically or less than 3 years of education periods were excluded.

Results: Compared to age-matched controls, the younger group of PD patients (less than 60 years old of onset) showed more frequent abnormalities in the memory domain than in the frontal domain (30.1% vs 26.1%). On the contrary, the older group showed more frequent abnormalities in frontal than in memory domain (72.2% vs 55.6%). SPM analysis of perfusion SPECT images showed temporo-parietal hypoperfusion in younger group and frontal in older group. Akinetic-rigid type of patients showed better performance on the visuospatial function and attention than tremor-dominant patients.

Conclusion: Non-demented patients with PD showed different pattern of cognitive impairment depending on the age of disease onset, supported by perfusion SPECT images. Patients with tremor dominant type showed worse performance on some domains of neuropsychological tests.

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Effect of Phenserine Treatment on Cerebral Glucose Metabolism, Brain and CSF Amyloid and Cognition in AD Patients

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Background and aims: Phenserine is a selective, non-competitive acetylcholinesterase inhibitor, which in experimental studies in cell lines and animals lowers Aß levels via translational regulation of APP synthesis. In this study, we investigated the effect of phenserine treatment in Alzheimer patients on PIB binding, cerebral glucose metabolism

(CMRglc) as well as CSF, plasma biomarkers and cognition. Methods: Twenty patients with mild AD (MMSE 20-26) were recruited for a 6 months study. Ten patients received phenserine (30mg/day) for 6 months and another 10 patients received initially placebo for 3 months followed by donepezil (5mg/day). The patients underwent PET examination to measure brain amyloid binding (11C-PIB) and glucose metabolism (18F-FDG) at baseline and after 3 and 6 months treatment. CSF, plasma sampling and cognitive tests were performed throughout the study. Results: After 3-months treatment, there was a significant improvement in glucose metabolism in frontal and parietal cortex and cognitive test of attention in the phenserine treated group (all $p<0.05$). At this time-point, the CMRglc positively correlated with the level of plasma Aß 40 as well as with attention test (all $p<0.05$). The plasma Aß40 level also positively correlated with attention ($p<0.05$). The cognitive improvements were maintained in the phenserine treated patients at 6 months. At this time-point, the PIB retention negatively correlated with the CSF Aß 40 level ($p<0.03$) for the whole group of patients, while the CMRglc positively correlated with attention ($p<0.05$). At 6-months, the CSF Aß 40 levels positively correlated with results of attention test in the patients ($p<0.02$).

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Quantitative Measurement of Axonal Transport Integrity in the Mouse Brain in Vivo

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Deficits of the axonal transport system have been identified as a key feature of neurodegenerative diseases including Alzheimer's disease. Axonal transport rates can be assessed in vivo using T1-weighed MRI. However, the full description of quantitative measurements of axonal transport integrity in vivo has not been reported to date. The aim of this study was to develop an in vivo method and to measure axonal transport rates in a quantitative manner using manganese enhanced MRI (MEMRI) that can be readily used for studying tau pathophysiology of AD.

Manganese chloride solution was administered into the nasal cavity of mice and amyl acetate odor was presented to the animals for 10 min. Animals were imaged using the developed fast multi-slice T1-mapping method in combination with a specially designed RF coil to provide high-resolution T1 maps of the mouse brain in a short scan time (125 x 125 x 400 μm3, 2.5min) at 9.4T.

A progressive contrast enhancement, shortening of T1 values, was clearly visible in vivo in the odor specific regions of the olfactory bulbs of the mice, indicating the manganese uptake at the epithelial cells in the nose, transport through olfactory neurons and accumulation at the olfactory neurons.

Current preliminary study demonstrates the feasibility of fast, high-resolution T1 measurements to follow the time course of manganese accumulation at the olfactory bulbs, which eliminates the dependence on B1 and T1 variations. This technique will be a valuable tool to characterize axonal transport deficits in transgenic mouse models of neurodegenerative and neurological diseases.

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Hypometabolic Brain Regions in Corticobasal Degeneration -a SPM Analysis of FDG-PET

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Background: Corticobasal degeneration (CBD) is a rare parkisonism plus dementia syndrome characterized clinically by asymmetric parkinsonism, apraxia, myoclonus, supranuclear gaze palsy, and dementia. Only a few studies have investigated functional changes of 2-[18F] fluoro-2-deoxy-D-glucose positron emission tomography (18 FDG-PET) scan using a voxel-wise analysis, and their results are inconsistent. Methods: We compared the regional metabolic patterns of PET images obtained from 10 patients with CBD and 11 healthy subjects using a statistical parametric mapping (SPM). Results: Significant hypometabolism was identified in superior fontal gyrus including the supplementary motor area, superior parietal lobule including precuneus, thalamus, inferior frontal with insula, pons, and cerebellar hemisphere. All the patients had lateralizing signs including asymmetric akinetic rigidity, and all had more severe hypometabolism in the contralateral side of predominant neurologic abnormalities. Conclusion: Our results suggest that CBD is a degenerative dementia that primarily affects premotor cortex with supplementary motor area and superior parietal lobule with precuneus. The hemispheric asymmetry of hypometabolism was common in patient with CBD, which is well correlated with clinical feature. These clinical and FDG-PET findings would help differentiate CBD from other degenerative dementias.

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Differentiated SH-SY5Y Cells: A Cellular Model of Dopaminergic Neurotoxicity

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Background and aims: A hallmark of Parkinson's disease (PD) is progressive loss of dopaminergic neurons. A major unmet medical need for PD requires a therapeutic strategy that can modify the disease by protecting dopamine neurons from neurodegeneration. The primary aim of present studies was to characterize a cellular model that could facilitate the discovery and development of new therapeutic targets/agents that have the potential to slow the progression of PD.

Methods: Using the human neuroblastoma cell line, SH-SY5Y, a paradigm of differentiation into dopaminergic cells was established empirically using retinoic acid (RA) followed by TPA and MPP+ induced toxicity was assessed using cytotoxicity and apoptosis markers.

Results: Reproducible differentiation could be achieved by exposing the cells to RA for 6 days followed by TPA for 7 days. These cells express dopaminergic markers such as Nurr1, D2 dopamine receptor, tyrosine hydroxylase and

dopamine transporter. As expected, the cell viability assays demonstrated that the RA/TPA differentiated cells are more sensitive to the neurotoxin, MPP+, than undifferentiated cells. Furthermore, MPP+ produced an early and transient increase in cytochrome C levels followed by caspase 3 and caspase 9 activation. Surprisingly, N-acetyl cystein, vitamin E or the caspase inhibitor, Z-VAD, did not block the toxic effects of MPP+. In contrast, the pituitary Adenylate Cyclase Activating Polypeptide, PACAP-27, concentration-dependently blocked the toxicity induced by MPP+.

Conclusions: Together, these studies demonstrate that RA/TPA differentiated SH-SY5Y cells offer a good model of dopamine neuronal toxicity and will facilitate the discovery and validation of novel therapeutic targets for PD.

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Degeneration of Cardiac Sympathetic Nerve Begins in the Early Disease Process of Parkinson's Disease

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Background and Aim: We reported that cardiac sympathetic denervation occurs in Parkinson's disease (PD), which accounts for the decreased cardiac uptake of meta-iodobenzylguanidine (MIBG), a physiological analogue of noradrenaline (Orimo S. et al. Acta Neuropathol 2005). To determine whether the cardiac sympathetic nerve is involved or not in the early disease process of PD, we examined cardiac tissue, sympathetic ganglia, and medulla oblongata at the level of the dorsal vagal nucleus.

Methods: We immunohistochemically examined each sample of 20 patients with incidental Lewy body disease (ILBD), which is thought to be a presymptomatic stage of PD, and 10 control subjects using antibodies against tyrosine hydroxylase (TH) as a marker for sympathetic nerves and neurofilament (NF) as a marker for axons.

Results: TH- and NF-immunoreactive nerve fibers of fascicles in the epicardium were well preserved in 10 of the 20 patients with ILBD as well as in the control subjects. In contrast, TH-immunoreactive nerve fibers had almost entirely disappeared in 6 and moderately decreased in 4 of the 20 patients with ILBD. Neuronal cell loss in the dorsal vagal nucleus and the sympathetic ganglia was not detectable in any of the ILBD patients examined.

Conclusions: These findings suggest that degeneration of the cardiac sympathetic nerve begins in the early disease process of PD, which precedes neuronal cell loss in the dorsal vagal nucleus.

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Role of Sirt1 in the Neuroprotective Effects of Resveratrol on Mpp+-Induced Apoptosis in Neurons

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Resveratrol, is a natural phytophenol present in in red grapes and wine. Apart of its antioxidant properties is able to activate the silent information regulator 2 (Sir 2) families of proteins (sirtuins). In this study, the neuroprotective effects of RES on MPP+ -induced cell viability loss in CGNs was assessed by counting the condensed nuclei. Neurons treated with MPP+ (200μM) condensed nuclei increases up to 50.9± 1.7 (mean ± SEM n= 6) (control value 0.5; mean ± SEM n= 6). The effect of MPP+ on loss of cell viability was±8.8 attenuated by RESV in a concentration dependent manner. Treatment with different M and 100μM of RESV rescued the MPP+-induced increased of μ concentrations 1μM, 50 1.7%, respectively.DNA± 0.8% and 14.5± 1.8%, 15.1 ±condensed nuclei by 51.8 fragmentation measured by flow cytometry was prevented by RESV. Next experiments were carried out to evaluate if the neuroprotective effects of resveratrol are mediated through the SIRT1 activation. To answer this question we add to cell cultures sirtinol an inhibitor of SIRT1. Our flow cytometric results demonstrated that sirtinol was not able to counteract the antiapoptotic effects of resveratrol. This data suggest that SIRT1 activation is not involved in the neuroprotective properties of RESV. We suggest that the antiapoptotic effects of RESV are probably due to its antioxidant properties.

This study was supported by grants SAF2005-01604 and SAF-2005-05179 from Ministerio de Educación y Ciencia ,PI 041300 and Centros de Investigación Biomédica en Red (CIBER) from Instituto de Salud Carlos III. Generalitat de Catalunya 2005/SGR00893.

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A Metaphonological But Not An Epiphonological Disturbance: Evidence That Parkinson's Disease and Normal Ageing Affect Phoneme Manipulation But Not Rhyming

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We show that Parkinson's disease (PD) and, to a lesser extent, normal ageing affect the ability to identify and manipulate phonemes. Using a series of phonological awareness tasks – including rhyme detection, rhyme production, phoneme deletion, phoneme exchange and common-unit identification - we show that a group of older adults diagnosed with PD perform significantly worse than a

group of healthy older adults (HOA). We also show that normal ageing has an affect on phonological awareness such that the HOA participants performed at a significantly lower level than healthy younger adult participants on some of the tasks. We also concluded that epiphonological awareness (the awareness of phonological units that develops earlier in childhood) is relatively spared compared to metaphonological awareness (the more high-level awareness of phonemes that develops later in childhood, usually when schooling begins). These findings suggest that there is an inverse relationship between the development of phonological awareness in childhood and the degeneration of phonological awareness in old age. In general we found that as the level of difficulty of the tasks increases (i.e. from the epiphonological to the metaphonological level) both people with PD and people ageing normally make more errors, make more omissions and respond less quickly. We argue that high-level language functions such as metaphonological awareness rely heavily on the integrity of the frontal-striate systems for working memory; these are often disturbed in normal ageing and to an even greater extent in PD.

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Neuropsychological Findings of Huntington's Disease

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Background: Huntingtion's disease(HD) is an autosomal dominant neurologic disorder characterized by chorea, dementia and psychiatric disturbances.

Neuropsychological deficites have been reported in visuospatial tasks, visual memory, language tasks, tactile-motor coordination, peformance of a visualmotor skill, and stimulus perception and encoding.

Case: A-57-year-old female patient presented choreic movement, progressive cognitive and behavior disturbances from about 13 years ago.

Neuropsychological tests demonstrated severe deficits in memory, language tasks, visuospatial tasks and executive function.

Brain MRI showed striking atrophy of caudate nucleus, focal enlargement of both frontal horn of lateral ventricles and diffuse atrophy.

Cerebral perfusion SPECT revealed hypoperfusion in both frontotemporoparietal area.

Conclusion: I report neuropsychological findings of Huntingtion's disease.

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The Familial Alzheimer Disease-Linked Presenilin-2 Mutation T122r Reduces the Calcium Level of Intracellular Stores in Different Model Cells

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Background And Aims: Wild type (wt) presenilin-1 (PS1) and presenilin-2 (PS2) have been recently proposed to function as Ca2+ leak channels in the endoplasmic reticulum (ER) (1). We previously showed that over-expression of different PS1 and PS2 mutants, as well as of the wt PSs, reduces the ER and Golgi apparatus (GA) Ca2+ level in various models (2-4).The present study focuses on the type of Ca2+ dysregulation which is induced by the FAD-linked PS2-T122R, among the strongest mutants affecting intracellular Ca2+ stores.

Methods: Ca2+ dynamics at the cytosolic, ER and GA level were studied upon transient expression of PS2-T122R together with suitably targeted aequorins in MEF clones knockout for both PSs or only PS2. Moreover, SH-SY5Y stable clones were employed to define the role of the PS2-T122R mutant under a low expression level in an endogenous PS background.

Results And Conclusions: Transient expression of PS2-T122R decreases the ER and GA Ca2+ content in both MEF models, whereas in stable SH-SY5Y clones the PS2-T122R effect was found at ER level. Additional experiments were designed to exploit the involvement of IP3/Ryanodine receptors, Ca2+ ATPase and translocon as possible PS targets which could directly be responsible for the reduced Ca2+ level. Preliminary results indicate that the PS2 mutant affect both Ca2+ uptake and Ca2+ leak at the ER level.

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A New Way to Investigate Antiapoptotic Compounds

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Background: Apoptosis in neurodegenerative diseases is regulated by a number of interacting pro- and antiapoptotic proteins. These interactions run in defined pathways, wherein the PI3K/AKT is one of the strongest survival pathway. AKT is a kinase, which plays a central role in this procedure. Extra- and intracellular effects can stimulate or inhibit this pathway. Several substances are known to influence this pathway, resulting in increased survival.

Methods: Beside standard methods like MTT for viability, fluorescence measurement of caspase-3 and caspase-9 activity, western blot for detecting the phosphorylation of AKT we use PI3K/AKT or apoptosis specific macroarrays, that show the changes of the RNA transcription level. In this study we investigated the effect of different compounds like beta-synuclein derived peptides and other neuroprotective compounds.

Results: The data shown were obtained with human neuroblastoma cells (SH-SY5Y) and primary cultured chicken neurons. Apoptotic cell death is induced by Rotenone or MPP+ Iodide. We use human IGF-1, an extracellular stimulant, for defining a positive standard value and

LY294002 for the negative value in this model, which are compared with data from the compounds. Measuring the effects after several time periods, our model shows improved viability due to the induction of the survival pathway. Caspase-3 activity is decreased, AKT is activated and several antiapoptotic proteins are overexpressed.

Conclusion: Taken together these results give a detailed view on the molecular mechanisms of apoptotic neuronal models influenced by several kinds of substances.

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Identification of Oxidized Thiol Proteases in Alzhiemer's Disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder affecting millions of people worldwide. In addition to the presence of plaques and tangles, affected regions of Alzheimer's disease are highly oxidized compared to age-matched controls. Thiol-proteases such as the calpains, caspases and cathepsins are part of a family of redox-sensitive enzymes that are implicated in the regulation of AD relevant proteins such APP and tau. Recently we showed that thiol-protease activity in AD brain is oxidatively inhibited but recoverable by addition of thiol reducing agents such as dithiothreitol (DTT). In the present study, we used DCG-04, a chemical probe linked with biotin that specifically binds to the reduced active site cysteine of thiol-proteases to identify the oxidized proteases. DCG-04 binding was measured in hippocampal samples of AD and age-matched controls obtained post-mortem. Slot blot analysis showed cysteine proteases profiled by DCG-04 from AD brains had ~40% lower reactivity than age-matched controls. This decrease in binding was abolished by DTT, demonstrating that active site cysteines are oxidized in AD. Quantitative analysis of two-dimensional SDS-PAGE electrophoresis showed that individual proteases had varying degrees of oxidation based on DCG-04 binding. Using mass-spectrometry, DJ-1 and ubiquitin carboxy-terminal hydrolase L1 were identified as highly oxidized. These data suggest that certain thiol-proteases are more sensitive to the redox environment associated with AD than others. We hypothesize that redox-mediated inhibition of specific thiol-proteases contributes to dysregulation of their protein substrates such as tau and APP which may play a role in the formation of AD pathological hallmarks.

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The c-Abl Tyrosine Kinase / p73 Signal Transduction Pathway in AD Neurodegeneration

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In Alzheimer's disease (AD), amyloid beta-protein (Abeta) accumulation has been causally implicated in the neuronal dysfunction and neuronal loss that underlies the clinical manifestations of AD. However there is not clear understanding about the signal transduction pathways involved in this neuronal death and the genesis of the cytoskeleton alterations.

Herein we discuss data that support that the kinase c-Abl/p73 protein system plays a central role during Abeta induced neurodegeneration. Our in vitro studies show the Abeta peptide fibrils induce activation of the c-Abl/p73 signal pathway associated to the preferential stabilization of pro-apoptotic p73 isoforms. In in vivo studies we find that injection of Abeta fibrils in the hippocampus induces an increase of c-Abl and p73. The c-Abl inhibitor STI571 not only preventes the apoptosis and tau phosphorylation but also significantly reduces the rat behavioural deficit induced by Abeta fibrils injection. Our in vitro studies support that the mechanisms underlying the effect of STI571 on tau phosphorylation and apoptosis induced by Abeta fibrils is the inhibition of the c-Abl/p73 pathway. These results strongly suggest that the c-Abl/p73 signal transduction pathway plays a central role in neurodegeneration induced by Abeta fibrils.

This work was supported by FONDECYT 1040782

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Protein Phosphatase 2a Methyltransferase Links Homocysteine Metabolism With Tau and Amyloid Precursor Protein Regulation

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Alzheimer's disease (AD) neuropathology is characterized by the accumulation of phosphorylated tau and amyloid-beta peptides derived from the amyloid precursor protein (APP). Elevated blood levels of homocysteine are a significant risk factor for many age-related diseases, including AD. Impaired homocysteine metabolism favors the formation of S-adenosylhomocysteine, leading to decreased activity of methyltransferases and inhibition of methylation reactions. Here, we show that incubation of neuroblastoma cells with S-adenosylhomocysteine inhibits protein phosphatase 2A (PP2A) methyltransferase (PPMT), resulting in demethylation of PP2A, a major brain Ser/Thr phosphatase. PP2A demethylation promotes the downregulation of Balpha-containing PP2A holoenzymes, thereby affecting PP2A substrate specificity. It stimulates tau phosphorylation at several epitopes. It enhances APP phosphorylation at the Thr-668 site, which favors amyloid-beta production. Remarkably, hyperhomocysteinemia induced in wild-type and cystathionine-beta-synthase (Cbs) +/- mice by feeding a high-methionine and low-folate diet is associated with increased brain S-adenosylhomocysteine levels, PPMT downregulation, PP2A demethylation, and tau and APP phosphorylation. Significantly, we have previously reported that downregulation of neuronal PPMT and PP2A methylation occur in affected brain regions from AD patients. The link between homocysteine, PPMT, PP2A methylation and key

CNS proteins involved in AD pathogenesis provides new mechanistic and therapeutic insights into this disorder.

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Circulating Beta Amyloid Deposition in Brain Tissues is Enhanced by Arterial Hypertension

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Although AD has been considered for long to be of non-vascular origin, many recent studies have indicated that cardiovascular risk factors, like arterial hypertension, could be involved in the pathophysiology of AD. We aimed to investigate the role of high blood pressure in deposition of AbetaP into cerebral tissues. We used two independent mouse models of high blood pressure. In both models the analysis of blood brain barrier (BBB) revealed an increased permeability in cortex and hippocampus. Interestingly, the hypertensive mice showed a marked positivity to anti-AbetaP antibodies as compared to control mice. This positivity was particularly relevant on vessels wall and surrounding tissues, suggesting a vascular source of such amyloid deposits. To investigate this issue we analyzed hypertensive-mice after passive immunotherapy with IgG against AbetaP, which hamper AbetaP migration from blood to brain. A group of hypertensive mice, and relative control, received intraperitoneal injection of anti-AbetaP IgG for one month. We observed that hypertensive mice treated with passive immunotherapy showed a markedly reduced AbetaP immunopositivity as compared to hypertensive mice treated with vehicle. Thus, our study demonstrates that hypertension determines an impairment of the BBB permeability with deposition of AbetaP. Moreover, passive immunotherapy is able to rescue hypertensive brains from AbetaP deposition, suggesting a circulating source of this latter. From these results we can infer that hypertension is not only a risk factor for cerebrovascular accidents such as stroke, but, associated with other risk factors, could trigger the pathophysiological cascade that finds its final expression in the AD onset

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Sphingomyelinase-Generated Ceramide Mediates the Nadph Oxidase and Subsequent Oxidative Damage During Neuroinflammation

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Inflammation and its mediators are implicated in the orchestration and progression of chronic degenerative pathologies of the central nervous system (CNS). In particular, oxidative stress plays a major role in the progression of CNS inflammation. Dysfunction of the neuronal cytoskeleton represents a crucial step in many degenerative CNS pathologies. The integrity and dynamics of the neuronal cytoskeleton is pivotal for growth cone motility, axon outgrowth, regeneration, and dendritic spine dynamics. In this study, we explored neurodegenerative mechanisms in SH-SY5Y neuroblastoma cells, and various primary neuronal cultures from chicken embryos exposed to inflammatory cytokines or the amyloid beta fragment. We elucidated a molecular pathway linking neuroinflammation to the destruction and dysfunction of the actin cytoskeleton. Inflammatory mediators stimulated an increase in the bioactive sphingolipid ceramide by activating a neutral sphingomyelinase. Increases in ceramide further resulted in an activation of a neuronal NADPH oxidase (NOX) activity. Subsequent NOX activity was shown to result in irreversible oxidative damage to the actin cytoskeleton and ultimately cellular dysfunction.

Together, our results implicate ceramide as a key intermediate of neuroinflammatory-mediated NOX activation in neuronal cells. Moreover, this investigation links neuronal NOX activation with irreversible oxidative damage to the actin cytoskeleton. Thus, inflammatory processes associated with chronic pathologies such as Alzheimerâ€™s disease could directly contribute to the degeneration of the CNS.

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Neuroprotective Effects of AAD-2004 Against Ischemic Brain Injury: Comparison With Memantine

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Memantine, a low-affinity, uncompetitive NMDA receptor antagonist, has been approved as a neuroprotective drug for the treatment of AD. Its neuroprotective action has been demonstrated in various animal models including transient forebrain ischemia. We examined if AAD-2004, an anti-oxidant and anti-inflammatory drug derived from salicylate, would protect the hippocampal CA1 neurons from transient forebrain ischemia in rat. Administration of AAD-2004 as well as memantine significantly attenuates delayed degeneration of the hippocampal CA1 pyramidal neurons following forebrain ischemia for 10 min. Moreover, AAD-2004 prevents blood-brain barrier disruption following transient forebrain ischemia. The neuroprotective effects of AAD-2004 were also verified in animal model of focal cerebral ischemia. AAD-2004 prevents the ischemic neuronal death primarily by blockade of free radical production. The present findings suggest that AAD-2004 protects against ischemic brain injury and can prevent inflammation (our companion study, Im et al.).

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Endogenous Production of Plastic Composite Material in AD Brain: Role of Copper Ions

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Background and aims: Acrolein, the lipid peroxidation byproduct found in the Alzheimer disease (AD) brain, polymerizes in the presence of amyloid-beta (Abeta) peptide (Seidler and Squire, Biochem Biophys Res Commun, 2005;335:501). The product formed is a plastic composite material consisting of polyacrolein-Abeta, which, we hypothesize, promotes neurodegeneration and leads to plaque formation. Our working model includes the contributing effects of Abeta's surfactant property.

Methods: We investigated the role of divalent cations (iron, copper and zinc), contributors to redox biology, in the deposition of Abeta in the polymerized matrix. We quantified the effect of these metal cations on the in vitro production of polyacrolein-Abeta by homogenizing dehydrated samples in trifluoroethanol and hexafluoropropanol solvents, and examined the novel polymers under light microscopy.

Results: In micromolar concentrations, both iron and copper increased the formation of insoluble plastic composite material. The organization of the polyacrolein-Abeta structure that was produced in the presence of iron ions resembled a honeycomb-like arrangement of layers. Copper ions exhibited a greater effect than iron. Contrary to the effects of iron and copper, zinc ions in micromolar concentrations suppressed the formation of polyacrolein-Abeta.

Conclusions: Our observations, which suggest a neurodegenerative role for lipid-derived acrolein and copper, are particularly interesting in light of the recent findings associating AD pathogenesis with intakes of dietary fats and copper (Morris et al., Arch Neurol 2006;63:1085).

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Modifications of Platelet From Alzheimer Disease Patients: A Possible Relation Between Membrane Properties, No Metabolites and Personality Traits

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Background, aims and methods: Alzheimer disease (AD) is a chronic neurodegenerative disorder characterised by a progressive loss of memory and cognitive functions. The formation of nitric oxide (NO), by astrocytes, has been suggested to contribute to the neurodegnerative process and some studies have described the participation of different isoforms of NOS in the progression of AD. The present work was conducted in order to investigate the role played by NO and peroxynitrite in platelets from AD patients, the possible correlation with Na+/K+-ATPase activity and the intracellular Ca2+ concentration in the same group of patients as well as NOS isoenzymes and nitrotyrosine expressions as markers of NO synthesis and reactive protein nitration, respectively.

Results: NO production was significantly elevated in the platelets from AD patients compared to controls as well as L-arginine/NO-dependent ONOO-. Membrane Na+/K+-ATPase activity was significantly decreased in patients than in controls while intracellular Ca2+ concentration shows an opposite trend. Platelet from AD patients showed a significantly increased TMA-DPH and DPH fluorescence anisotropy compared with controls. Western blot analysis using anti-iNOS and eNOS antibodies demonstrated that both isoforms were detectable as well as nitrotyrosine more pronounced in AD patients than controls.

Conclusions: the increased expression and activity of nitrergic system may produce platelet membrane alteration or vice versa. These modifications may contribute further to the neurodegenerative process in AD because the abnormal function of Alzheimer platelets play an important role in the pathogenesis of the disease. Studies are in progress to verify any possible correlation with personality traits.

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Mitochondrial Dysfunction Potentiates Endoplasmic Reticulum Stress. Studies on Alzheimer's Disease Cybrids

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AD is a progressive and fatal disorder of the central nervous system with relevant social impact, characterized by progressive memory loss and deterioration of cognitive functions. An increasing body of evidence supports that endoplasmic reticulum (ER) stress and mitochondrial dysfunction have active roles in the neurotoxic mechanisms that lead to this pathology. This study was aimed at investigating whether impaired mitochondrial function affects the cell response to ER stress induced by thapsigargin or brefeldin A or by the AD-associated amyloid-beta peptide (Abeta1-40 isoform) using, as a cellular disease model, cytoplasmic hybrid (cybrid) cells made from mtDNA of non-familiar AD subjects or age-matched controls. AD cybrids display inhibition of complex IV of the mitochondrial respiratory chain, increased oxidative stress and perturbations in calcium homeostasis.

Results show that AD cybrids are more sensitive than control cybrids to ER stress induced by the AD-associated Abeta1-40 peptide or by the classical ER stressors thapsigargin and brefeldin A. GRP78 levels and caspase-4 activity, two ER stress markers, were significantly increased in AD cybrids when compared to controls. Furthermore, the activity of caspase-3 and the subsequent cleavage of poly-ADP-ribose polymerase (PARP) were higher in treated-AD cybrids. Finally, the decrease in cellular viability triggered by Abeta1-40, thapsigargin or brefeldin A was potentiated in AD cybrids when compared to control cybrids.

Altogether, results demonstrate that the mitochondrial dysfunction present in AD cybrids render these cells more sensitive to ER stress and support the close communication between ER and the mitochondria in the neuronal death that occurs in AD.

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Proline-Rich Polypeptide Complex (Prp) From Ovine Colostrum Regulate Activity of Antioxidant Enzymes

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Proline-rich polypeptide complex (PRP) was isolated from ovine colostrum by Janusz et. al. PRP shows an immunoregulatory and procognitive activities. In the form of orally administered tablets called Colostrinin® containing 100 μg of PRP, it improves the outcome of Alzheimer's disease (AD) patients. The mechanism of action of PRP/Colostrinin in AD is not yet clarified. It is known that the immune system plays an important role in neurodegeneration. Neurodegenerative processes may be enhanced by oxidative stress and overproduction of NO and proinflammatory cytokines. It was previously shown that PRP regulates the secretion of Th1 (IFN, TNF-alfa) and Th2 (IL-6, IL-10) cytokines and inhibits NO release (in vivo). Nowedays, the effect of PRP on the activity of antyoxidant enzymes - superoxide dismutase (SOD), catalase and glutathione reductase was examined. It was shown that PRP didn't change the activity of SOD, but when applicated together with PMA, the activity of SOD was inhibited about 40%. This results correlated positively with the hydrogen peroxide release.

In the case of catalase and glutathione peroxidase activity PRP showed also differential regulatory activity. The results presented suggest, that the benefitial effects of PRP/Colostrinin observed in AD patients may be due to regulatory activity of antioxidant enzymes and inhibition of overproduction of reactive oxygen species (ROS), which promote neurodegenerative processes.

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Studies on the Mechanism of a Proline-Rich Polypeptide (PRP)- Effect on Cytokine Induction

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The proline- rich polypeptide (PRP) isolated from ovine colostrum exhibits multifunctional properties. It modulates T cell functions, cytokine, reactive oxygen species and nitric oxide release both in mice and in cultures of human whole blood cells and isolated leukocytes. There properties pointed us to propose PRP as a potential drug in the case of Alzheimer'a disease. The beneficial effect of PRP in the form of orally administered tablets- colostrinin was shown in multicenter clinical studies. Soluble mediators such as cytokines are able to modulate the growth and function of cells within the central nervous system. HL60 and THP1 cell lines were used as a microglial cells model. Differentiation into monocyte/ macrophages was initiated by use of vitamine D3. Cytokines: IL-1β, IL-6, IL- 10, TNF-α, and IFN- γ induced by LPS, PHA or PRP were measured at the mRNA level (Real Time PCR). PRP shows different effects on induction of mRNA for cytokines. The effect depended on the stage of differentiation of the studied cells. TNF-α and IL-1β but not IL-6, IL- 10 or IFN- γ; mRNA was detected.

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Inverse Correlation Between Cancer and Alzheimer's Disease

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Cancer and dementia are high-prevalence diseases, but their relationship is controversial: autoptic and clinical series shown a negative association between cancer and AD, while cancer has been proposed as risk factor for dementia. Epidemiological evidences demonstrated negative correlation between PD and cancer.

Medical cancer history of 467 subjects including 202 AD, 87 MCI, 50 VaD, 24 FTLD, 28 non-degenerative dementias and 76 healthy controls was retrospectively evaluated to assess the frequency of malignancy in patients with MCI and dementia and the possible relationship with dementia subtypes.

21.05% of healthy subjects, 9.19% of MCI, 16.83% of AD, 29% of FTLD, 14% of VaD and 25% of other dementias had cancer hystory; to eliminate age bias we then considered only the 265 subjects over 75 (Italian "cancer-free" life expectancy). In this subgroup a significant lower prevalence of malignant neoplasia was observed in MCI (X2: 5.59, $p<0.02$) and in AD (X2: 3.84, $p<0.05$) compared with controls. Patients with FTLD seem to have more cancer in their medical hystory (37.5%).

This preliminary study offers interesting basis to further investigations, extended also on the biological and genetic side, considering the evidence of alteration of cell-cycle in AD brain, leading to apoptosis and neuronal death. These alterations, probably linked to an Ubiquitin Proteasome System (UPS) dysregulation, have been observed also in PD. Recent evidences also show alteration of progranulin gene espression, involved in cell survival, in FTLD. Larger studies will be useful to clarify if the higher cancer prevalence in FTLD may have a genetic base.

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Up-Regulation of Tissue Inhibitor of Metalloproteinases-3 in AD Brain: A Putative Link to Neuronal Apoptosis

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We have performed proteomics to identify proteins that mediate neuronal apoptosis induced by serum deprivation. Tissue inhibitor of metalloproteinases-3 (TIMP-3) was

identified as a potential target underlying serum deprivation induced apoptosis (SDIA). TIMP-3 was significantly increased within 2 hr following serum deprivation and sustained over 16 hr. Administration of an active MMP-3 prevented activation of Fas, caspase-8, and caspase-3 and neuronal death following serum deprivation but did not attenuate neuronal cell necrosis induced by excitotoxicity or oxidative stress. Western blot experiments revealed up-regulation of TIMP-3 in brain tissues of AD patients compared with age-matched control group. Neurons immunoreactive to TIMP-3 were positive to TUNEL staining. The present study provides evidence that TIMP-3 is up-regulated in neurons undergoing degeneration in AD brain and mediates neuronal apoptosis.

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Computer-Assisted 3d Reconstructions of the Human Substantia Nigra – A Tool for Neurodegenerative Studies

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Background and aims: The human substantia nigra is a characteristic nuclear complex of the mesencephalon. Its rostral pole extends to a plane connecting the commissura posterior to the mammillary bodies, whereas its caudal pole reaches as far as the inferior colliculus. It´s a concensus that the substantia nigra comprises the pars compacta consisting of cell-dense clusters of neuromelanin-rich neurons and the pars reticulata part with large widely dispersed neuromelanin-free nerve cells. The pars compacta can be further subdivided into rostral and caudal principal parts. Depending on the plane of section, highly complex nuclear profiles consisting of cell strands and islands can be identified allowing the pars compacta parcelation into more than 20 subnuclei. Different neurodegenerative diseases may either to affect or to spare some of these subnuclei, in a particular manner. Methods: Computer-assisted 3D reconstructions of the pars compacta clusters based on 360 to 440 µm thick Nissl-stained serial sections of two infant and five adult human substantiae nigrae. Results:The reconstructions revealed that the nerve cell of the caudal part form a perforated ridge, rather than clusters. Several cell strands radiate from the caudal part into a rostral direction. Therefore, the rostral and the caudal principal parts of the pars compacta are forming a unit resembling the fingers of a hand. Conclusion: Our investigation is intended to provide a normative basis of the human substantia nigra in order to assess and to locate foci of neuropathological changes in neurodegenerative diseases.

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Orthographic Regularity and Its Effect on the Spelling of Alzheimer's Patients

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There are few studies dedicated to investigate groups of patients with the same pathology across different languages. In this study, we compared one group of Spanish patients with other group of French patients (both with Alzheimer´s disease) in a writing-to-dictation task. Spanish has a more transparent orthography than French because writers in the first language can use phoneme-to-grapheme correspondences to spell words correctly.

We tested 20 Spanish patients, 20 French patients and a control group of 20 healthy participants for this research. The three groups were matched in age, sex and education level, and the average of the two groups of AD patients in the MMSE was identical. The participants were asked to write 30 words orally presented by the examiner. The words were orthographically identical in both languages, but 15 of them were regular and the other 15 were exceptions to the orthographic rule.

An interaction between the regularity and the language was observed: Spanish patients spelled regular words more accurately than irregular words, while French patients spelled irregular words more accurately than regular words. We propose that this reflects the impact of the relative transparency of the orthography on how spelling is learned and, in consequence, on how spelling knowledge is preserved as dementia progresses. A language like French, will tend to require the acquisition of more extensive knowledge concerning lexical orthography whereas a language like Spanish, with few exceptions, will tend to require the acquisition of more extensive knowledge concerning phoneme-to-grapheme correspodences.

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The Innate Immune System Clears Brain Amyloid-Beta But is Deficient in Patients With Alzheimer's Disease

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Background: Brain amyloidosis is the principal lesion of Alzheimer's disease (AD). Our neuropathological studies showed that blood-borne monocytes penetrate the blood-brain barrier in AD brain, invade neuritic plaques, up-load amyloid-beta, and may have a pivotal role in amyloid-beta clearance. Unlike the spinal fluid, peripheral blood monocytes can be easily obtained for diagnostic studies. Our previous results showed that normal monocytes and monocyte-derived macrophages phagocytize amyloid-beta (1-42, dissolved in DMSO) in vitro, but, in a majority of patients, these immune cells are poorly able to perform amyloid-beta phagocytosis. Methods: To examine the physiology and pathology of neuroprotection by the innate immune system, we exposed sections of AD brain to peripheral blood mononuclear cells of control or AD subjects and immunostained monocytes, neurons, glia and amyloid-beta. Results: Control subjects' monocytes avidly up-loaded and became saturated with amyloid-beta after 4 days, whereas AD patients' monocytes showed lesser avidity. To improve the AD innate immune system, we treated AD macrophages by curcuminoids, which were effective in 50% patients in enhancing amyloid-beta phagocytosis. Conclusions: A healthy innate immune system appears capable of clearing amyloid-beta from the brain. Therapeutic strategies should investigate enhancement of amyloid-beta phagocytosis by macrophages of AD patients.

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Anaerobic Glycolysis Protection Against 1-Methy-4-Phenylpyridinium (MPP+) Toxicity in C6 Glioma Cells

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The neurotoxin 1-methy-4-phenylpyridinium (MPP+) is used for its' capacity to induce Parkinsonism through its inhibitory effects on mitochondrial complex I. This inhibition disrupts cellular energy formation and aerobic glycolysis. The objective of this study was to demonstrate that the mitochondrial aerobic inhibition with MPP+ can be reduced by stimulating anaerobic glycolysis using glucose supplementation. In this study, C6 Glioma cell viability was examined in the presence of different concentrations of MPP alone and with the addition of glucose. Cell viability measurements by Resazurin showed a significant increase ($p<0.001$) in cells treated with glucose and MPP+ verses MPP+ treated alone. Fluorometric analysis using 100 uM Rhodamine 123 indicated mitochondrial membrane potential was not restored in MPP+ treated cells with glucose; however, normal cell viability was confirmed using 2 ug/ml Fluorescein diacetate. This dual fluorescence indicated mitochondrial damage from MPP+ while glucose augmented cell survival. Further confirmation of cell survivalwas evident in TUNEL staining. Positive staining was prominent only in MPP+ treatment groups alone, while control and co-treated groups exhibited little to no TUNEL staining. ATP measurements of all MPP+ treated groups exhibited a significant ($p<0.001$) decrease verses control. Groups co-treated with MPP+ and glucose revealed a significant increase (250 µM group: $p<0.001$) in ATP. It was conclude from this study that glucose supplementation was able to sustain cellular viability and ATP production through anaerobic glycolysis despite the inhibitory effect of MPP+ on aerobic glycolysis. (Supported by NIH grant RR03020)

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Differential Profiling of Proteins of the Mitochondrial Oxidative Phosphorylation System in Alzheimer's Disease

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Most cellular energy in the form of ATP is produced by mitochondria. Mitochondria also produce free radicals which can damage cellular structures. There is increasing evidence that mitochondrial dysfunction plays an important role in the pathology of a number of neurodegenerative diseases, including Alzheimer's disease (AD). Dysfunction of mitochondrial energy metabolism would lead to reduced ATP production, altered Ca2+ buffering, and increased production of free radicals. Our hypothesis is that the proteins of the mitochondrial energy production system are reduced or damaged in AD brains. To test this hypothesis, we are using a quantitative proteomics approach to compare the levels of the mitochondrial proteins in AD versus normal brain during the course of the disease. We are examining this issue using genetically altered triple transgenic mice (3xTg-AD; APPSwe, PS1M146V, tauP301L) which develop age-dependent accumulation of both amyloid plaques and neurofibrillary tangles, as well as age-associated memory impairments. Quantitative proteomics will be achieved using two dimensional differential gel electrophoresis (2D DIGE), followed by mass spectrometry analysis. Differential profiling of mitochondrial proteins will be carried out using mitochondria isolated from the cortex and hippocampus of wild-type and 3xTg-AD mice at three stages of AD progression: before (2 months old); during (6 months of age); and after (14 months old) the appearance of amyloidal and tangle pathology and cognitive impairment. The findings from these studies should help further define the role of mitochondrial energy dysfunction in the pathogenesis of AD.

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Inflammation Sensitive Proteins Effects on Amyloid-Beta Aggregation and Toxicity

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Background and aims: Recent evidence strongly implicates the inflammatory response in the pathogenesis of Alzheimer's disease (AD). AD is considered to be associated with a low-grade chronic inflammation where activated glial cells produce pro-inflammatory cytokines and Reactive Oxygen Species that over long periods of time create a pro-oxidative state favouring the beta-amyloid (Aß) peptide fibrillation and aggregation leading to senile plaque formation and disease progression. The first reaction to immunological challenge is the innate response: the pro-inflammatory cytokines are released, and also other inflammatory mediators such as non-specific acute-phase reactants (inflammation-sensitive proteins) are involved. Inflammation sensitive proteins represent important yet non-specific markers of inflammation that can be used for assessing reactive processes, especially when both positive and negative acute phase variables are investigated.

Methods - We studied the effects of several inflammation-sensitive proteins on Aß 25-35 induced fibrillar aggregation and cytotoxicity, investigating both the so-called positive and negative acute phase reactants. Results - Both reactants were able to interact with the Aß-peptide but with differential effects and mechanisms on fibrillar aggregation and toxicity. Conclusions -Taking into account the results here reported and the previously demonstrated presence of inflammation-sensitive proteins in the senile plaques, it is possible to hypothesize a model where these proteins can act in a first phase as Aß-protecting or Aß -interacting substances that, however, in later phases, may themselves be involved in the aggregation process thus explaining their co-deposition together with Aß within the senile plaques.

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Calcium Binding Proteins in Inferior Olivary Nucleus in Alzheimer's and Parkinson's Diseases

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Calcium binding proteins (CBPs) are markers for neuronal viability, and their stability is shown to be impaired both in aging and Alzheimer's disease (AD), depending on brain region. In AD, neurons in the principal nucleus of the inferior olive (PO) are lost but the underlying pathomechanisms are not known. Normal levels of CBP's are needed to maintain Ca2 homeostasis, neuronal excitability, rhytmicity and synchronization. In this study we estimated the proportion of neurons that maintained or lost expression for three CBPs (calretinin (CR), calbindin (CB) and parvalbumin (PV)) during normal aging, AD and Parkinson's diseases (PD). An unbiased stereological approach was used with vertical and horizontal sections, and the percentage of positive and negative cells were calculated. In normal aging there was no reduction of CR or CB, while PV was lost after 40 years of age. Moreover, the number of CB-positive cells, but not CR-positive cells increases in relation to age. The PD brains showed a similar staining pattern, as in controls, for both CR and CB. In the AD group a significant decrease of CR- and CB-positive neurons and an increase of negative neurons were estimated. In AD brains, the final results of horizontal and sagittal sections differed significantly, indicating that the orientation of structure and cutting plane become important. Since CBPs have a multi-functional role in neurons, especially in the olivo-cerebellar loop, we can conclude that the neurons in the PO loose CBPs in AD but not in PD, which may indicate a disease specific neuronal impairment.

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Distribution of LIF and LIF-Receptor Beta in Alzheimer Disease, Parkinson Disease and Control Brains

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Background and aims: Leukemia inhibiting factor (LIF) is a member of the (IL)-6 family of cytokines and is involved in neuronal and immune responses to injury. LIF signals through the LIF-receptor (LIFR), which is composed of two components, LIF-receptor-beta (LIFRB) and gp130. LIF expression has been detected in Alzheimer's disease (AD). Expression of LIFR in AD and expression of LIF and LIFR in Parkinson's disease (PD) is unknown. The aim of the study was to quantify and study the distribution of LIF and LIFRB expression in AD, PD and control brains. Methods: Brain specimens from 34 patients with AD, 40 patients with PD and from 40 healthy controls were studied. DNA was prepared from brain tissue and stored at -70ºC. LIF and LIFRB copy number was determined by quantitative real-time PCR. The distribution of LIF and LIFRB expression was investigated by immunoperoxidase staining of brain sections. The difference between the groups in LIF or LIFRB expression was analyzed using the Student's t-test. Results: LIF expression in temporal cortex and hippocampus was highest in AD and in anterior cingulate cortex in PD. LIFRB expression was highest in AD hippocampus. The difference between LIF copy numbers in AD temporal lobe region in comparison with control temporal lobe region was statistically significant ($p=0.04$) Conclusions: LIF is involved in AD pathogenesis either as a factor promoting neuronal survival or as a mediator of the inflammatory reaction seen in AD.

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Presenilin 2 is Secreted in Mouse Primary Neurons

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Background and aims: Presenilin 1 (PS1) and presenilin 2 (PS2) encoding genes are genetically associated with familial Alzheimer's disease (AD), a neurodegenerative disorder characterized by the death of neurons in brain regions involved in cognition. The main objective of our work is to characterize PS2 metabolism under physiological and pathological conditions. Methods: Intracellular and extracellular levels of PS2 CTF were analyzed in mouse primary neurons and in N9 cell line both in control and apoptotic conditions. Cells were treated with 1 uM staurosporine (Sts) in serum deprivation condition. The PS2 intracellular and extracellular protein levels were detected by Western blot. Results: As previously reported by our group for PS1, here we demonstrated that, in mouse primary neurons and microglial cells, PS2 CTF is released by shedding into the extracellular compartment as a soluble form and that this release is increased during apoptosis. Confocal imaging analysis on mouse primary neurons showed that, in physiological condition, PS2 is localized mainly in the cell body and in its growth cone; after Sts treatment, the PS2 staining was partially redistributed to the plasma membrane, along neuronal processes and to shedded vesicles. Conclusions: In our experimental paradigm, PS2 CTF fragment seems to play a role in the extracellular compartment. The understanding of biological events regarding proteins demonstrated to be causative of genetic forms of AD might add new targets in the difficult search for therapeutics for sporadic forms of the disease.

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Calcium Binding Protein Expression in the Human Hippocampus and Its Relation to Tau Pathology

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Background and aims: The pathological findings in Alzheimer's disease (AD) are partly attributed to alterations in calcium-binding protein (CBP) functions. We evaluated

immunoreactivity of secretagogin, a recently cloned CBP, in hippocampus of 30 neuropathologically examined post mortem brains (female, 18; mean age, 79.8 ±15.1 years; 15 cases, definite AD).

Methods: Sections were incubated with secretagogin-specific antibodies and the number of immunoreactive neurons as well as staining intensities in both neurons and neuropil were assessed.

Results: Both cellular and neuropil immunoreactivity were restricted to subiculum and Ammons horn. Cellular immunoreactivity was further restricted to pyramidal neurons and showed a hierarchical distribution: the mean percentage of immunoreactive neurons was highest in sector CA3 (64.41%), followed by CA2 (44.09%), CA4 (34.38%), CA1 (10.9%), and the subiculum (2.92%; $P<0.001$, except CA2-CA4, $P>0.05$), while it did not differ significantly between groups with different degrees of AD pathology. Double staining for both tau and secretagogin (immunofluorescence) revealed that both proteins rarely co-localize since 5.3% of tau and 2.9% of secretagogin positive neurons, respectively, showed such co-localization and secretagogin.

Conclusions: The pattern of secretagogin immunoreactivity resembles that of calcium sensor proteins as it is restricted to a subset of neurons and therefore secretagogin could serve highly specialized tasks in neuronal calcium signalling. The lack of an association between tau burden and the density of secretagogin expressing neurons together with the low co-localization rate of both tau and secretagogin in the human hippocampus, suggest that secretagogin expressing neurons are largely resistant to neurodegeneration in AD.

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Peripheral Inflammation and Neuroprotection: Subcutaneous Injection of Complete Freund Adjuvant Reduces 6-Hydroxydopamine Toxicity in a Rodent Model of Parkinson Disease

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The aim of this study was to investigate whether pre-conditioning with a peripheral, pro-inflammatory stimulus is able to modify the progression of the neurodegenerative process, in a rodent model of Parkinson's disease (PD). For this purpose, Complete Freund's adjuvant (CFA) - a water/oil emulsion containing inactivated M. tuberculosis - was inoculated, subcutaneously, to Sprague-Dawley rats prior to the intrastriatal injection of 6-hydroxydopamine (6-OHDA). Animals were sacrificed 7 and 28 days following 6-OHDA injection; neuronal damage, glial activation and cytokine levels, within the nigrostriatal system, were then investigated. Nigrostriatal degeneration induced by 6-OHDA was accompanied by early microglial and astroglial activation, which preceded the onset of dopaminergic cell loss, in the SNc, without significant changes in cytokine levels. CFA pretreatment markedly reduced the SNc neuronal loss and associated microglial activation, as well as the rotational response to apomorphine. These changes were associated with moderate, transient increases in the nigrostriatal levels of glial-cell derived neurotrophic factor (GDNF) and pro-inflammatory cytokines, including interleukin (IL)-1alpha, IL-1beta and IL-6. Our results show that prior delivery of a peripheral, pro-inflammatory stimulus induces neuroprotection, in a rodent model of Parkinson's disease, possibly through the modulation of cytokine production at the nigrostriatal level.

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Effects of MPTP and Rotenone on Dopaminergic Neuronal Growth in Organotypic Culture

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Background & Aims: Complex I inhibitors such as N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and rotenone have been shown to reproduce features of Parkinson's disease, including selective nigrostriatal dopaminergic (DAergic) degeneration. Using organotypic cultures from day 5-6 Sprague-Dawley rat pups, we established growth of dopaminergic neurons up to 7 weeks in vitro without any trophic supplementation or special neuronal medium.

Methods: Optimal growth of tyrosine hydroxylase (TH+ve) neurons was observed from 3 to 5 weeks with neurons displaying axons and dendritic branching. Acute oxidative stress was induced by treatment with MPTP or rotenone on 7 and 14 day cultures for 24 hours at various concentrations. Growth and recovery period was then assessed at 1, 2 and 3 weeks-post treatment.

Results: Both neurotoxins induced degeneration of DAergic nerve cells and neuronal loss in a dose-dependent manner. At high doses of MPTP or rotenone, significant destruction of TH-labelled DAergic neurons was observed compared to controls and vehicles. Week 1 DA cultures showed some recovery with significant regrowth following toxin exposure but in week 2 cultures showed significant reduction of TH+ve cells and very reduced regrowth after neurotoxin. These results indicate that DAergic neurons grown for 14 days in vitro are more susceptible to the complex I inhibitors than 7 day cultures and do not recover as well as 7 day cultures.

Conclusion: We hypothesize that the increased differentiation and innervation of their striatal target of 2 week postnatal DAergic neurons enhanced their susceptiblity to the effects of the neurotoxins and their reduced regrowth compared to 1 week cultures.

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Modulation of BACE-1 Expression and Beta-Secretase Activity in Primary Cultures From Brain

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Background and aims: After the identification of BACE-1, the beta-secretase playing a key role in the generation of beta-amyloid peptides, several papers have described experimental conditions in which changes of this enzyme expression take place in neurons, astrocytes and brain tissue. Based on these results, and in line with the observation of changes in the secretase levels in brains from Alzheimer patients, the hypothesis has been put forward that alterations in the control of BACE-1 expression may be involved in the pathogenesis of this disease. Testing of this hypothesis appears as a crucial task, not only to further our understanding of the molecular mechanisms that govern BACE-1 expression, but also for the development of new therapeutic strategies.

Methods: Our work was carried out in rat primary cultures of neurons and astrocytes exposed to the experimental treatments that had been shown to modulate BACE-1 expression and/or beta-secretase activity.

Results: In our experimental conditions such changes were not detected, except for a significant reduction in beta-secretase activity observed in astrocytes exposed to a mix of cytokines.

Conclusions: No changes in BACE-1 expression could be obtained in primary neurons exposed to environmental stress.

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SorLA and APP Trafficking

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SorLA is a membrane protein highly expressed in neurons of the brain. Its homology to intracellular sorting receptors that shuttle proteins between endosomes and Golgi, suggests a related function for sorLA in neuronal trafficking processes. We demonstrate selective loss of sorLA expression in the brain of patients suffering from Alzheimer's disease (AD) indicating a causal role for the receptor in the pathogenesis of this disease. Our studies show that sorLA interacts with the amyloid precursor protein (APP) in vitro and in cells, and that both proteins co-localize in endosomal and Golgi compartments. SorLA protects against the proteolytic breakdown of APP to amyloid b-peptide (Ab) by controlling APP maturation and Golgi transport. In addition, we have identified sorting signals within the cytoplasmic region of sorLA important for its intracellular function as a sorting receptor. By mutation of these motifs, we show that mislocalization of sorLA greatly affect APP processing, and can either lead to increase or decrease of Ab production dependent on the subcellular localization of the receptor mutants. Ablation of sorLA expression in knockout mice results in increased APP processing and Ab levels as well as enhanced amyloid plaque deposition similar to the situation seen in patients with spontaneous AD.

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A Potential Role for Micrornas in Alzheimer's Disease

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MicroRNAs are small endogenous non-coding RNAs that have emerged as universal regulators of gene expression at the post-transcriptional level. Although essential for life, recent studies point to a pathogenic role for microRNA deregulation in disease. Recent genetic evidences suggest a role for gene expression misregulation in Alzheimer's disease (AD). We therefore addressed the question whether microRNA deregulation could contribute to AD pathology. Using bioinformatics, in vitro systems and cellular models, we have obtained direct evidence for the regulation of genes in the amyloid cascade by microRNAs. Global microRNA expression profiling from sporadic AD brains showed several microRNAs that are specifically misexpressed in disease. In a small cohort of AD patients, we found a correlation between protein expression and changes in particular microRNA levels. Last, we provide support for the regulation of AD-related genes by microRNAs under physiological conditions in vivo. Our results demonstrate that AD-related genes can be targets for microRNA regulation. We hypothesize that by loss or gain of function mechanisms, microRNAs could contribute to AD pathology and possibly other neurodegenerative disorders.

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Interferon-Gamma and Tumor Necrosis Factor-Alpha Regulate Amyloid-Beta Plaque Deposition and Beta-Secretase Expression in Swedish Mutant APP Transgenic Mice

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Background: Reactive astrocytes and microglia in Alzheimer's disease (AD), surround amyloid plaques and secrete pro-inflammatory cytokines that affect neuronal function. Relationship between cytokine-signaling and amyloid-beta peptide (Abeta) accumulation is poorly understood.

Methods: We created a novel Swedish beta-amyloid precursor protein mutant (APP) transgenic mouse where the interferon (IFN)-gamma receptor type I was knocked out (APP/GRKO). Animals were tested at 14 months of age by biochemistry and immunohistochemistry. Primary cultured astrocytes, microglia, and neurons from wild type (WT) or GRKO mice were also tested for their Abeta production, pro-inflammatory cytokine production, and BACE expression.

Resutls: IFN-gamma signaling loss in the APP/GRKO mice reduced gliosis and plaques (both diffuse and compact)

in cortex and hippocampus. IFN-gamma- elicited tumor necrosis factor (TNF)-alpha secretion in WT but not GRKO microglia and co-cultured astrocytes. Both IFN-gamma and TNF-alpha enhanced A_eta production from APP-expressing astrocytes co-cultured with microglia or APP-expressing cortical neurons, which was attenuated by anti-TNF-alpha neutralizing antibodies. TNF-alpha directly stimulated beta-site APP cleaving enzyme (BACE1) expression and enhanced beta-processing of APP in astrocytes. The numbers of reactive astrocytes expressing BACE1 were increased in APP compared to APP/GRKO mice in both cortex and hippocampus. IFN-gamma and TNF-alpha activation of WT microglia suppressed Abeta degradation, while GRKO microglia had no changes.

Conclusion: These results support the idea that glial IFN-gamma and TNF-alpha enhance Abeta deposition through BACE1 expression and suppression of Abeta clearance. Taken together, these observations suggest that pro-inflammatory cytokines are directly linked to AD pathogenesis.

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Novel Neuroprotective Action Mechanism of the Ginkgo Biloba Extract EGb761: Potential Implications for Cerebral Amyloid Angiopathy, Chronic Hypoperfusion and Stroke

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Background: Ginkgo biloba extract (EGb761) is a well-standardized extract with origins in traditional Chinese medicine. The extract constituents are likely to have synergistic effects and have been shown to protect against cell death. In the CNS, EGb761 can protect neurons exposed to oxidative stress injury. Although it is thought that EGb761 has anti-oxidative properties, its mechanism remains unclear. We tested the hypothesis that EGb761 affords cellular protection by inducing the endogenous antioxidant enzyme heme oxygenase (HO). HO is the rate-limiting enzyme for the degradation of free heme, a pro-oxidant derived from the degradation of numerous hemoproteins. It also generates carbon monoxide, which at physiological levels can act as a vasodilator.

Methods and Results: Using cortical neuronal cultures, we first observed that EGb761 is one of the most potent inducers of HO-1 and that this induction is sufficient to provide neuroprotection against induced oxidative stress and excitotoxicity. The neuroprotection is blocked significantly by an HO inhibitor or a de novo protein synthesis inhibitor. We further examined whether EGb761 is protective against a mouse model of transient ischemia. After being treated for 7d with EGb761, the mice had a 48% smaller infarct volume and an associated 51% better neurobehavioral outcome than mice that received vehicle. Interestingly, when mice lacking the HO-1 gene were treated, the EGb761 therapeutic effects were almost completely abolished.

Conclusion: Together, the data suggest that EGb761, by inducing HO-1 enzyme, can provide the brain with resistance to various acute and/or chronic neurodegenerative conditions, such as stroke and Alzheimer disease.

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Dihydropyridine-Type Calcium Antagonists and Cognitive Function in Elderly Patients: Analysis of Controlled Clinical Studies

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Dementia disorders of vascular origin are included in the heterogeneous nosographic entity of vascular dementia (VaD). Arterial hypertension is a main risk factor for cerebrovascular disease, cognitive decline and dementia. Treatment of VaD except than in terms of prevention of risk factors still represents a challenge.

Data from recent trials suggest that antihypertensive treatments may contribute to prevent dementia disorders. Calcium antagonists of dihydropyridine-type are the class of antihypertensive drugs decreasing more significantly the risk of cognitive impairment independently of the blood pressure level. This suggests that these antihypertensive agents may have a neuroprotective activity.

Dihydropyridine derivatives investigated for cognitive decline and dementia were nimodipine, nitrendepine and nicardpine. Nimodipine was of some benefit in the treatment of patients with features of dementia. More recent studies have reported a benefit of treatment with nimodipine in subcortical VaD. In subjects over the age of 60 years with isolated systolic hypertension, treatment with nitrendipine reduced the incidence of dementia. Nicardipine is the dihydropyridine derivative investigated in the larger number of patients, having been studied in more than 5,000 subjects suffering from VaD. The drug was effective for treating mental deterioration of vascular origin and delayed worsening of cognitive decline.

The documented effects of the above dihydropyridine-type calcium antagonists in patients with VaD support the rationale for their evaluation in dementia disorders associated with cerebrovascular disease.

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Holistic Approach to Manage the Alzheimer Disease

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Alzheimer's disease presents a formidable challenge to doctors and patients. First World Conference on Alzheimer's disease in Washington, in July 2000, found acupuncture to be very helpful in treating anxiety, depression associated with Alzheimer's, and in slowing the progressive deterioration of cognitive function.

The study is of 8 elderly patients, average age 66 years, diagnosed with mild to moderate Alzheimer's. They were on medicines for last1-3 yrs but were not satisfied. They were treated with disposable sterilized acupuncture needles, daily for 10 days and then 2-3 times per week for 3 months along with electrical stimulations. Each treatment, done under strict aseptic conditions lasted for 20-25 minutes, medicines continued as before. Periodic evaluation and assessments of depression, sleep problems and ability to function in activities

of daily living were done by team of psychiatrist, acupuncturist, and family caregivers.

Acupuncture is found very helpful in reducing anxiety and depression in Alzheimer's. The patients felt increased energy and pain relief from other age related conditions. The cognitive functions appeared to remain stable over 4-5 years follow ups. There were no negative side effects of acupuncture. All patients expressed satisfaction and enjoyment during acupuncture treatments, with no dropouts from study. Holistic/ acupuncture treatment is safe and useful in Alzheimer and is not antagonist to medicines.

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Reversal of Symptoms of Alzheimer's Disease Following Omentum Transposition to the Brain

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Background and Aims: To show that patients who have Alzheimer's disease can have their cognitive and neurological symptoms reversed by placing the omentum directly on the brain.

Methods: The omentum is separated from the transverse colon and from the proximal portion of the stomach, leaving the gastroepiploic vessels intact within the omental apron. The omentum is then surgically lengthened and brought up through a subcutaneous tunnel developed along the chest and neck and behind the ear. A craniotomy is performed, the dura opened, and the omentum laid on the brain.

Results: One-third of patients who have had the procedure have had their symptoms reversed, most probably because of increased CBF following omental transposition (OT) to the brain. In Alzheimer patients, it is theorized that improvement in cognitive symptoms results from increased blood flow and biochemical substances originating from the omentum, which allow viable but deteriorating neurons to be "rescued" by augmenting neuronal energy (ATP production) that leads to cognitive improvement.

Conclusion: It is possible to reverse cognitive and neurological symptoms of biopsy-proven AD patients as a result of OT to the brain.

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Combination Therapy of Acetylcholinesterase-Inhibitor and Vitamin E in Alzheimer's Disease

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Alzheimer's disease is a neurodegenerative disease with progressive cognitive decline including predominent memory loss and impairment of functions of daily living. Treatment strategies include acetycholine-esterase inhibitors to increase the availability of acetylcholine hence improve memory function, NMDA-antagonists to diminish excitotoxic glutamatergic effects and antioxidants to prevent from free scavengers. However until today almost no data on combination therapy of two or more of these substances are available. In a retrospective design 41 patients diagnosed with mild to moderate Alzheimer's disease (AD; NINCDS-ADRDA criteria; age 68.9 ± 8.1 years [mean ± standard deviation]) could be separated into patients receiving either an acetylcholin-esterase inhibitor alone (28 patients, MMSE 23.5 ± 3.6) or in combination with high-dose vitamin E (13 patients, MMSE 21.8 ± 5.3). Mean observational time was 14.5 month. In no patient therapy had to be discontinued. The group receiving vitamin E had a significant less decrease of the MMSE (0.5 points vs. 3.1, mean difference 2.8 ± 1.0, 95% confidence intervall 4.9 – 0.7, p=.01). ApoE, age and type of acetylcholine-esterase inhibitor did not have a significant confounding effect on the results. It was for the first time that a beneficial effect of combination of an acetylcholine-esterase inhibitor and vitamin E to an acetylcholine-esterase inhibitor alone could be demonstrated. Limitation is the retrospective design and the small sample size. Further studies with a randomized placebo-controlled prospective design are required.

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Hyaline Atherosclerosis and Cognitive Decline in a Large Clinicopathological Series

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Background and Aims: It is still a matter of debate how vascular changes, specially the microscopic ones are related to cognitive status. To assess which kind of vascular change is more associated with cognitive status independently of AD pathological changes

Methods: Randomic sample of a neuropathological series belonging to the Brazilian Brain Bank of the Aging Brain Study Group consisted of 76 retrospectively studied cases. Those that fulfilled CERAD criteria or had Braak criteria suggestive of AD (N=20) were excluded. The remaining subjects (N=56) were classified according to the CDR.into CDR=0 (N=26), CDR=0.5 (N=12) and CDR≥1 (N=18). The association between certain vascular changes (infarcts, hyaline arteriolosclerosis, siderocalcinosis, circle of Willis atherosclerosis) with cognitive decline was tested both separately and combined. Data were analyzed with chi-square tests using the statistical software Stata v.9.0. Differences were considered significant if $p<0.05$)

Results: Brain infarcts were observed in 4 subjects, whereas 12, 7 and 19 subjects presented hyaline arteriolosclerosis, siderocalcinosis and circle of Willis atherosclerosis, respectively. The only significant association was found between the presence of hyaline arteriolosclerosis and CDR >0 ($p<0,011$ and $p<0,005$, if considering CERAD or Braak staging exclusion criteria).

Conclusions. Hyaline atherosclerosis was the vascular change most associated with cognitive decline in a sample of cases without pathological evidence of AD. This finding supports the evidence that microvascular changes, not only of brain infarcts, may independently cause cognitive decline

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Expression of Gga1 is Altered in Alzheimer's Disease and Regulates the Generation of Amyloid ß-Peptide

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Background and Aims: Aß is a major component of the Alzheimer disease (AD)-associated senile plaques and is generated by sequential cleavage of APP by ß-secretase (BACE1) and γ-secretase. Since BACE1 initiates Aß generation, it is important to understand the molecular mechanisms that regulate its cellular metabolism, transport and activity.

We and others showed that GGA proteins (Golgi-localized-gamma-ear-containing-ARF-binding) interact with BACE1 and are involved in the subcellular trafficking of BACE1. Since their role in generation of Aß and the pathogenesis of AD is not known, we wanted to investigate whether GGA1 affect APP processing and to examine the expression of GGA1 in human brain.

Results and Conclusions: We demonstrate that GGA1 is preferentially expressed in neurons of the human brain. Notably, the expression of GGA1 was found to be significantly decreased in AD brains. Functional analyses with cultured cells demonstrate that GGA1 is implicated in the proteolytic processing of APP. Over-expression of GGA1 or a dominant-negative variant reduced cleavage of APP by BACE1 as indicated by a decrease in CTF-ß generation. Importantly, the secretion of Aß was also reduced, while RNAi mediated suppression of GGA1 increased the generation of Aß. The modulation of APP processing by GGA1 is independent of a direct interaction of both proteins. Since total cellular activity of BACE1 was not affected by GGA1 expression, our data indicate that changes in the subcellular trafficking of BACE1 or other GGA1-dependent proteins contribute to changes in APP processing. Thus, GGA proteins might be involved in the pathogenesis of AD.

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The Physician and Pharmacist as Teachers in a Specialized Alzheimer's Facility

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The establishment of a facility exclusively for persons with Alzheimer's disease has afforded a unique teaching opportunity. The merging of academia and the service sector can be a positive experience, promoting Alzheimer's competency through more effective educational programs, improving patient care, and providing a forum for the exchange of information between students and staff. Students in a variety of health and related fields have an opportunity to experience the effects of this disease on individuals and families, and learn how to best manage their care by participating in weekly physician and pharmacist rounds. In this session, participants will experience a teaching program in a facility that specializes in the care and study of individuals with Alzheimer's disease and dementia. Topics include: 1) defining the educational mission; 2) essential content of an affiliation agreement; 3) defining roles and relationships; 4) creating an environment for success; 5) benefits for staff, students, families and residents; 6) promoting positive student experiences; and, 7) facilitating mutually beneficial research. Over the past 17 years, over 2000 students from a variety of fields such as medicine, nursing administration, pharmacy, health care administration, gerontology, social work, geropsychiatry, etc., have participated in this program, generating more effective educational programs and clinical experiences. In addition, several research projects are in progress due to the educational exposure. Program benefits touch many including students, staff, residents and families, who believe it to be a valuable experience for all involved.

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Benefits of a Research Program in a Specialized Alzheimer's Facility

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Increasingly, research is being conducted in long-term care to examine the benefits of specific programs as well as the effects of pharmacological treatment, particularly as related to Alzheimer's disease. The Alois Alzheimer Center, a free-standing Alzheimer's disease and dementia facility, began conducting research in 1988. While there was information in the literature about Alzheimer's disease related therapies at that time, little was research based. This 102 bed facility, housing a continuum of care, was an ideal setting for research with sufficient numbers of participants and individuals in various stages of the disease process. A protocol review and research procedures were developed. Existing university affiliations for education and research enhanced the long-term care research capacity. The research conducted over the past 20 years has been of benefit to all individuals, including residents, families and staff. The program has grown to include collaborative efforts with other individuals, organizations and institutions to enhance the number of projects and learning for all. All information is shared by Alois Alzheimer Center and partners through national and international presentations and publications.

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Effect of Indomethacin on Alzheimer's Disease Progression: A Randomized Controlled Trial

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Background and Aims: Previous research indicates that inflammation plays a role in the pathogeneses of Alzheimer's disease (AD), and nonsteroidal anti-inflammatory drugs (NSAIDs) may retard the progression of the disease. The objective of this study was to determine whether treatment with (the nonselective NSAID) indomethacin slows the cognitive decline in patients with mild-to-moderate AD.

Methods: A double-blind, randomized, placebo-controlled multicenter trial was conducted in which 51 patients received 100 mg indomethacin or placebo daily for 12 months. Additionally, all patients received omeprazole. The primary outcome measure was the change from baseline after one year on the cognitive subscale of the AD Assessment Scale (ADAS-cog). Secondary outcome measures included the Mini-Mental State Examination, the Clinician's Interview Based Impression of Change with caregiver input, the noncognitive subscale of the ADAS, the Neuropsychiatric Inventory, and the Interview for Deterioration in Daily life in Dementia.

Results: 19 of 25 patients in the placebo group, and 19 of 26 patients in the indomethacin group completed the study. No significant differences between treatments were found on the ADAS-cog (indomethacin = –7.8 ± 7.6; placebo = -9.3 ± 10.0; p value = 0.61), or on any of the secondary outcome measures. More serious adverse events were reported in the indomethacin group than in the placebo group.

Conclusion: The results of this study do not support the hypothesis that indomethacin slows the progression of AD. However, since treatment numbers are too small, an effect of indomethacin cannot be excluded.

1003

Physical and Emotional Environment as An Effective Treatment in Alzheimer's Disease

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Individualized programs serve to benefit residents as much as pharmaceutical treatments. Assessing specific individual needs and providing continuum-based dementia care provides person-centered care, consistent staff and programs, and modifications in the emotional atmosphere/physical environment based on disease progression. As residents progress at different rates and in unique ways, so must programming and environment change to meet resident needs. This presentation describes a 20 year-old continuum-based, dementia program within a 102-bed dedicated facility. A sample of residents will be described with regard to behavioral functioning, cognitive characteristics and personal care needs across four distinct, secure units. Residents were selected purposively to be representative by unit, including 76% of residents in the facility. Measures include the Reisberg Global Deterioration Scale (GDS), Cohen-Mansfield Agitation Inventory (CMAI), Haycox Dementia Behavioral Scale (DBS), and demographic items of age, diagnosis and prior residency. Mean unit GDS ranged from 4.08 to 6.00. Mean unit CMAI scores ranged from 1.23 to 1.5, with the highest incidence of behaviors exhibited in the third of four units, most often related to wandering. Mean unit DBS scores ranged from .96, with the greatest between unit changes for continence, dressing and grooming. Given these measures and direct observation of residents, staff and environment, residents will be characterized by unit, with a description for level of care and stimulation, atmosphere and physical environment. Beneficial treatment results identified in individuals with movement to new units will be discussed. Results are useful in terms of targeting specific populations and tailoring programs/environments along the care continuum.

1004

The Generation Effect as a Means to Discriminate Patients With Mild Cognitive Impairment From Those With Alzheimer Disease

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Previous research has shown that people learn and remember more easily self-generated information than the information they have been given by the experimenter. This is known as the generation effect, and has proved to be effective to improve the learning and memory in healthy adults and in some neurological disorders (e.g. multiple sclerosis, brain injury). But in other diseases such as the Alzheimer dementia (AD), patients don't seem to benefit from this strategy. We report recent research designed to investigate if the generation effect occurs in people presenting symptoms of mild cognitive impairment, as it is a middle stage between the health and the cognitive disorders typical from the AD.

Three groups (control, AD and mild cognitive impairment) were asked to read a list of 32 sentences. In 16 of them the last word was missing (generation condition), and the patients had to complete each sentence with a suitable word. For the other 16 sentences (provided condition) the last word was underlined, and the participants were simply asked to read them aloud. Then the examiner asked the participants to recall and recognize the 32 words (either missing or underlined) immediately, after a delay of 30 minutes and a week later.

The presence of the generation effect in patients with mild cognitive impairment could be a useful factor that contributes to differentiate this disease from the AD patients.

1005

Vagus Nerve Stimulation in Alzheimer's Disease: Follow-Up Results Through 12 Months

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Background and Aim: Cognitive-enhancing effects and safety of vagus nerve stimulation (VNS) have been reported during 6 months of treatment in a pilot study in Alzheimer's disease (AD). Data through 12 months are now reported.

Methods: All patients (n=17) met standard criteria for probable AD. Responder rates for the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) and Mini-Mental State Examination (MMSE) were measured as improvement or absence of decline from baseline. Global change, depressive symptoms, and quality of life were also assessed. Cerebrospinal fluid (CSF) levels of total tau, tau phosphorylated at Thr181 (phospho-tau), and Abeta 42 were measured using standardized ELISA.

Results: VNS was well tolerated. After 1 year, 41.2% (7/17) and 70.6% (12/17) of patients improved or did not decline from baseline on the ADAS-cog and MMSE, respectively. Twelve of 17 patients were rated as having no change or some improvement from baseline on the Clinician Interview-Based Impression of Change (CIBIC+). No significant decline in mood, behavior, or quality of life occurred during 1 year of treatment. The median change in CSF tau at 1 year was a reduction of 4.8% (p=0.057), with a 5.0% increase in phospho-tau (p=0.040; n=14). All patients continued treatment with VNS beyond 12 months.

Conclusion: The results support long-term tolerability of VNS in patients with AD and warrant further investigation.

1006

Design and Baseline Data for a Study of Lipitor's Effect in Alzheimer's Dementia (Lead-E)

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Introduction: Statin use is associated with a reduced risk for Alzheimer's Disease (AD). Cholesterol may influence the development and progression of AD by increasing amyloid precursor protein processing and associated beta-amyloid production. Lowering cholesterol with statins can reduce beta-amyloid production and could modify disease progression. The LEADe study tests the hypothesis that statin (atorvastatin-ATV) with cholinesterase inhibitor (donepezil-DPZ) benefits cognition and global functioning over DPZ alone.

Study objectives: To evaluate the efficacy and safety of combining ATV 80mg + DPZ 10mg vs. DPZ alone on cognition and global functioning in mild-moderate AD patients. Other clinical outcome measures include: activities of daily living, neuropsychiatric symptoms, global dementia severity.

Methods: This is a 20-month multicenter (100 sites), double-blind, randomized (1:1), parallel-group study of 600 mild-moderate AD patients (MMSE 13-25). Inclusion criteria were: men/women 50-90 yrs, stable DPZ 10mg $\geq$ 3 months, LDL-C 95-195 mg/dL. Co-primary endpoints are changes in AD Assessment Scale-Cognitive subscale (ADAS-Cog) and AD Cooperative Study-Clinical Global Impression of Change (ADCS-CGIC) Scale scores. Evidence for disease-modifying effects of ATV + DPZ are evaluated by ATV withdrawal and measuring brain volume and metabolism (MRI/MRS) and AD biomarkers.

Current status: Enrollment is complete. The baseline mean data are: age 74+/-8 years, 53% females, MMSE 21+/-3, ADAS-Cog 32+/-11, ADFACS 13+/-9, NPI 10+/-11. Mean prior DPZ treatment was 409+/-407 days.

Conclusion: This large placebo-controlled RCT will be completed by the end of 2007 and will provide a more definitive evaluation of the potential for statins in the treatment of people with AD.

1007

Use of Simvastatin is Associated With a Reduced Incidence of Dementia and Parkinson's Disease in Non-Hypertensive Subjects

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Aim: To investigate whether statins are associated with a reduction in the incidence of dementia and Parkinson disease.

Methods: Analysis of data from the decision support system of the US Veterans Affairs database, which continues diagnostic, medication and demographic information on 4.5 million subjects. The association of lovastatin, simvastatin and atorvastatin were examined with Cox proportional hazard rates for subjects taking statins compared to subjects taking cardiovascular medications other than statins, after adjusting for co-variates associated with dementia or Parkinson disease.

Results: Simvastatin is associated with a significant reduction in the incidence of dementia in subjects 65 years or older after adjustment for hypertension, cardiovascular disease, diabetes and obesity. The hazard ratio for incident dementia for simvastatin is 0.694 (CI 0.658 – 0.732, $p<0.0001$). Atorvastatin showed a reduction in the incidence of dementia that was weaker and borderline significant 0.900 (CI 0.795 – 1.019, $p=0.0974$), while lovastatin was not associated with a reduction in the incidence of dementia. The effect of simvastatin was sensitive to the presence of hypertension. The hazard ratio for incident dementia in subjects without hypertension was (HR 0.240, CI 0.204 – 0.282, $p<0.0001$) and with hypertension was (HR 0.900, CI 0.851 – 0.951, $p=0.0002$). Simvastatin also exhibited a reduced hazard ratio for newly acquired Parkinson disease among non-hypertensive subjects (HR 0.360, CI 0.299 – 0.434, $p<0.0001$).

Conclusion: Simvastatin appears to be associated with a strong reduction in the incidence of dementia and Parkinson disease among non-hypertensive subjects.

1008

The Involvement of Brain 12/15lipoxygenase in AD Pathogenesis

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Alzheimer's disease (AD) is a chronic neurodegenerative disorder whose initiating events are not known. Consistent data show that oxidative stress and inflammation are two important feature of AD pathology. 12/15Lipoxygenase (12/15LO) is widely expressed in the central nervous system (CNS) and by oxidizing fatty acids synthesizes hydroperoxyacids, which are potent oxidant and pro-inflammatory mediators. Previously, we have shown that this metabolic pathways is increase at the earliest stages of human AD

In the present paper we explore the effects of genetic deletion and pharmacologic inhibition of 12/15LO on the AD-like phenotype of the tg2576 mice.

Absence of this enzyme, or its pharmacologic inhibition was well tolerated by the animals, and resulted in a significant reduction in amyloid beta (Abeta) formation and deposition. This was associated with no change in total levels of APP, but a significant reduction in sAPP beta and C-terminal fragment beta, but not alpha. In vitro studies showed that the molecular target of this enzymatic pathway is the modulation of beta secretase, which would results in a reduced formation of Abeta.

These findings support the novel hypothesis that blockade of 12/15LO in the CNS could be an effective therapy for preventing or treatment of AD.

1009

Epigenetic Mechanisms Lead to the Recovery of Learning and Lost Long-Term Memories in An Animal Model for Neurodegeneration

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Neurodegenerative diseases of the central nervous system are often associated with impaired learning and memory eventually leading to dementia. Although the term learning and memory is often used as a single expression in the literature, individuals suffering from neurodegenerative diseases display impairments that draw a distinction between learning and memory. An important aspect that has not been addressed extensively in pre-clinical research, is the loss long-term memories and the exploration of strategies to re-establish access to those memories. By using a mouse model that allows temporal- and spatial-restricted induction of neuronal loss, we show here that environmental enrichment reinstated learning behavior and re-established access to long-term memories after significant brain atrophy and neuronal loss had already occurred. Environmental enrichment correlated with epigenetic changes, namely increased histone-tail acetylation. Moreover, increased histone acetylation by inhibitors of histone-deacetylases induced sprouting of dendrites, an increased number of synapses, and reinstated learning behavior and access to long-term memories. This data suggests that epigenetic approaches might be a suitable therapeutic avenue for neurodegenerative diseases associated with learning and memory impairment and raises the possibility of recovery of long-term memories in demented patients.

1010

X11 Adaptor Proteins Improve Cognition and Neuronal Function in Appswe (Tg2576) Mice

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Background: X11 adaptor proteins bind APP and reduce Aβ production. In APPswe/X11a double transgenic mice cerebral Aβ levels and amyloid deposition are significantly decreased compared with APPswe mice. We tested whether deficits in memory and neuronal function in APPswe mice are similarly ameliorated

Methods: We studied cognition and LTP in young (3-4 months), middle-aged (11-12 months) and elderly (16-18 months) APPswe/X11a mice and littermates. Spatial memory was tested using the Morris water maze: 3 days visible platform testing then 9 days testing with a hidden platform, including probe trials on days 4, 7 and 10. Afterwards, we studied LTP in vivo in the hippocampal CA1 region.

Results: Elderly X11a mice performed similarly to wild-type littermates, while APPswe mice did worse, displaying increased latency to platform ($p=0.026$) and reduced memory retention on Day 10 probe trials ($p=0.950$, target vs. opposite quadrant). In contrast, APPswe/X11a mice performed better than APPswe littermates, displaying latencies ($p=0.031$) and memory retention ($p=0.001$) similar to wild-type littermates. Middle-aged APPswe mice also displayed a reduction in memory retention ($p=0.190$), while APPswe/X11a littermates displayed good retention ($p=0.011$) as did wild-type mice ($p<0.001$). In older APPswe/X11β transgenic mice, LTP during the 2nd and 3rd hours post-tetanus was greater than in APPswe mice ($p<0.0001$ and $p<0.05$, respectively) and was not significantly different from wild-type and X11β littermates.

Conclusions: Overexpression of X11 proteins, resulting in decreased cerebral load of Aβ, reduces both cognitive impairment and LTP deficits in middle-aged and elderly APPswe mice.

1011

A Molecular Chaperone Inducer Protects Neurons From ER Stress

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The endoplasmic reticulum (ER) stress response is a defense system which treats an accumulation of unfolded proteins in the ER lumen. Recent reports showed that ER stresses are involved in the pathology of many diseases, such as some neurodegenerative diseases and cerebral ischemia. In a screen for compounds that induce the ER-mediated chaperone BiP/GRP 78 (BiP), we identified BiP inducer X (BIX). BIX induced only BiP in a dose-dependent manner without induction of other molecules of ER stress response: BIX is a specific inducer of BiP rather than an ER stressor. The induction of BiP mRNA by BIX was mediated by activation of ER stress response element (ERSE) through ATF6 pathway. Pretreatment of SK-N-SH neuroblastoma cells with BIX reduced cell death induced by ER stress. Intracerebroventricular pretreatment with BIX reduced the area of infarction of brain and neuronal deficits due to focal cerebral ischemia by occlusion of the middle cerebral artery of mice. In the penumbra of BIX-treated mice, the number of TUNEL-positive cells was reduced with the reduction of CHOP messages. BIX induces BiP to prevent neuronal death by ER stress, suggesting that it may be a potential therapeutic agent for cerebral diseases caused by ER stress.

1012

Therapeutic Potential of Potent Marine Neuroprotectants

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A growing body of evidence implicates oxidative stress in the cellular mechanisms leading to neuronal cell death associated to certain neurodegenerative conditions such as AD, PD or ALS. Thus, anti-oxidative therapies constitute promising approaches to slow the progression of some neurodegenerative processes. The marine environment represents an enormous resource for the discovery of potential chemotherapeutic agents. Within a systematic screening program aimed at isolating compounds from marine organisms showing biological activities relevant for preventing and/or treating neurodegenerative diseases, we have identified a family of natural compounds showing showing potent neuroprotectant activity against oxidative stress. Two of these marine compounds are able to protect human neuroblastoma cells from H2O2-induced cell death with EC50s in the nM range. This neuroprotective effect is also observed when cell death is induced by other extracellular toxic insults such as 6-hidroxidopamine or the Ab25-35 peptide, in the sub mM-nM range as well. In a first attempt to study the mechanism of action of this family of compounds, we have observed that they do not show free radicals scavenger properties in vitro, nor they have any direct effect on the in vitro superoxide dismutase (SOD) or catalase activities. An active synthesis program has been developed in Neuropharma, leading to several naturally-derived, synthetic lead compounds with better potencies and ADME properties. Some of these compounds are currently being tested in several in vivo proof of concept studies, in order to know whether they show any significant neuroprotection activity in several animal models and are worthy of further development.

1013

Peptidomimetics as Potential Therapeutic Agents in Alzheimer's Disease

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As the amyloidogenic processing of APP is crucial for the development of AD, focussing on APP and endeavouring to find ways of restoring its function is a rational approach for development of new neuroprotective strategies.

We reported that in animals rendered amnestic by Aβ memory was rescued by tripeptide Arg-Glu-Arg (RER), part of the growth-promoting domain of APP. A protected form of RER, when injected peripherally, is rapidly transported across the blood-brain-barrier, and protects against memory loss induced by Aβ1-42 and Aβ25-32.

In efforts both to cast light on the mode of action of the peptide, and to increase its efficacy as a potential cognitive enhancer, we have now studied the effects of various D/L-forms of the peptide. Here we report that rER (where the lower case indicates the D-isomeric form of the amino acid), protects against Aβ induced memory loss when injected peripherally up to 12hr prior to the training task, and thus becomes of considerable interest as the basis for a potential therapeutic agent in the early stages of Alzheimer's Disease.

Peptidomimetics derived from the amyloid precursor protein (APP) might become of considerable interest as potential therapeutic agent in the early stages of Alzheimer 's disease.

RM and SPRR are named as inventors of the tripeptides described (UK Patent Number GB2391548 owned by The Open University). Research was partially funded by Talisker.

1014

A Hybrid Peptide Colivelin for a Potential Drug Candidate for Alzheimer's Disease

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Colivein is a hybrid-peptide that is composed of ADNF and one of the Humanin derivatives, AGAC8R-HNG17. Humanin is neuroprotective against all Alzheimer's disease

(AD) related insults so far examined, including Abeta. We identified Humanin cDNA as a gene encoding a novel 24 amino acid peptide through functional expression screening. We have obtained several Humanin derivatives with more potent nerutoprotective activities. Among them, AGAC8R-HNG17 is the most potent one. ADNF(Activity-dependent neurotrophic factor, SALLRSIPA) is a glia-delived peptide and neuroprotective at femtomolar concentrations and protects neurons from death caused by Abeta, one of the AD related insults. For unknown reason, the neuroprotective activity of ADNF abolishes at 1nM or higher concentrations. We found, however, that Colivelin suppressed neuronal death caused by Abeta and AD related insults in a dose dependent manner, without declined activity at higher concentrations. We hypothesized that increasing the neuroprotection of AGAC8R-HNG17 from 10pM to 100 fM just by attaching ADNF to the N-terminal of HN derivative is through dimerization of the molecule, as Humanin was reported to exert its neuroprotection by dimerization. Circular dichroism (CD) spectra results suggested that Colivelin is not a simple mixture of the ADNF and HN derivative structures, but a new structure, different from them. We are currently examining the dimerization hypothesis by sedimentation equlibrium experiment.

1015

Enhanced Clearance of Alzheimer's Beta-Amyloid Peptide Across the Blood-Brain Barrier Caused by Environmental Enrichment

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AD is accompanied by a defective function of the neurovascular unit, leading to impaired clearance of Aβ peptide. We have recently demonstrated that enriched housing in TgCRND8 mice results in reduction of Aβ plaques and lower extent of amyloid angiopathy (Am J Pathol, 169:544-52). Based on these results, we questioned whether enrichment affects the neurovascular unit and brain to blood Aβ clearance. Mice were housed in standard or enriched cages (n=18) until 5 months of age. DNA microarray analysis and angiogenesis "profiler" arrays revealed simultaneous downregulation of antiangiogenic as well as upregulation of proangiogenic genes due to enrichment. Moreover, cerebral vessel density was significantly increased (29%, p=0.04). There was additionally a strong trend (93%, p=0.1) towards an increased Aβ40 blood plasma content and a strong negative correlation (r=-0.97) between the magnitude of cerebral plaque burden and Aβ40 levels in blood, both of which indicating enhanced Aβ transport from brain to blood. In accordance with this Aβ shift, RAGE, promoting Aβ influx in the brain, was suppressed by 60% (p=0.004), whereas Lrp1, facilitating Aβ efflux was upregulated by 384% (p=0.05). Furthermore, transcriptional levels of Aβ binding proteins ApoE and A2M mediating Aβ efflux by Lrp1 revealed significant increased expression by 42% (p=0.006) and 50% (p=0.02), respectively. These data indicate that environmental enrichment improves Aβ clearance across the blood brain barrier by induction of angiogenesis and differential regulation of specific Aβ transporters.

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Interaction Between TNF-alpha and IGF-I in Alzheimer Disease: Pathogenic and Therapeutic Implications

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Recent experimental data suggest that the signalling pathways of tumor necrosis factor-alpha (TNF-alpha) and insulin-like growth factor I (IGF-I) are functionally interrelated and contribute to the pathogenesis of Alzheimer´s disease (AD). It has been demonstrated that TNF-alpha counteracts the capacity of IGF-I to promote neuronal survival and antagonizes brain amyloid-beta clearance induced by serum IGF-I, whereas IGF-I reverses neuronal apoptosis mediated by TNF-alpha.

Interactions between peripheral IGF-I and TNF-alpha systems, as well as the effects of the treatment with a neurotrophic compound (Cere) on these factors were investigated in AD patients.

Enhanced TNF-alpha levels, reduced IGF-I concentrations and a negative correlation between circulating IGF-I and TNF-alpha values were found in the serum of AD patients. This correlation was not evidenced in control subjects or in mild cognitive impairment cases. Serum levels of soluble TNF Receptor I (sTNF-RI) and IGF binding protein 3, both having pro-apoptotic activity, were also found to be increased in AD patients as compared with controls. However, sTNF-RII and IGFBP-1 showed no changes in AD sera.

In mild to moderate AD patients enrolled in a 24-week, double blind, placebo-controlled trial with three dosages of Cere, we found that Cere reduced circulating TNF-alpha and sTNF-RI levels and increased free IGF-I concentrations with respect to placebo in a dose-dependent manner.

These results showing a negative interaction between serum TNF-alpha and IGF-I suggest that the opposite influence of inflammatory and growth factors on AD pathology is having a peripheral reflect, which can be modified with neurotrophic drugs like Cere.

1017

Dutch Easycare Study: Geriatric Home Visiting Programme Shows Improvement in Cognitively Impaired and Vulnerable Older Adults in a Randomised Trial

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Background: Earlier we showed beneficial effects of the Dutch Geriatric Intervention Programme (DGIP), a programme that consisted of problem based home visits by geriatric nurses, in cooperation with GP, and geriatrician. In this study we analysed how much cognitively impaired and frail patients did benefit.

Methods: 151 participants (mean age 82.2 years, 74.8% female) were included in an observer blinded randomised trial

with six months of follow up for the primary outcomes: 85 patients were visited, and 66 received usual care. A subset of 49 patients (26 intervention, 23 usual care) had strong indications of cognitive impairments (Mini Mental State Examination ≤ 21; MMSE). Primary outcomes were functional abilities (Groningen Activity Restriction Scale-3; GARS-3: 18-54) and mental well-being (subscale mental health MOS-20; MOS-20MH: 0-100). Intention-to-treat analyses focused on differences between treatment arms in changes in GARS-3 and MOS-20MH over time. An interaction analysis was done with MMSE high/low as stratifying factor.

Results: Overall, after three months the beneficial effects were on GARS-3 2.2 points [95% CI 0.3;4.2], and on MOS-20MH 5.8 points [0.1;11.4], and 1.6 [-0.7;3.9] and 9.1 [2.4;15.9] after 6 months respectively. The interactions of treatment with MMSE were not significant. In the cognitively impaired subset results were: GARS 4.6 [0.6;8.5] and 6.2 [0.5;11.9], and MOS20MH 1.3 [-10.1;12.7] and 14.1 [-2.7;30.9], at 3 and 6 months respectively.

Conclusions: Despite non-significant interactions, a problem based home intervention – already proofed to be effective – may be even more effective for improving functional abilities of cognitively impaired and frail older patients.

1018

How Beta-Amylod Proteins and Pro-Inflammatory Cytokines Might Collaborate to Produce the Alzheimer Brain

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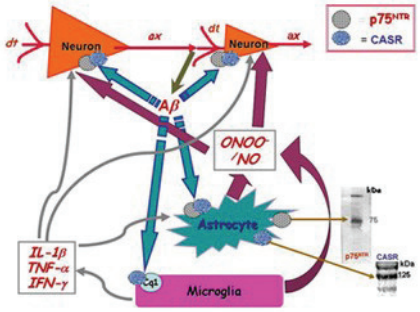
Background and aims. Abetas released from neurons are byproducts of the cleavage in active synapses of APP bound to CPEB (cytoplasmic polyadenylation element binding) protein, which stimulates local protein synthesis to maintain and reinforce synaptic connectivity. These Abetas', accumulating excessively in the aging or mutant brain, kill neurons by triggering a mutually enhancing cytocidal package of events that we are currently explicating.

Methods. We have studied via morphologic and biochemical means Abetas- and/or cytokines-induced apoptosis and NO production in cultured human neuroblastoma cells expressing all or parts of the p75NTR receptor and normal adult human astrocytes.

Results: Abetas directly killed neuroblastoma cells through signals from p75NTR receptors' death domains (DD). By activating Cq1 and CaSR (Ca2+-sensing receptor) receptors, Abetas also induced microglial macrophages to make NO and its cytocidal ONOO- derivative as well as pro-inflammatory IFN-gamma, TNF-alpha, and IL-1beta cytokines. These cytokines together caused astrocytes to express NOS-2 and make NO/ONOO-. By also activating the astrocytes' p75NTR and CaSR receptors, Abetas collaborated with the three pro-inflammatory cytokines to amplify NO/OONO- production and consequently neuronal damage and death.

Conclusions. Abetas excessively accumulating in an ageing or mutant brain trigger a vicious cytocidal cycle of pro-inflammatory cytokines, p75NTR and CaSR receptor signaling, and massive NO/ONOO- release neuron-astrocyte in functional units (Fig.1).



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Nonpharmacolocical Treatment for Persons With Alzheimer's Disease

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Retrogenic principles can be productively used to improve the quality of life of dementia patients in conjunction with pharmacological treatments.

Retrogenesis has been defined as follows: Retrogenesis is the process by which degenerative mechanisms reverse the order of acquisition in normal development. There is extensive evidence for the occurence of this process in Alzheimer's disease (AD) and related disorders. For care and training methodology the recognition of the retrogenic process provides an opportunity to draw upon and be inspired by a wealth of methods traditionally used to help developing humans to reach their full potential. Persons in the different stages of AD have different abilities and different needs, therefore different training curricula need to be developed.

We have developed a stage specific training methodology following retrogenic principles.

For this purpose we defined three different training groups with different curricula : Group 1: Highly functioning and MCI (Global Deterioration Scale (GDS) stages 2,3); Group 2 : Cognitively disturbed persons with mild to moderate dementia (GDS stages 4,5) and Group 3: Cognitively severely disturbed persons with severe dementia (GDS stages 6 and 7).

The training methods draw primarily from two different domains : (a) physical activation and (b) cognitive activation. The training methodology can be described as „comprehensive" because several brain areas are activated.

The results of a randomised controlled trial show significant positive effects in the domains cognition and functioning for persons in the treatment group. The effects are comparable to the effects of cholinesterase inhibitors.

1020

Interleukin-18: A New Player in Alzheimer's Disease Pathogenesis?

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Inflammation plays a major role in the pathogenesis of Alzheimer's Disease (AD), since the senile plaque of AD brain, mainly made of Amyloid-beta peptides (Abeta), are associated with an ongoing chronic inflammatory process, characterized by microglia activation and release of pro-inflammatory soluble factors, such as cytokines. Interleukin (IL)-18 is a pleiotropic pro-inflammatory and immunomodulating cytokine, able to mediate neuroinflammation and neurodegeneration in several disorders of CNS. So far, the association of IL-18 to AD is not clear yet, but convergent results are being arising towards such direction. We previously observed that some IL-18 promoter polymorphisms can be relevant for AD, and that myeloid dendritic cells, the peripheral analogues of microglia cells, when differentiated in vitro in the presence of Abeta, acquire an inflammatory phenotype and produce high amounts of IL-18. In the present study, we have determined the levels of IL-18 and its natural inhibitor, IL-18BPa, in serum and in culture supernatants of LPS-treated peripheral-blood mononuclear cells (PBMCs) from healthy donors (HD) and AD patients. Although the serum levels of total IL-18 and IL-18BPa did not show any statistically significant difference between the studied groups, the circulating free form of IL-18 was significantly higher in AD than in HD. Furthermore, AD-derived PBMCs released significantly higher amounts of IL-18 in culture medium, comparing to HD. These results indicate that a dysregulation of IL-18/IL-18BP system occurs in AD patients, suggesting that IL-18 is indeed implicated in AD pathogenesis.

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Molecular Approaches Towards Alzheimer Disease

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Alzheimer disease (AD) is the most common neurodegenerative disorder, affecting the quality of life of a large number of people causing memory loss. This disease is characterized by the accumulation of β amyloid (Aβ) peptides as senile plaques and neurofibrillary tangles (NFTs). Senile Plaque formation by β amyloid (Aβ) peptides increases in the presence of enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). NFTs are build up of paired helical filaments (PHFs), produced as a result of hyperphosphorylation of tau protein. Tau protein is a microtubule associated protein (MAP) and the phosphorylation of tau protein is involved in promoting assembly and stability of microtubule. In Alzheimer's diseased brain, tau protein is abnormally hyperphosphorylated due to several protein kinases but the most common in this disease include cyclic AMP dependent protein kinase (PKA). Hence inhibition of cholinesterases, and protein kinase A with natural and synthetic inhibitors is a promising therapeutic goal to prevent and treat AD and related tauopathies.

We are currently screening a large number of natural and synthetic compounds for their enzyme inhibitory activities against AChE, BChE and PKA. Biochemical and structural basis of enzyme inhibition will be determined by enzyme kinetics and computational methods such as molecular docking. The effect of positive hits on cultured cells will be studied. This study is expected to result in the identification of interesting leads for the prevention and treatment of AD and related neurodegenerative disorders.

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Studies of Beta-Amyloid in Postmortem Brain Tissue From Alzheimer Patients

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β-Amyloid (Aβ) depositions are one of the major histopathological hallmarks of Alzheimer's disease (AD). The amyloid-imaging positron emission tomography (PET) tracer [11C]PIB (N-methyl[11C]2-(4'methylaminophenyl)-6-hydroxy-benzothiazole) is used in the assessments of amyloid plaques in the human brain. The exact nature of the [11C]PIB-amyloid binding interaction is unknown, although it appears to bind to fibrillar forms including both neuritic and non-neuritic plaques. The presence of amyloid depositions at an early stage of the disease progress has been reported and recent evidence suggests that Aβ may be important in producing clinical symptoms and early functional deficits. We have studied the [11C]PIB-amyloid interaction in the brain, by investigating [11C]PIB-labeled amyloid depositions in postmortem tissue, by classical in vitro autoradiography and radioligand binding assay with [11C]PIB in the frontal, parietal, temporal cortices and cerebellum of human brain of patients with AD and healthy controls. The levels of guanidinium soluble Aβ40 and Aβ42 has been measured with ELISA and correlated to [11C]PIB-amyloid binding. The binding assays show significantly higher [11C]PIB binding in cortical regions from AD patients as compared to cerebellum and to control subjects. However, [11C]PIB binding could be detected in the cortex of a few of the older controls. The levels of Aβ40 and Aβ42 were significantly higher in all measured regions with the exception of Aβ42 in the cerebellum. The findings of the binding properties of [11C]PIB together with Aβ-measurements in different brain regions of AD patients

compared to controls will facilitate in elucidating the mechanisms behind plaque formation in the brain.

1023

Deglycosylated Antibody for Abeta Sequestration

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Accumulation of amyloid beta (Abeta) is a pathological hallmark of Alzheimer's disease, and lowering Abeta is a promising therapeutic approach. Abeta binding agents present in the blood reduce brain Abeta load by enhancing Abeta efflux from the brain to the periphery (Abeta sequestration). Intact anti-Abeta antibodies induce Abeta sequestration, but they also evoke immune reactions which are not involved in sequestration. The glycochain of immunoglobulin is critically involved in interactions with effectors including the Fc receptor and complement c1q; deglycosylation eliminates these interactions, while binding affinity is maintained. In this study, we investigated the efficiency of deglycosylated antibody in Abeta sequestration as well as its effect on microglial phagocytosis and neuroinflammation. After enzymic deglycosylation, undigested antibody (intact antibody) was not detected and deglycosylated antibodies maintained Abeta binding affinity. Deglycosylated antibodies did not enhance Abeta phagocytosis or cytokine release in primary cultured microglia, while intact antibodies significantly enhanced phagocytosis and cytokine levels. Intravenous injection of deglycosylated antibody elevated plasma Abeta level to a similar or greater degree compared to intact antibodies. Deglycosylated and intact antibodies showed comparable short-term kinetics. Overall, deglycosylated antibodies effectively induced Abeta sequestration without provoking neuroinflammation. Thus, deglycosylated antibodies may be optimal for sequestration therapy for Alzheimer's disease.

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Molecular Approaches Towards Alzheimer Disease

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Alzheimer disease (AD) is the most common neurodegenerative disorder, affecting the quality of life of a large number of people causing memory loss. This disease is characterized by the accumulation of β-amyloid (Aβ) peptides as senile plaques and neurofibrillary tangles (NFTs). Senile Plaque formation by β-amyloid (Aβ) peptides increases in the presence of enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). NFTs are build up of paired helical filaments (PHFs), produced as a result of hyperphosphorylation of tau protein. Tau protein is a microtubule associated protein (MAP) and the phosphorylation of tau protein is involved in promoting assembly and stability of microtubule. In Alzheimer's diseased brain, tau protein is abnormally hyperphosphorylated due to several protein kinases but the most common in this disease include cyclic AMP dependent protein kinase (PKA). Hence inhibition of cholinesterases, and protein kinase A with natural and synthetic inhibitors is a promising therapeutic goal to prevent and treat AD and related tauopathies.

We are currently screening a large number of natural and synthetic compounds for their enzyme inhibitory activities against AChE, BChE and PKA. Biochemical and structural basis of enzyme inhibition will be determined by enzyme kinetics and computational methods. The effect of positive hits on cultured cells will be studied. This study is expected to result in the identification of interesting leads for the prevention and treatment of AD and related neurodegenerative disorders.

1025

Cholinergic Precursors in the Treatment of Cognitive Dysfunction Associated With Cerebrovascular Disease: A Review of Controlled Clinical Studies

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Cholinergic dysfunction is involved in the pathophysiology of cognitive impairment associated with cerebrovascular disease. Cholinergic precursor loading therapy was the first approach tried to relief cognitive impairment in dementia disorders. Negative results of clinical trials with choline or phosphatidylcholine (lecithin) were considered indicative of the ineffectiveness of cholinergic precursors in the treatment of cognitive dysfunction associated with dementia disorders. This conclusion is probably not justified for selected precursors such as citicoline and choline alphoscerate. Citicoline (cytidine diphosphate choline, CDP-choline) is a precursor in the synthesis of phosphatidylcholine and other phospholipids. Choline alphoscerate (alpha-glyceryl phosphoryl choline, GPC) is a precursor in the biosynthesis of brain phospholipids. To date it is the most effective cholinergic precursor increasing the bioavailability of brain acetylcholine. Elderly patients with chronic cerebrovascular insufficiency treated with CDP-choline showed improvements in attention and memory deficits. Intramuscular or oral CDP-choline improved cognition, behavior, and memory in patients with multi-infarct dementia in relatively small and uncontrolled studies. Studies with GPC in elderly patients with vascular dementia and with sequelae of acute cerebrovascular disease have reported improved memory and attention, as well as of affective and somatic symptoms. Comparative studies

gave Sandoz Clinical Assessment Geriatric Scale scores more favourable to GPC than to CDP-choline. In spite of limits of studies using cholinergic precursors in the treatment of cognitive dysfunction associated with cerebrovascular disease, available data suggest the interest of deepening the clinical role of selected precursors in newer appropriate clinical trials.

1026

Short-Term Treatment With the Low-Molecular Weight Heparin Nadroparin Improves Y-Maze Performance in a Mouse Model of Alzheimer's Disease

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Alzheimer's disease (AD) is the major amyloid disease of the brain characterized by parenchymal and cerebrovascular deposition of the amyloid β-peptide (Aβ). Heparan sulfate proteoglycans (HPSG) and glycosaminoglycans (GAG) are invariable components of amyloid deposits and have been shown to be involved in amyloidogenesis including Aβ fibril formation and deposition. GAG-based substances like low molecular weight (LMW) heparins and GAG mimetics have previously been suggested to be potential therapeutic agents for the treatment of AD. In the present study, we have investigated the effects of a short-term treatment with the LMW heparin Nadroparin in the SweAPP/PS1 mouse model of AD. Treatment with Nadroparin led to significant increases in Y-maze performance without affecting soluble Aβ levels in the brain. In three month-old mice, we observed a delay in Aβ deposition as revealed by reductions in formic acid soluble Aβ and reduced numbers of Aβ deposits. In seven to eight month-old mice, however, short-term treatment with Nadroparin showed no effects on Aβ deposits and formic acid soluble Aβ levels. We conclude that short-term treatment with the LMW heparin Nadroparin can reverse hippocampus-dependent cognitive deficits in the SweAPP/PS1 mouse model of AD. Moreover, treatment with Nadroparin appears to have prophylactic effects on Aβ plaque deposition.

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The Magnetic Field and the Ultratonetherapy of the Treatment of Initial Stages of Parkinson's Disease

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Background and Aims: The effect of the low-frequent variable magnetic field and the ultratonetherapy on the muscle rigidity of the patients having initial stages of Parkinson's disease was investigated.

Method: 86 patients aged from 60 to 75 (38 females and 48 males) having initial stages of Parkinson disease (PD) were observed. We chose the patients with prevalent muscle rigidity. The patients were divided into two groups. The first group (52 patients) received in addition their basic medication and physiotherapy with ultratonetherapy - variable sinusoidal higth-tension (4 - 5 kV) higth-frequent (22kHertz) low-intensive current (power 1 - 10 Vatt) and low-frequent variable magnetic field (frequency to 100 Hertz, magnetic induction 27mTesla) treatment of upper and lower extremities, with taking turn each other. The ultratone exposure was 12 - 15 min. The low-frequent variable magnetic field exposure was 10 - 12 min. The complete course was 10 - 12 procedures. The second group (control, 34 patients), received only the basic medication.

Results: The muscle rigidity and subjective sensation of constraint extremities of the patients in the first group was reduced after 18 - 20 days of treatment (78,8% patients) compared to the control group, where muscle constraint reduced after 22 - 24 days of treatment (55,8% patients), $p<0,05$.

Conclusion: The addition of the complex (low-frequent variable magnetic field and ultratonetherapy) to the treatment of initial stages of PD resulted in earlier reducing of the muscle rigidity and the subjective sensation of constraint extremities.

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Diagnostics and Treatment Control of Parkinson's Disease With Analysis of Saccadic Eye Movement

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Aim of current research is to estimate the validity of the analysis of saccadic eye movement parameters as one of the methods of objective diagnostics and treatment control of Parkinson's disease (PD).

Methods: Patients with a diagnosis of PD (n=20) were examined and compared with control subjects (n=20). The UPDRS scale was evaluated. During the following treatment all the patients were given a dopamine D2/D3 agonist (piribedil). Also, the patients and the control subjects took part in the electrooculographic investigation, which included eye movements recording during visual stimuli presentation.

Results: The saccadic reaction time means increased in the patients with PD (188 ± 2 ms) compared to the control group (163 ± 2 ms, $p<0.001$). Also, the patients demonstrated a longer mean time of the gaze shift ($p<0.001$). After an additional analysis we found the cause of the mean time increase of the gaze shift. As it turned out, the percentage of the particular saccades (multisaccades) rose in the patients with PD. In these cases not one but two or three saccades were performed with smaller amplitudes.

After four weeks of piribedil therapy the UPDRS score decreased by 57%. The saccadic reaction time and the percentage of the multisaccades also decreased.

It is to say that the percentage of the multisaccades is the simplest quantitative indicator of the saccadic system dysfunction during PD ($r = 0.6$, $p<0.05$).

Conclusions: Our results show that saccadic eye movement registration may be used as a promising technique for diagnostics and medication control of PD.

1029

Deep Brain Stimulation in Parkinson Disease Under General Anasthesia Without Electrophysiological Guidance Initial Experience

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Objective: We propose a DBS implantation method for Parkinson's disease treatment under general anaesthesia with entirely MRI based direct anatomical targeting.

Patients and methods: Between 2001 and 2005, 24 patients have been billateraly implanted:14 in the Gpi and 10 in the STN. The criteria for surgery included an idiopatic PD, DOPA sensibility superior to 33%, severe motor fluctuations or dyskinesias and a UPDRS score of minimum 30 on and maximum 30 off. All patients were evaluated by a neurologist and had a psychiatric and neuropsychological evaluation prior to surgery. Target was selected after direct visual recognition of STN or Gpi boundaries on transverse axial MR sections without referencing to Atlas or AC-PC line. Clinical evaluation was done following the CAPSIT protocol including the application of the UPDRS 30 days preoperatively and postoperatively each 3 months in medications on and off states with the stimulator on and off so.

Results: 10 patients out of the 14 implanted in the GPi have been at least followed up for 1 year and 7 patients out of 10 implanted in the STN have been at least followed up for 6 months.

In Gpi treated patients, percentage of improvement was 36.7% in Off medication condition and On stimulation condition and 51.8% in Off medication and On stimulation condition in STN patients.

Conclusion: DBS treatment for PD in the Gpi and STN nucleus can be performed safely and with good clinical results with an entirely anatomical MRI based technique under general anaesthesia.

1030

Mechanisms of Neuroprotective Function of DJ-1 in a Cellular Model of Parkinson's Disease

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Background and Aims: Parkinson's disease (PD) is a neurodegenerative disorder that involves a loss of dopaminergic neurons in the substantia nigra. Mitochondrial dysfunction, proteasome inhibition, and protein aggregation play roles in the PD pathogenesis. The loss-of-function mutations in the gene encoding DJ-1 have been linked to familial PD. In this study we examined whether DJ-1 protects dopaminergic neurons from various insults related to PD and explored the underlying mechanisms.

Methods and Results: Expression of human wild-type DJ-1 rescues dopaminergic neurons in primary midbrain cultures from selective toxicity induced by rotenone, proteasome inhibitors, and mutant α-synuclein. Mutant DJ-1 involved in familial PD exhibited decreased neuroprotective activity, and DJ-1 silencing sensitizes dopaminergic neurons to all three insults. We found that wild type DJ-1 increases the levels of (i) cellular glutathione and the antioxidant transcriptional regulator Nrf2 under oxidative stress; (ii) heat shock protein 70 (Hsp70) under conditions of proteasome inhibition and mutant α-synuclein overexpression. By comparing the neuroprotective ability of DJ-1 and the antioxidant compound N-acetyl cysteine, we observed that the antioxidant function of DJ-1 was critical for protection against oxidative stress. In contrast, by comparing the effects of overexpressing DJ-1 and Hsp70, we found that the chaperone activity of DJ-1 was necessary and sufficient for protection against mutant α-synuclein. The antioxidant and chaperone activities of DJ-1 were both important for protection against proteasome inhibitors, presumably because proteasome inhibition is coupled to oxidative stress.

Conclusions: Our data suggest that DJ-1 plays a central role in dopaminergic neuron survival by regulating various pathways.

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Gene Therapy of Parkinson's Disease: Use of a Mutant 5-Ht4 Receptor Stimulated Solely by Synthetic Ligands

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We propose to use a receptor inducing time and space controlled-cAMP synthesis as a tool to palliate the degeneration of dopaminergic neurons in PD, taking into account cAMP neuroprotective and anti-inflammatory effects.

We have engineered a Receptor Activated Solely by Synthetic ligands (RASSL) derived from serotonin Gs-coupled receptors. 5-HT4-RASSL is generated by a single mutation, is insensitive to serotonin and still responds to synthetic ligands. These ligands have affinities in the range of nanomolar concentrations for the mutant receptor. They also exhibit full efficacy and can pass through the blood brain barrier.

5-HT4-RASSL selectively expressed in defined regions could locally and temporally increase the levels of intracellular cAMP upon administration of synthetic agonists. This receptor continues to remain silent to endogenous serotonin. In the case of PD, activation of 5-HT4-RASSL by synthetic ligands and the resulting local increase of cAMP level should lead to several positive effects. cAMP could save degenerating dopaminergic neurons from death, due to its neuroprotective effects, and preserve or restore their activity. In these neurons, cAMP would also stimulate tyrosine hydroxylase formation, thus increasing dopamine synthesis. Moreover, cAMP has anti-inflammatory effects that can reduce the inflammatory process that arises in PD.

Canine adenoviruses containing the 5-HT4-RASSL will be injected unilaterally in substantia nigra or striatum of rodents. After induction of the experimental model of PD and activation of cAMP production, we will analyze the neuroprotective effects on dopaminergic neurons.

Our work should unequivocally determine whether cAMP is neuroprotective in animal models of PD.

1032

Pharmacological Basis for the Medicinal Use of Turmeric in Alzheimer's Disease

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Background; Turmeric is considered useful for Alzheimer's disease (AD). Acetyl cholinesterase (ACE) inhibitors and calcium channel blockers (CCB) are known to be beneficial in AD.

Aims; This study conducted to see if Turmeric contains constituents with combination of ACE inhibitory and CCB activities.

Methods; ACE inhibitory activity was measured by spectrophotometric method. For CCB activity, 2 cm long segments of rabbit jejunum were incubated in Tyrode's solutions at 37 oC, aerated with carbogen. Inhibitory responses against K+ (80 mM) were measured on Bioscience oscillograph.

Results; Curcumin (Sigma, USA) and curcuminoids (Sami Labs, India) well known constituents of Turmeric were studied for their possible ACE inhibitory and CCB activities. Curcumin (62.5 µM) caused less than 50 % inhibition while curcuminoids exhibited dose-dependent inhibition of ACE with IC50 value of 21.90 µM (20.15-25.02, 95% CI). In jejunal preparations curcumin caused dose-dependent inhibition of spontaneous and K+-induced contractions with respective IC50 of 8.01 µM (7.01-10.60) and 1.57 µM (0.8-2.56) suggestive of CCB effect. Similarly, curcuminoids caused inhibition of spontaneous and K+-induced contractions with respective IC50 of 30.80 (29.26-35.21) and 13.26 (10.26-16.28). The CCB activity was confirmed when pretreatment of tissue with curcumin and curcuminoids caused rightward shifts in the Ca++ dose-response curves, similar to that caused by verapamil. Curcuminoids was less potent for its CCB effect as opposed to the pattern of inhibitory effect on ACE.

Conclusions; These data indicate that Turmeric constituents possess unique combination of activities (CCB and ACE inhibitory), which might be the contributing factor towards its usefulness in AD.

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Comparison of the SCOPA-Cog, MMSE and Mattis Dementia Rating Scale in Parkinson's Disease Patients and Age-Matched Controls

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Background And Aims: Cognitive disturbances are diagnosed in 39% of Parkinson's disease (PD) patients. Evaluation of cognitive performances in a routine clinical setting is quite difficult and time-consuming. The Mattis score is time-consuming. The SCOPA-COG (Scales for Outcome in Parkinson's Disease-Cognition) has been developed and validated for application in PD patients. We compared the MMSE, Mattis and SCOPA-COG and also correlated mental decline with their motor severity according to the Hoehn and Yahr (I and II=1 mild; III=2 moderate and IV + V=3 severe).

Methods: Thirty healthy subjects (mean age 65+ 9.7) and 59 PD pts (mean age 66.5+ 9.8 y; disease duration 8.1+ 5.6y, motor severity 1: 21 pts; 2: 22 pts; 3: 16 pts) were included. PD pts were classified as non demented (PDNDEM; n=50) and demented (PDDEM; n= 9) according to the Clinical Dementia Rating scale [CDR] and MMSE.

Results: No difference was found between controls and PDNDEM in comparing MMSE (p= 0.007), SCOPA-COG (p=0.007) and Mattis (p= 0.23,) although a trend of differentiation was evident. MMSE results did not correlate with motor decline (p=0.12). On the contrary, the SCOPA-COG (p=0.031) and Mattis (p=0.039) showed similar correlation with a lower cognition score for patients with advanced PD.

SCOPA-COG was usually performed within 20 minutes (+ 3); Mattis usually took 40 minutes (+ 6 minutes).

Conclusions: SCOPA-COG is a simple, sensitive, validated test, which should be incorporated in the tests for the evaluation of PD patients.

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The Combined Therapy at the Syndrome "The Cervical Arterial Hypertensia" at Workers of Locomotive Brigades With Cervical Myofascial the Painful Syndrome

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The purpose of research was to study influence of the combined therapy on a cerebral blood-groove and arterial pressure at workers of locomotive brigades with cervical myofascial pain¬ful syndrome (CMPS).

Material and methods of research. Under supervision there were 146 patients with CMPS, men, workers of locomotive depot (train drivers and assistants of train drivers) Kuybishev the railway, in the middle age 34 years. At all observable workers of locomotive brigades monitoring arterial pressure was carried out daily, and as ultrasonic dopplerography before course of treatment.

Results of research. The combined therapy of workers of the locomotive brigades sick CMPS with a syndrome «cervical arterial hypertension» (CAH), was carried out in view of the basic pathogenetic factors forming a stage of disease:

• Treatment of patients cervical CMPS 1-2 stages was carried out by not medicamental methods;

• At 3 stage of CMPS combined therapy was carried out. A problem elimination of psy¬cho-vegetative infringements and AH is special. At 25 patients with the phenomena chronic vertebra-basilar insufficiency in addi¬tion applied Actovegin in a doze of 200 mg.

The conclusion. The revealed features of a daily structure the AP at patients cervical CMPS have allowed to allocate a syndrome CAH at railwaymen. At patients on 3 stages CMPS displays vertebra-basilar the insufficiency accompanying with an ischemia and hypoxia are not rare. In this situation use Actovegin - a highly active stimulator of synthe¬sis and

recycling of oxygen and glucose in conditions of an ischemia and hypoxia, an improving brain blood-groove.

1035

Specific Features of Secondary Parkinsonism in Neuroborreliosis

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Background and objective: Lyme disease is a polysystemic natural focal transmissive disease. It is characterized by stage-wise development with the predominant damage of the nervous system in up to 30% of cases. The spectrum of neurological disturbances in Lyme borreliosis is broad and includes damages of both the peripheral nervous system (neuropathies) and the central one (extrapyramidal syndromes, encephalopathy).

In the literature there are single reports on the development of the parkinsonian syndrome several years after a tick bite and the stage of migrating erythema.

Methods: In our work among the examined 260 patients with significant diagnosis of Lyme disease with damage of the nervous system there was revealed the syndrome of parkinsonism in 23 patients. Clinically they differed from patients with Parkinson disease by a rapid augmentation of extrapyramidal symptomatology with a weak response to DOPA drugs (or its complete absence). In addition to typical motor disturbances in these patients we revealed progressive encephalopathy that was accompanied by marked changes in cognitive evoked potentials (EP P300) in the form of increased latency, changed shape of wave as well as weak visualization of P300 component. MRT of the brain in the subcortical structures revealed hypointensive foci in T1 and hyperintensive foci in T2 regimes.

Results: After the conducted pathogenetic therapy including antibiotics (cephtriaxon) and immunomodulators (interferons) a positive dynamics in the neurological status as well as improvement of the basic parameters during EP P300 and neuropsychological testing were noted in all patients.

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Dynamic on-the-Brain Mapping of Neuronal Impairment

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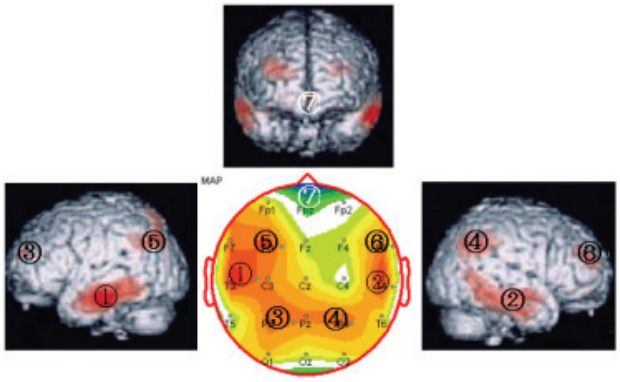
Purpose: Since impaired neuronal activities become unstable as found by Musha et al. (T. Musha et al., Clin. Neurophys., 113 (2002) 1052-1058), the normalized power variance (NPV) of the 21-channel spontaneous EEG in frequency range 13~80 Hz is used as a measure of neuronal activities related to that local potential. We aim at establishing an inexpensive, non-invasive (without exposure to radiation), sensitive, and easy-to-operate differential diagnosis tool of brain disorders. Method: A difference NPV map of an individual AD patient from the mean NPV map averaged over 56 age-matched normal controls was made and it was averaged over 33 moderately sever AD patients. The resulting difference NPV map is shown below together with the corresponding SPECT maps. Results: Similarity between these maps is seen. An individual difference NPV map can be calculated in every second, and we trace a dynamic change of the impairment pattern caused by unstable activities of impaired neurons. The difference NPV map visualizes the efficacy of medication and rehabilitation. The movie of the dynamic change will be presented.



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Naturalistic Long Term Follow-Up of Psychotic Parkinson's Disease Patients Treated With Either Clozapine or Quetiapine

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Background And Aims: The objective of this study was to evaluate the long-term outcome of quetiapine (QTP) and clozapine (CLOZ) use in psychotic Parkinson's Disease patients (PPDP).

Methods: Thirty five PPDP (mean age 76, mean disease duration 10.3 y; 19 of them with dementia DSM IV criteria) received QTP (mean daily dose 93+74 mg) and were followed up for 2 years. Thirty two PPDP (mean age 73, mean disease duration 12.2 y; 14 with dementia) received CLOZ (mean daily dose 20 mg) and were followed for 5 years.

Results: In the QTP group, 11 patients (31%) continued treatment for 2 years (7 of them with dementia). Complete resolution of symptoms was observed in 7 patients and partial resolution in 4. Treatment was stopped in 24 patients: 15 (42%) lack of effect, 3 (9%) resolution of symptoms; 1 severe somnolence; 3 personal reasons; 2 patients died for reasons unrelated to QTP. In the CLOZ group 19 patients (59%) continued treatment for 5 years (14 with dementia). Thirteen patients (40%) stopped treatment: 9 (28%) because of resolution of symptoms; 3(9%) somnolence; 1 personal reasons. No correlation in either groups was found between age, sex, duration and severity of disease, presence of dementia and LD dose.

Conclusions: In this study, CLOZ was more effective and better tolerated. However, due to the necessity of continuous blood monitoring there is still a tendency in clinical practice to

first try QTP. In resistant patients, CLOZ is undoubtedly a useful alternative.

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Ablative Stereotactic Surgery Improves Manual Performance Time in Parkinson's Disease

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The objective of this study was to determine the influence of stereotactic ablative surgical interventions on the time required for the performance of manual tasks (i.e.performance time) in patients with Parkinson's disease (PD). Matherial and methods: we studied 28 patients after pallidotomy and pallido-thalamotomy who were evaluated at four timepoints: before the operation, and 2 days, 3 and 6 months postoperatively. The speed of performance of handwriting and drawing were assessed by means of a chronometer using certain parts of an international standard scale (modified by Fahn). The patients were also assessed according to the Unified Parkinson's Disease Rating Scale (UPDRS) part III. The patients were divided into 2 groups. Those in group A had relief of all main parkinsonian symptoms after pallidotomy including tremor. The patients in group B had no relif of tremor staight after pallidotomy. For them the pallidotomy was completed by thalamotomy in the same sitting, which had resulted in cessation of tremor. Results: The time of performance of the manual tasks diminished significantly in all cases in both groups (Student's T test: $p< 0.0001$). Complications: No complications developed following pallidotomy. Pallido-thalamotomy caused transient adverse effects in 2 patients, and 1 patient developed permanent adverse effects such as dysarthria and dysequilibrium. Conclusions: significant improvements ensued in the speed of handwriting and drawing in both groups, but pallido-thalamotomy was accompanied with complications.

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Bilateral STN Dbs Improves Manual Performance Time in Parkinson's Disease

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The objective of this study was to evaluate the influence of deep brain stimulation (DBS) of the subthalamic nucleus (STN) on the time required for the performance of manual tasks in patients suffering from Parkinson disease. Material and methods: 13 patients were evaluated before the operation and 3 months after surgery. The speed of performance of handwriting and drawing were assessed by means of a chronometer using certain parts of an international standard scale (modified by Fahn, Tolosa). The patients were also assessed according to the Unified Parkinson's Disease Rating Scale (UPDRS) part III. Results: Postoperatively the time of performance of the manual tasks (Student's T test: $p< 0.01$), and the motor scores (UPDRS III) diminished significantly in all cases. Complications: in one case transient and in other case permanent complication developed following STN DBS implantations. Conclusions: bilateral STN DBS significantly improves manual performance time in Parkinson's disease. These improvements were strongly correlated with improvements in motor function, primarily with regard to bradykinesia.

1040

Potential Mechanisms of Action of Electroconvulsive Therapy in Parkinson's Disease

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Background and Aims: Electroconvulsive therapy (ECT) is a widely used and effective treatment for mood disorders. ECT also appears to have positive effects on the motor symptoms of Parkinson's disease (PD), improving rigidity and bradykinesia for weeks to months, even in non-depressed patients. Two of the most consistent effects of electroconvulsive shock (ECS) in animals are upregulation of monoamine receptors and enhancement of neurotrophic factors in limbic brain regions. We hypothesized that these effects may also occur in the parkinsonian striatum after repeated ECS treatment. Methods: We treated unilateral 6-hydroxydopamine-lesioned rats with ECS or sham every day for 10 days. Forty-eight hours after the last treatment, the animals were sacrificed and their brains were processed for dopamine receptor binding or neurotrophic factor concentrations. Results: Repeated ECS treatment significantly increased D1 and D3 receptor binding in the lesioned and non-lesioned striatum, without affecting the D2 receptor. Both BDNF and FGF-2 protein concentrations were significantly increased in the striatum of ECS-treated animals. In addition, ECS-treated rats showed improved performance on a beam-walking test. Conclusions: Taken together, these results suggest that the mechanism of action of ECT in PD may be similar to its hypothesized mechanism of action in depression, i.e. upregulation of monoamine receptors and/or enhancement of specific neurotrophic factors, and these effects may underlie the ECT-induced improvement in motor function in PD patients. Our results support the continued use and study of ECT as an adjunctive treatment for PD.

1041

Dynamics in Emotional Features and Efficiency of Neuropsychological Rehabilitation in PD

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Background and aims: Emotional disturbances are not so evident as motor symptoms in PD, but they deeply influence patients' activity and quality of life. The study was aimed to find out the relations between patients' emotional and personal concepts and the efficiency of their neuropsychological rehabilitation (gait correction through external visual cues).

Participants and Methods: 11 patients with PD aged from 49 to 78 (9 men and 2 women, with mean Hoehn-Yahr stage from 1,5 to 3) were assessed before and after rehabilitation cycle through comprehensive neuropsychological Luria battery that evaluates (qualitatively and quantitatively) patients' cognitive functions. Emotional state and the self-appraisal were studied with the tests of Wylie and Spilberger, Geriatric depression scale and original Self-appraisal scale. Gate disturbances and their dynamics were estimated by traditional gate tests (step length, walking speed and speeding-up ability) and by objective instrumental methods - podometry and stabilometry.

Results: Patients with less cognitive disturbances, lower level of depression, of anxiety state and trait and with moderate level of self-appraisal show better results in gate correction. They are more motivated to participate in rehabilitation program, more optimistic about its efficiency, and they appropriate better the program. Mutual influence of gait improvement with emotional symptoms decrease was revealed.

Conclusions: Personal and emotional state is an important criterion of patients' rehabilitation efficiency and prognostics. The levels of depression and anxiety tend to normalize during gait correction. Emotional and personal reactions to the disease in PD should be one of the main orientations in psychological rehabilitation.

1042

Continuous Transdermal Delivery of Apomorphine Hydrochloride for Advanced Parkinson's Disease Using the Passport(Tm) System: Preclinical and Phase 1 Clinical Results

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Apomorphine hydrochloride is a highly potent drug for treating advanced Parkinson's disease. Its efficacy is comparable to levodopa, but its lack of oral bioavailability and rapid systemic clearance limit its use. Continuous subcutaneous infusion of apomorphine HCl may dramatically reduce "off" time in patients with motor fluctuations. However, infusion pump therapy can be complicated, inconvenient, and uncomfortable. We are developing a transdermal patch product to provide continuous apomorphine HCl delivery without these drawbacks. The patch is based on the PassPort System, a technology for delivering water soluble drugs and macromolecules through microscopic channels in the stratum corneum. In hairless rats, apomorphine HCl was delivered transdermally at rates exceeding 0.2 mg/cm2/hour. Using simple aqueous formulations, plasma levels reached apparent steady state approximately 4 hours after patch application and remained constant until patch removal at 24 hours. To improve bioavailability from the patch, polymer film formulations containing apomorphine HCl in solid form were developed. Transdermal delivery rates from polymer films were comparable to or greater than the aqueous formulations. Phase 1 clinical studies were performed to demonstrate transdermal apomorphine HCl delivery and to estimate delivery rates in healthy human volunteers. The ultimate goal of these studies is to develop a transdermal therapeutic that delivers apomorphine HCl at rates from 1 – 4 mg/h for up to 24 hours, using small convenient skin patches with functional areas of 2 – 8 cm2. This product has the potential to offer substantial clinical benefit to patients with advanced Parkinson's disease.

1043

Induction of the Redox Protein Thioredoxin for Protection Against Neurotoxicity Caused by MPP+ the Toxic Metabolite of MPTP

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New therapies for managing Parkinson's disease have been developed for symptomatic relief. Observations indicate that selegiline, rasagiline, and pramipexole might delay the need of L-DOPA for early patients. Selegiline and rasagiline prevent neurodegeneration caused by 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) via mechanisms other than inhibition of MAO-B.

Thioredoxin (Trx, 200nM) blocked neurotoxic effects of 1-methyl-4-phenylpyridinium (MPP+) in human SH-SY5Y neuroblastoma cells. Preconditioning up-regulation of Trx reduced the apoptosis caused by either oxidative stress or MPP+. Our results obtained from experiment using Trx antisense infer that selegiline directly protected against MPP+-induced neurotoxicity via the induction of Trx. Other report indicates that Mice over-expressed Trx are less vulnerable following MPTP. We thus investigated further multifaceted neuroprotective mechanisms of Trx.

It has been suggested that Trx is a redox protein which modifies protein–SH for redox chain reactions. In addition to its known antioxidative property our findings revealed that Trx is a potent inhibitor of caspases and thus blocks oxidant-induced apoptosis. Trx activated the transcription factor cMyc and the induction of mitochondrial Bcl2 for enhancing survival. Interestingly, Trx induced neuron specific proteins leading to neuronal plasticity such as neurite outgrowth (i.e., NF160, NF200, and syntasin-1) and synaptogenesis (i.e., phosphoactivation of GAP, MAP-2, and SNAP-25). Additional experiments of knock down of Trx levels by antisense confirmed such an important role for Trx in not cell survival but also neuronal plasticity. In conclusion, Trx may reduce oxidative stress and repairs neuronal damage following oxidative stress caused by pro-oxidants in the brain of PD and perhaps AD. <chiueh@tmu.edu.tw>

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Cerebrolysin Promotes Gabaergic Neuronal Differentiation of Cultured Ng2-Expressing Progenitor Cells Isolated From Adult Rat Hippocampus

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Recent evidence have revealed that about 15% of newly generated neurons in the adult dentate gyrus might be GABAergic and partly derived from NG2 proteoglycan-expressing progenitor cells (NG2 cells). We have recently established a novel method to isolate and expand NG2 cells in serum-free medium from diverse regions of adult rat brain including the hippocampus. These cells were multipotential and differentiate into neurons or glias depending on the environmental cues in vitro. In the present study, we investigated the role of Cerebrolysin (CL), a neurotrophic drug shown to improve cognition and mood of patients with Alzheimer Disease or ischemic stroke, on the GABAergic neuronal differentiation of adult hippocampal NG2 cells (HNCs) in vitro. CL significantly increased GABA, MAP2a/b, synapsin I, and vesicular GABA transporter immunoreactivities in HNCs. CL also increased the number of HNCs by reducing spontaneous apoptosis. Combined treatment with CL and platelet derived growth factor (PDGF) further increased the GABA and GAD67 expression in HNCs in association with decreased NG2 expression and with the neuron-like morphological changes. Interestingly, these effects were most obvious in HNCs located in the periphery of the colonies, suggesting that cell-cell contact might also play roles in the GABAergic neuronal differentiation of HNCs. Taken together, our data is highly suggestive that CL promote the GABAergic differentiation of HNCs in vitro especially when it is combined with PDGF. Our in vitro culture system may be a useful tool to investigate the molecular mechanisms of adult GABAergic neurogenesis both in the physiological and pathological conditions.

1045

Gene Transfer of HGF by Microbubble-Enhanced Ultrasound Improve Abeta-Induced Memory Impairment in Mice

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Background- Alzheimer disease (AD) is characterized by Amyloid beta (Abeta) containing plaques in the neocortex and hippocampus and neurofibrially tangles, synaptic and neuronal loss along with progressive cognitive impairment. HGF (hepatocyte growth factor) is a unique multifunctional growth factor which can not only induce angiogenesis, but also act as a neurotrophic factor. Recently, we have reported that HGF improved memory and learning function after cerebral infarction in rats. In this study, we investigated whether HGF gene transfer could improve memory deficit in Aβ-injection model.

Methods and Results- We used male ddY mice (6-8weeks old). Abeta(1-40) was injected by intracerebroventricular (i.c.v.) administration into mice. 2 week after Abeta(1-40) injection into cerebroventricle, mixture of HGF gene/or BDNF gene and microbubbles were injected into cerebroventricle. After the injection, ultrasonic irradiation was aplied to each mice. 1 week after the administration of HGF gene, the water finding task and Y-Maze test were carried out according to the established procedure. Mice treated with HGF gene or BDNF gene significantly had better performance in water finding task than mice treated with control vector. We confirmed upregulation of BDNF after BDNF gene transfer, and unexpectedly found that HGF also upregulated BDNF in brain. We demonstrated DHE staining to evaluate oxidative stress and confirmed that HGF reduced oxidative stress induced by Abeta.

Conclusions- Ultrasound-mediated gene transfer of HGF improved Abeta-induced memory impairment in mice associated with upregulation of BDNF and downregulation of oxidative stress. HGF gene therapy might be a novel therapeutic strategy for Alzheimer's disease.

1046

Model Systems for Lentiviral Vector Mediated Therapeutic Gene Delivery for the Treatment of Parkinson's Disease

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Gene therapy is a promising technology for the treatment of neurodegenerative diseases such as Parkinson's disease. Prerequisites for detailed evaluation of potential therapeutic genes are appropriate gene delivery systems as well as eligible in vitro and in vivo models. Ideal candidates for efficient gene delivery into differentiated non-dividing cells are lentiviral vectors facilitating stable integration and long-term expression of transduced genes. In the present study, we have evaluated these vectors to deliver distinct transgenes into cells of primary mesencephalic and organotypic slice cultures of the mouse brain. Optimised conditions for infection and analysis were defined and finally efficient infection of neuronal cells could be demonstrated. In addition, the lentiviral vectors were pseudotyped using envelope proteins derived from five different heterologous viruses. Concentration by ultracentrifugation facilitated generation of high titer virus stocks which were successfully used for stereotactic injection into the mouse brain. Immunohistochemical analyses followed by confocal laser microscopy were performed using cell-type specific markers to evaluate transduction efficiency and target cell specificity of these vectors. In this respect, vectors pseudotyped with the surface protein of the vesicular stomatitis virus revealed best efficiencies for infection of neuronal cells. Currently, distinct therapeutic genes, such as ß-synuclein and parkin, are evaluated in context of the established lentiviral vectors in the respective model systems.

Data from these studies will be presented and discussed in detail.

1047

Effect of Human Mesenchymal Stem Cells on Neuronal Death and Memory Deficits Induced by Trimethyltin in the Rat Hippocampus

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Trimethyltin (TMT) is a potent toxicant that selectively kills cells in the central nervous system and immune system. Within the central nervous system, TMT selectively destroyed neurons in the neocortex, amygdale, and olfactory tubercle, but its most striking effects were in the hippocampal formation. In the present study, we examined the effect of human umbilical cord blood (hUCB)-derived mesenchymal stem cells (MSCs) on TMT-induced hippocampal cell death and impairments of learning and memory in Morris water maze in rats. The hUCB-MSCs were grafted into the hippocampus (CA1) 1 week after TMT (6.0mg/kg, i.p.)-induced neurodegeneration. We identified that hUCB-MSCs survived and differentiated in TMT-induced rat brain by analysis of Hoechst dye, bromodeoxyuridine (BrdU) and neuronal marker NeuN immunofluorescent. In TMT exposed rats hUCB-MSCs grafts improved spatial learning and memory in water maze, suggesting that grafts can in some circumstances reduce spatial deficits on the central nervous system after TMT-induced neurodegeneration. Our results suggest hUCB-MSCs grafted into the TMT-induced rat brain are able to differentiate into neurons and to improve spatial recognition.

1048

Small Molecule SGS1704 Promotes Neuronal Differentiation in Neural Stem Cell Culture

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Research on neural stem cells(NSCs) have raised possibilities of cell-based therapy of damaged brain tissues. Specific biochemical molecules that can stimulate neurogenesis of quiesent NSCs are of special interest to potential clinical applications. Our recent bioactivity-guided screening for natural compounds identified small molecule SGS1704, as an potent neurogenesis inducer. we investigate the effects of SGS1704 on the proliferation and differentiation of NSCs in cultures. SGS1704 inhibits proliferation of NSCs induced by EGF in dose dependant manner; SGS1704 was also found to promotes the neuronal differentiation of NSCs. The treatment of SGS1704(10μM) increased the numbers of Map2ab and β -TUJ1 positive neuronal cells and the expression level of neural gene and protein β -TUJ1. SGS1704-induced neuronal differentiation was partially reversed by treatment with CNTF(100ng/ml); SGS1704-treatment attenuates the stat3 phosphorylation level induced by CNTF as confirmed by ELISA assays and western blot analysis; Furthermore, SGS1704 can decrease the expression of Notch1, and its downstream effectors genes Hes1 and Hes5 during differentiation of NSCs. These results show that SGS1704 can regulate cell fate choice of NSCs, and promote the neuronal differentiation in the NSCs cultures via modulating the Notch/Hes signaling.

1049

Decreased Frequency of Circulating CD34+ Hematopoietic Stem Cells in Early Alzheimer's Disease

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Hematopoietic stem cells (HSC) contribute to mammalian brain tissue regeneration by transdifferentiation processes suggesting that a regenerative interface exists between the nervous and hematopoietic organ systems. As transdifferentiation of HSC might also be relevant for the diagnosis and therapy of neurodegenerative diseases we evaluated the HSC frequency in the blood of patients with early Alzheimer's disease (AD). The early AD group (n=23) fulfilled the NINCDS-ADRDA criteria for probable AD and the diagnosis was supported by cerebrospinal fluid (CSF)-based neurochemical dementia diagnostics. Spouses or caregivers served as non-demented age, sex and environmentally matched controls (n=25). CD34+/CD45R0low HSC were assessed by flow cytometry. We found decreased counts of circulating HSC in early AD (p=.01), which significantly correlated with age (r=-.661; p=.001), cerebrospinal fluid Aβ1-42 (r=-.467; p=.025) and most pronounced the Aβ42/40 ratio (r=-.688; p=.005). The correlations between HSC counts and total tau or phospho-tau protein were not significant. Decreased HSC counts may reflect an accelerated aging process and a premature exhaustion of the stem cell pool suggesting a deficient regenerative hematopoietic support for the central nervous system in early AD.

1050

Mesenchymal Stem Cell Transplants Improve Motor Disorders of Rats After Chemical Lesion of Striatum as a Model of HD

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Introduction: Neurodegenerative inherited motor disorders are due to progressive neuronal degeneration in the

neostriatum and neocortex. There is currently no therapeutic method with significant effects for this disorders. In recent years, cell transplantation has received a great deal of attention as a potential restorative therapy for a variety of neurodegenerative deficits. Method: The present study considered effects of transplantation of bone marrow derived mesenchymal stem cells in wistar rats that have been chemically induced as animal model of motor disorders such as HD. Stem cells were derived and purified from bone marrow of 4-6 weeks old rats. These cells were transplanted into a unilateral lesion(quinolinic acid induced)of striatum.For better tracking, the incorportaion of cells was performed by standard methods.The ability of the transplants to improve QA-induced motor function deficits was analyzed during 8 weeks after transplantation. Apomorphine induced rotation test and cylinder test were used in this regard. The results showed that mesenchymal stem cell transplants significantly improved motor function deficits in comparison with control animals. Histological analysis with nissl staining for tracing migration and differentiation of transplanted cells was performed. Conclusion: we suggest using bone marrow derived mesenchymal stem cells as new perspective of several abilities of these cells in treatment of HD experimental model. Therefore, cell therapy using adult bone marrow derived stem cells might be useful for therapeutic purposes of some neurodegenerative disorders in near future.

1051

Effect Size as a 'Best in Class' Outcome Measure in Clinical Trials of Dementia

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Objective: In reporting of clinical data, there is often focus on statistical significance, rather than size of treatment effect. In exploratory drug trials, numerous comparisons may be made to find an effect producing problems of multiplicity. Furthermore, trials designed to detect effects of compounds on cognition often employ a variety of assessments to sample across a range of cognitive domains. These are often based on different measurement units, making internal and external benchmarking difficult. Cognitive Drug Research (CDR) provides assessment of cognitive function in clinical trials and has the unique advantage of 20 years experience in working with over 300 compounds, including numerous cognitive enhancers. Using our database of compound effects, we can determine primary outcome measures for assessing particular classes of compound. This information can be used to power future trials, guide dose selection and establish primary outcome measures by timepoint and cognitive test.

Methods: We have reanalysed historical data to determine effect size and established a hierarchy of peak cognitive effect for a range of anti-dementia compounds such as nicotinic agonists and anti-cholinesterase inhibitors, in both healthy volunteers and various patient groups including Alzheimer's disease, Dementia with Lewy Bodies and Parkinson's disease dementia.

Results: This technique has enabled us to establish 'best in class' cognitive tests for many compounds, and provide detailed recommendations for single primary outcome measures in clinical trials of dementia.

Conclusion: This technique provides a valuable way of determining cognitive effect and in aiding the selection of primary cognitive outcome measures in clinical trials.

1052

Transcranial Electrostimulation Via the Nexalin Device for Treatment of the Parkinson's Disease

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In contrast to deep brain stimulation, the information concerning transcranial electrostimulation (TES) for treatment of Parkinson's disease (PD) is limited. TES is based on the activation of opioidergic systems of the brain. Disturbances in the production of endogenous opiates and serotonin could be one of the mechanisms underlying the development of PD. Presently, TES is widely used to treat depression, insomnia, and pain. The aim of this study was to evaluate the efficacy of the TES via "NEXALIN" device for treatment of PD.

Twenty patients with mild and moderate PD were enrolled in a 5 week open label trial. Repeated clinical assessments, testing with UPDRS, and "Up and Go" were conducted during baseline (2 weeks), treatment (7 consecutive days; each session: 40 minutes, average current 15 mA) and follow up (2 weeks). All patients maintained their regular pharmacotherapy.

Statistical and clinical improvements were noted in decreasing symp-toms of hypersalivation, depression, and bradykinesia ($p<0.05$). Also ob-served were improvements in postural unsteadiness, stiffness, carriage, sleep, and mood. Decreases were noted in freezing when waking, facilitation of cut-ting of food and writing, and pain levels.

The analysis of the "Up and Go" demonstrated decreased time ($p<0.0001$). Number of steps reduced after 7 sessions and the first week of follow up ($p<0.0001$). At final follow up the number of steps returned to the base level.

No side effects were registered.

The results of treatment of PD via the "NEXALIN" device proved some effectiveness of TES and could be the basis for future trials.

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Autocoid Mediated Drug-Cyclodextrin-Liposome Combination for Management of Parkinsonism: A Novel Approach

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In the present study it was hypothesized to prepare solid inclusion complex of l-DOPA with Î²-CD and its derivative. The prepared complexes were then incorporated in unilamellar liposomal vesicles. These liposomes were composed of egg phosphatidylcholine cholesterol and DSPE-PEG 2000 in optimized molar ratio and were prepared by dehydration-

rehydration method. The molar ratio for the l-dopa: Î²/HP-Î² CD inclusion complex was estimated to be 1:1 using the continuous variation method based on the dissolution studies and 1H NMR data. The prepared long-circulating liposome containing drug-CD complex were further conjugated with dopamine and were characterized for stability, particle size, particle number etc. The results obtained were then analyzed for in-vivo effectiveness probenecid-MPTP induced parkinsonism model. The data for Rota rod model, behavioral analysis and step down model suggests a significant (30%) increase in the efficacy of drug-cd-liposome-conjugate combination when compared to liposomal formulation of l-dopa. The results also pave the way for further utilization of this strategy for antiparkinsonism as well as antitumor drugs for effective targeting incase of brain related disorders

1054

Extent of Involvement and Interest of Religious Organisations to Provide Services to Caregivers

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The gerontological literature describes the stress-buffering role of religious involvement for caregivers. The desire to institutionalize is greatest when caregivers experience high levels of stress and when caregiving is physically and emotionally burdensome.

The purpose of this study was to examine the extent of involvement by religious congregations in the provision of social services to older adults. The study also assessed the interest of clergy in developing programs for members and non-members. This study explored if there were differences in service provision by religious congregations based upon theological orientation, ethnicity, age of clergy, education of clergy, and percentage of older adult congregants. Interest in providing services was also examined.

The methodology included the sampling of 3400 congregations and 600 survey instruments were sent out. The survey instrument was developed, based upon Knapp's 2001 survey of United Churches of Christ to assess extent of involvement and extent of interest to become involved in provision of social services and programs. Results indicated that clergy have an interest in developing programs and services for older adults. Clergy also reported an interest in attending workshops and seminars on ministry for older adults and a lack of resources to fund programs and services for older adults. Future research should explore sources of funding for religious congregations to provide services to caregivers.

1055

Mild Cognitive Impairment, Depression and Personality Disorder : A Case

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Background and aims: Mild Cognitive Impairment (MCI) is a recently described syndrome that involves more substantial cognitive and memory decline than normal aging and represents a significant risk factor for the development of dementia (about 15% per year). Given into account that patients with MCI are often reffered for a depression and that cognitive dysfunction can be also observed in depression, the two clinical entities clinically may co-exist.

Methods: We report the case of a woman ,79 years old ,she was first diagnosed with depression , after her husband had died, and since then she was under antidepressant meditation. The initial symptoms , according to her family were: apathy, appetite loss, anxiety, dysphoria , time disorientation and recent memory problems.

Results: She was first seen at our hospital on March 2006 as external resident . The strict clinical , neuropsychological and psychiatric examination showed: instead of depression , a passive-narcissistic personality disorder with normal neuroimage but MMSE 24.

Conclusions: MCI is not only a simple syndrome but a clinical entity which may be underestimated and misdiagnosed. The reality of pure depressive pseudodementia seems doubtful. MCI must be a differential diagnosis to every case of persistent depression in the elderly.

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in Vivo and Post Mortem Clinicoanatomical Correlations in FTDP-17

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Frontotemporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17) is associated with mutations in the Microtubule Associated Protein Tau (MAPT) gene or in Progranulin (PGRN). Cases with MAPT mutations are invariably characterized by the widespread deposition of hyperphosphorylated tau protein in the central nervous system (FTDP-17T). Cases with PGRN mutations are instead associated with tau-negative ubiquitin-positive neuronal inclusions in the frontotemporal cortex, striatum and dentate gyrus of affected individuals (FTDP-17U). In spite of these genetic and pathological differences, FTDP-17T and FTDP-17U share a largely overlapping clinical phenotype.

Here we present the clinical findings of cases of FTDP-17T associated with either the newly reported G335S MAPT mutation or the exon 10+3 mutation as well as those of individuals affected by FTDP-17U associated with distinct PGRN sequence variations (A9D, IVS6-2A>G, and R493X). MRI and/or PET studies were obtained along the disease courses in order to analyze in vivo clinicoanatomical correlations.

FTDP-17T cases were associated with a symmetric frontotemporal involvement. Behavioral changes constituted the predominant clinical presentation.

Conversely, an asymmetric degenerative process was seen in all of our PGRN cases. A corticobasal syndrome was noted in the case associated with the A9D mutation while

severe language deficits accompanied the FTD symptoms in the cases with the IVS6-2A>G and R493X mutations.

These data are compared to the findings observed at the postmortem neuropathological examination. This multidisciplinary approach provides valuable insights on the natural history of these disorders and tests the possibility of an early differentiation of the FTDP-17T and FTDP-17U phenotypes.

These studies were supported by PHS P30 AG010133 and intramural research programs of the National Institutes of Health, National Institute of Neurological Disorders and Stroke.

Neuro–degenerative Diseases

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