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The 4th Kuopio Alzheimer Symposium

Microteknia, Kuopio, Finland
February 2-4, 2006

Program
and
Abstracts

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Dear Collegues and Friends,

You are cordially invited to participate into the Fourth Kuopio Alzheimer Symposium, which will be held in Kuopio, Finland, February 2-4, 2006.

Year 2006 will be the year of celebrations of dementia research and neuroscience in Finland. In November 1907 Alois Alzheimer presented the neuropathological features in a patient with an early-onset dementia, Auguste D, in a medical conference. The disease characterized with neuritic plaques and neurofibrillary tangles in the brain and slowly evolving dementia as a clinical phenotype has since been known as Alzheimer's disease and shown to be the most common form of dementia. As a result of an intense research the first drugs for symptomatic treatment have been emerging. In 1996 the first cholinergic drug, tacrine, was approved by Finnish authorities for treatment of Alzheimer's disease. The opportunities for drug treatment started a whole new era for medical community and particularly for patients suffering from dementia and their relatives. The focus since then has been on early diagnosis and organizing the multidisciplinary care and treatment for these patients.

The forthcoming meeting is the fourth meeting in series of Kuopio Alzheimer Symposiums. The Department of Neurology in Kuopio University Hospital were founded in 1976 and the meeting will celebrate the beginning of neuroscience in our area. The programme will focus on important topics concerning the possible causes of dementia, the genetics of dementing disorders, and risk factors and opportunities for prevention of dementia. We will also hear overviews on behavioural symptoms in dementia and the current status of therapeutic approaches in Alzheimer's disease. As before our goal is to bring experts together with young researchers and provide a forum for new ideas for future research.

I warmly welcome all to Kuopio to enjoy this exciting scientific meeting, which will also provide you an opportunity to experience the Finnish spring-winter.

Hilkka Soininen
Professor
Chairperson of the Organizing Committee

The Fourth Kuopio Alzheimer Symposium

Organized by Department of Neuroscience and Neurology, University of Kuopio, Kuopio, and Finnish Alzheimer's Research Society

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ORAL PROGRAM

THURSDAY, FEBRUARY 2, 2006

WORKSHOP IN GENETICS OF NEURODEGENERATIVE DISEASES

Organized by
Nordic Center of Excellence in Neurodegenerative Diseases
Department of Neuroscience and Neurology, University of Kuopio, Finland

09.00	**Opening**
Chairperson:	**Heikki Tanila, Kuopio**
09.10	**From phenotype to gene defect** *Tiina Paunio, Finland*
09.55	**From gene defect to function** *Mikko Hiltunen, Finland*
10.40	**Transgenic animals as tools for understanding pathogenesis of neurodegenerative diseases** *Deniz Kirik, Sweden*
11.25	**LUNCH**
Chairperson:	**Tuula Pirttilä, Kuopio**
12.25	**Genetics of dementia diseases – an overview** *Julie van der Zee, Belgium*
13.10	**Searching risk genes for Alzheimer's disease** *Andreas Papassotiropoulos, Switzerland*
13.55	**COFFEE BREAK**
Chairperson:	**Kari Majamaa, Turku**
14.15	**Role of mitochondrial genes in neurodegenerative diseases** *Kari Majamaa, Finland*
14.45	**Genetic testing and ethical aspects in neurodegenerative diseases** *Helena Kääriäinen, Finland*
15.15	**Questions and answers - Closing remarks**

THURSDAY, FEBRUARY 2, 2006

THE 4th KUOPIO ALZHEIMER SYMPOSIUM

18.00 **Welcome addresses**
Matti Uusitupa, Rector, University of Kuopio
Hilkka Soininen, Chair of the Organizing Committee

CURRENT STATUS OF ANTI-AMYLOID TREATMENT IN ALZHEIMER'S DISEASE

Chairpersons: **Christoph Hock, Switzerland**
Marc Baumann, Finland

18.15 **Brain amyloidosis – a protein conformational disorder**
Marc Baumann, Finland

18.45 **Immunization for Alzheimer's disease**
Christoph Hock, Switzerland

19.15 **Amyloid breakers**
Celine Adessi, Switzerland

20.00 – 22.00 **WELCOME RECEPTION**

Brain Amyloidosis – a Protein Conformational Disorder

Marc Baumann

Protein Chemistry/Proteomics Laboratory and the Neuroscience Research Program, Biomedicum Helsinki, P.O.Box 63 (Haartmaninkatu 8), 00014 University of Helsinki

Background: Alzheimer's - and Creutzfeld-Jacob disease are the only brain amyloidoses among more than 20 forms of human diseases associated with extracellular, fibrillar, protein deposits ("amyloid"). Nevertheless, protein deposits in AD and CJD share identical fiber structure as seen in other amyloidoses although originating from totally unrelated proteins. It is believed that the misfolding cascade leading to the fibril formation of these different proteins is the key initiator to disease symptoms in several, if not all amyloidoses.
Methods: We have studied the principles of misfolding in amyloidoses by several physico-chemical methods and used the aquired data to verify the effects of the found "amyloidogenic core properties" in some "non-amyloidosis" related proteins, as well as some risk factors to Alzheimer's disease.
Results: We show that amyloidogenic proteins share certain physico-chemical similarities which lead to fibril formation. We also show that the ability of a protein to misfold to an amyloid is usually caused by a small amino acid sequence motif: the "amyloidogenic core sequence". We further show that such motifs can be found in several non-amyloidogenic proteins and that these can form fibers identical to the disease related proteins. Moreover, we also demonstrate that several other interactions between amyloidogenic and non-amyloidogenic proteins or disease risk factors are mediated through identical epitopes.
Conclusions: The only common feature between amyloidogenic proteins is their ability to misfold to amyloid. In the present paper we demonstrate that conformational changes leading to amyloid are caused by identifiable physico-chemical properties which are rather common amongst different proteins. We further propose that these properties might be responsible for the numerous false interactions that amyloids go through with other proteins finally leading to a disease phenotype.

Amyloid Breakers

Celine Adessi[1] Lisbell Estrada[2] and Claudio Soto[2].

[1] Hoffmann-La Roche Ltd, CNS Pre-Clinical Research, Basel, Switzerland and [2]George and Cynthia Mitchell Center for Alzheimer's disease research, Depts of Neurology and Neuroscience & Cell Biology, University of Texas Medical Branch, Galveston, TX, USA.

Compelling evidence indicates that a hallmark event in Alzheimer's disease (AD) is the misfolding, aggregation and brain deposition of amyloid-beta (Aß) protein in cerebral amyloid plaques. Several strategies have been proposed for AD treatment based on inhibiting the amyloid pathway. One therapeutic strategy we have been focusing for the last years has been the rational design of peptide inhibitors capable to specifically prevent and reverse the Aß misfolding and aggregation. We proposed that short synthetic peptides able to bind Aß but unable to become part of a ß-sheet structure (ß-sheet breaker peptides) may destabilize the amyloidogenic Aß conformation and hence preclude amyloid formation. Several peptides with these characteristics were designed and evaluated. We identified a 5-residue peptide (iAß5p, Seq: LPFFD) able to inhibit and disassemble amyloid fibrils in vitro, to prevent Aß neurotoxicity in cell culture, and to arrest and dissolve amyloid plaques in several in vivo animals models. Treatment with iAß5p also inhibited neuronal death, brain inflammation and memory impairment in vivo. In addition, the compound showed low toxicity, low immunogenicity, high solubility and reasonably high brain uptake. These findings led to perform a phase I clinical study in healthy volunteers to begin the clinical evaluation of this compound for the treatment of AD. In this presentation, we will describe the current status of development of these compounds, as well as our structure-activity relationship studies and our efforts to produce a second generation of peptidemimetics with better drug-like properties.

FRIDAY, FEBRUARY 3, 2006

Main Theme **HEREDITARY AND RARE DEMENTIAS**

Chairpersons: **Peter St. George-Hyslop, Canada**
Patrik Brundin, Sweden

09.30 **Early-onset Alzheimer's disease**
Peter St. George-Hyslop, Canada

10.00 **Approaches towards finding novel genes for early onset dementias**
Kristel Sleegers, Belgium

10.30 **Frontotemporal dementias and other tauopathies**
Juha Rinne, Finland

11.00 **Huntington's disease**
Patrik Brundin, Sweden

11.30 **LUNCH BREAK**

Chairpersons: **James Ironside, Scotland**
Irina Alafuzoff, Finland

13.00 **Prion diseases**
James Ironside, Scotland, UK

13.30 **Hereditary Vascular Dementias**
Matti Viitanen, Sweden

14.00 **Genetic testing as part of routine diagnostics**
Andreas Papassotiropoulos, Switzerland

14.30 **COFFEE BREAK**

Approaches towards finding novel genes for early onset dementias

Kristel Sleegers MD PhD, Julie van der Zee Msc, Christine Van Broeckhoven PhD DSc

Neurodegenerative Brain Diseases group, Department of Molecular Genetics, Flanders Interuniversity Institute for Biotechnology, Institute Born-Bunge, University of Antwerp, Antwerpen, Belgium

The neurodegenerative dementias are characterized by different clinical and pathological phenotypes and are diagnosed according to specified criteria. In brain the pathology shows different neurodegeneration profiles due to the deposition of defined proteins in defined areas that are characteristic for the disease. Neurodegenerative brain diseases that lead to dementia are known to have a genetic etiology though multifactorial in nature meaning that both genetic and environmental factors contribute to the expression of the disease. Molecular and epidemiological geneticists are aiming at identifying the genetic variations that underlie the disease process. Initial results were obtained in families in which the disease was apparently only genetically since the disease was transmitted as an autosomal dominant trait. The proteins involved are being studies in detail and have resulted in important biological hypothesis that are currently being pursued for the development of suitable and more effective treatments. Not all families can be explained by a mutation in one of the known dementia genes indicating that other genes are still to be found. We systematical screen presenile dementia patients for mutations in five genes (APP, PSEN1, PSEN2, MAPT and PRNP). If no mutation is identified we initiate genealogy studies for patients that belong to multiplex families. Informative families are included in a genome wide scan to identify novel chromosomal loci and genes. This approach has already resulted in the identification of a novel locus for Alzheimer's disease at 7q36. In addition, this approach has shown that a clinical categorical approach in mutation screening of dementia patients is not advisable.

Frontotemporal Dementia and other Tauopathies

Juha O Rinne, MD, PhD

Turku PET Centre, University of Turku, Finland

Frontotemporal degeneration (or frontotemporal lobar degeneration) is divided into three clinical syndromes: frontotemporal dementia, primary nonfluent aphasia and semantic dementia which show different topographical distribution of major brain pathology. In these patients the underlying histopathology includes those with tau-positive inclusions, those with tau-negative ubiquitin-positive inclusions and those without tau-or ubiquitin-positive inclusions (often referred as dementia lacking distinctive histological features, DLDH). Each histological subtype can be associated with each clinical subtype. In frontotemporal dementia the most common histopathological feature is tauopathy. Other tauopathies include progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) with typical distribution of tau-positive inclusions. Also patients with argyrophilic grain disease and Alzheimer´s disease show tau-positive inclusions.

Tau is a microtubule associated protein, but recent evidence suggests that tau is multifunctional and also contributes to other neuronal functions than microtubule function. Phosphorylation is the major posttranslational modification of the tau protein and deposition of hyperphosphorylated tau in insoluble filaments in brain is characteristic to tauopathies. The tau deposits in different tauopathies consist of either 3- or 4-repeat tau isoforms. The human microtubule-associated protein tau gene (MAPT) is located on chromosome 17. Linkage to chromosome 17 was demonstrated first in families clinically presenting with frontotemporal dementia and parkinsonism (FTDP-17). Later mutations in MAPT have been found in these and other families with FTLD. This far, about 35 mutations in around 100 families have been identified which affect differently on tau function. The clinical phenotype may be variable even between cases sharing identical mutations, indicating that additional unidentified environmental and/or genetic factors contribute to this phenotypic variability. Tau haplotype H1 has been associated with PSP and CBD resulting in especially 4-repeat tau isoform accumulation.

Better understanding of the role of tau in frontotemporal dementia and other tauopathies will hopefully lead to effective therapies in these disorders which unfortunately are lacking at present.

Prion Diseases

Professor James W Ironside

National CJD Surveillance Unit, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, UK

Human transmissible spongiform encephalopathies or prion diseases are fatal neurodegenerative conditions that occur in sporadic, familial and acquired (transmitted) forms. All are associated with the accumulation of an abnormal isoform of the prion protein (PrP) in the brain, which is very closely associated with infectivity and may represent the entire infectious agent. The commonest of these is the sporadic form of Creutzfeldt-Jakob disease (CJD), which is a worldwide disorder occurring around 1-2 cases per million population per annum. There is a naturally occurring polymorphism at codon 129 in the PrP gene and homozygosity at this locus is a predisposing factor for sporadic CJD. Variant CJD, which was first described in 1996 and is associated with human exposure to the bovine spongiform encephalopathy (BSE) agent. Infectivity in variant CJD is detectable outside the CNS and accumulates in lymphoid tissues. Two cases of probable iatrogenic variant CJD infection have been identified in the UK in recipients of blood transfusions from asymptomatic donors who subsequently died from variant CJD. It is extremely difficult to predict the future numbers of variant CJD cases in the UK and elsewhere, so continuing clinical and pathological surveillance is essential for accurate diagnosis, epidemiological studies and health care planning. Although no proven treatments for prion diseases are currently available, there are several compounds that are being studied in the context of clinical trials in the UK and elsewhere.

Hereditary Vascular Dementias

Matti Viitanen[1], Hannu Kalimo[2]

1. University of Turku, Finland and Karolinska Institutet, Sweden, 2. University of Helsinki Finland and University of Uppsala; Sweden

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is the most common hereditary cause of vascular dementia. One third of patients suffer from migraineous headache beginning at teenage, when also the first changes in cerebral white matter (WM) may be detected in T2-weighted MRI. The onset of neurological symptoms dates to the thirties or forties and at the same time the cerebral blood flow begins to decrease. Cognitive decline develops slowly and leads to subcortical type of vascular dementia. Mutations in *NOTCH3* in chromosome 19 cause degeneration of vascular smooth muscle cells with subsequent progressive thickening of arteriolar walls and stenosis, which lead to leukoaraiosis and multiple lacunar infarcts in cerebral WM and deep grey matter.
Cerebral amyloid angiopathies (CAA) constitute another group of hereditary vascular dementias, in which the vascular changes are mostly cortical leading to microinfarcts and miliary haemorrhages. In some CAAs lobar haemorrhages are common. The most important amyloids deposited in CAAs are Aβ, ABri, ADan, ACys, AGel and APrP. Hereditary multi-infarct dementia described by Sourander and Wålinder, CARASIL and HERNS are rare causes of familial vascular dementia with unknown gene defects.

Genetic testing as part of routine diagnostics?

Andreas Papassotiropoulos

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Alzheimer's disease is a genetically complex trait which results from the interplay between genetic and environmental factors. High heritability estimates suggest that naturally occurring genetic variations (e.g. single nucleotide polymorphisms, SNPs) have a major impact on the development and the psychopathological manifestation of this devastating disorder. Therefore, the identification of these genetic factors may have important clinical implications for future diagnosis and potential personalized treatments. Recent advances in the development of high-density genotyping platforms along with the development of appropriate computerized algorithms now allow for the calculation of coherent SNP clusters -rather than isolated SNPs- with significant impact on disease risk. Systematic SNP analyses in genes related to such distinct pathogenetic pathways as amyloid precursor protein processing, β-amyloid degradation, tau phosphorylation, proteolysis, protein misfolding, neuroinflammation oxidative stress and lipid metabolism are expected to contribute to the characterization of functionally distinct genetic risk profiles related to AD risk and to AD-related endophenotypes. Individual allocation to predefined genetic clusters might improve our ability to estimate disease risk and to design tailored therapies.

FRIDAY, FEBRUARY 3, 2006

Main theme **BEHAVIORAL SYMPTOMS IN DEMENTIA**

Sponsored by Pfizer

Chairpersons: **Clive Ballard, UK**
Hannu Koponen, Finland

15.00 **Prevalence and Significance of Behavioral Symptoms**
Risto Vataja, Finland

15.30 **Assessment of Behavioral Symptoms**
Hannu Koponen, Finland

16.00 **Depression and Dementia**
Amos Korczyn, Israel

16.30 **Treatment Opportunities of Behavioral Symptoms**
Clive Ballard, UK

Evening Programme

19.00 – 23.00 **POSTERS AND GET-TOGETHER PARTY**

Prevalence and Significance of Behavioral Symptoms

Risto Vataja, MD, PhD

Kellokoski Hospital, Kellokoski, Finland

Background
Behavioral and psychological symptoms are very common in patients with dementia. The prevalence and significance of these symptoms in dementing disorders will be discussed.

Methods
Literature review.

Results
Up to 90% of patients with dementia suffer from neuropsychiatric symptoms at some stage of their illness. Depression, apathy and sleep disorders are the most common behavioral symptoms, with reported prevalence of around 60 % in patients at institutionalized care. Personality changes, disinhibition or apathy often precede cognitive problems in patients with frontotemporal dementias. Likewise, visual hallucinations, delusions and depression emerge early in patients with Lewy body disease. However, even in Alzheimer's disease and vascular dementia the presenting symptoms are often psychiatric. In patients with mild cognitive impairment (MCI) the behavioral symptoms are also common, predicting a faster cognitive decline and increasing the risk of conversion from MCI to Alzheimer's disease. Depression, anxiety and apathy are usually the first symptoms to appear, whereas psychotic symptoms and agitation typically complicate the later stages of dementia.
Behavioral and psychological symptoms are associated with functional decline, reduced quality of life and increased caregiver burden. They are more important predictors of institutionalization than cognitive symptoms, thus significantly increasing economical burden of dementia.

Conclusions
Emergence of psychiatric symptoms in elderly patients should be seen as a warning sign for incipient dementia. In dementia patients, these symptoms should be actively recognized and treated to improve the overall outcome.

Assessment of Behavioral Symptoms

Hannu J. Koponen, professor

Department of Psychiatry, University of Kuopio, P.O. Box 1777, FIN-70211 Kuopio, Finland. E-mail: hannujuhani.koponen@uku.fi

Behavioral symptoms related to dementia constitute an important symptom domain as they substantially increase the burden of relatives and caregivers and endanger daily outpatient living. They also predispose for prolonged hospitalization and thus are of marked financial interest. Nowadays various pharmacological and non-pharmacological treatment options for behavioural symptoms exist and thus the detailed evaluation of the nature and severity of the symptoms is important for diagnostic work-up, proper treatment selection and follow-up.

As symptoms of anxiety and depression are more common in the mild to moderate forms of dementia and overt aggression and psychotic symptoms in the more severe forms of dementia, several rating scales have been developed for the different symptom domains. Some of the most commonly used assessment methods are the Cornell scale and Geriatric Depression Scale for depression, Cohen-Mansfield Agitation Inventory for agitation and the Neuropsychiatric Inventory for psychotic symptoms. In this presentation the strengths and limitations of the current assessment methods for behavioural symptoms of dementia are discussed.

NOTES

SATURDAY, FEBRUARY 4, 2006

Main Theme **EMERGING TREATMENT OPPORTUNITIES**

Sponsored by Novartis

Chairpersons: **Howard Feldman, Canada**
Timo Erkinjuntti, Finland

09.00 **Treatment options of Mild Cognitive Impairment**
Howard Feldman, Canada

09.30 **Use of cholinesterase inhibitors in other dementias**
Timo Erkinjuntti, Finland

10.00 **Possibilities to influence cholinergic system**
Abraham Fisher, Israel

10.30 **COFFEE BREAK**

Use of Cholinesterase Inhibitors in Other Dementias

Timo Erkinjuntti, MD, PhD.

Department of Neurology, University of Helsinki, Finland.

In addition to Alzheimer's disease (AD) cholinergic system is involved in patients with vascular dementias (VaDs), dementia with Lewy –bodies (DLB) and Parkinson dementia (PD), as well as in AD patients with cerebrovascular disease (AD w\CVD). We summarize large randomized clinical trials (RCT) with cholinesterase inhibitors in these other types of dementia.

VaDs. Donepezil, showed in two 6-month RCTs (307: Stroke 2003;34:2323-2332. and 308: Neurology 2003;61:479-486) in possible or probable VaD significant improvements in ADAS-cog (5 mg and10 mg/d) and CIBIC-plus (5 mg/d) compared to placebo. However, the Activities of Daily Living (ADL) measure failed to show significant difference. Galantamine, in a 6-month RCT (GAL-INT-6: Lancet 2002;359:1283-1290) in patients with probable VaD showed a non-significant positive trend in improved cognition (ADAS-cog). In a subsequent similar RCT in patients with probable VaD (GAL-INT-26) the cognitive outcome was significant, but the study failed to show efficacy in global and ADL outcomes. Rivastigmine, a 24 wk RCT (Vantage) in patients with probable VaD is ongoing.

PD. Rivastigmine in a 24-week RCT (Express: N Engl J Med 2004;351:2509-18) showed superiority to placebo in all main (ADAS-cog, ADCS-CGIC)) and secondary (ADCS-ADL, NPI, MMSE, CDR attention, Verbal fluency, Ten point-clock) outcomes.

DLB. Rivastigmine in a 20-w. RCT (ENA-INT-03: Lancet 2000;356:2031-36) showed significant improvement in behavior and in a computerized cognitive assessment.

AD w\CVD. Galantamine, in a 6-mo. RCT (GAL-INT-6; Lancet 2002;359:1283-1290) showed efficacy on all main outcome measures (ADAS-cog, CIBIC-plus, ADL's), and positive responder analysis for cognition, global functioning and behavior.

Possibilities to Influence the Cholinergic System in Alzheimer's Disease (AD)

Abraham Fisher[1], Antonella Caccamo[2], Salvatore Oddo[2], Lauren M. Billings[2], Kim N. Green[2], Rachel Brandeis[1], Zippora Pittel[1], Nira Barner[1], Hanoch Elkon[1], Niva Natan[1] and Frank M. LaFerla[2]

[1]Israel Institute for Biological Research, Ness-Ziona, Israel; [2]Department of Neurobiology & Behavior, University of California, Irvine, CA, USA; Contact: fisher_a@netvision.net.il

The modest clinical benefit of FDA-approved acetylcholinesterase inhibitors (AChE-Is) in AD is attributed in part to a progressive degeneration of cholinergic innervations in AD. The postsynaptic M1 muscarinic receptor (M1 mAChR), preserved in AD, less dependent on such degeneration and involved in modulation of major AD hallmarks - is an alternative prime therapeutic target. While some muscarinic agonists were effective in AD, their clinical value was limited by lack of selectivity on M1 mAChR and by adverse effects. The M1 selective muscarinic agonists AF102B [EVOXAC™], AF150(S) and AF267B *via* M1 mAChR: elevated the secreted amyloid precursor protein-β, decreased β-amyloid (Aβ) and tau hyperphosphorylation, and blocked Aβ-induced neurotoxicity, *in vitro*; restored impaired cognition and cholinergic markers; and decreased tau hyperphosphorylation, *in vivo* - all these with a very wide safety margin [Fisher et al, J Neural Transm 62: 189, 2002; Farias et al, Neurobiol Disease, 17: 337, 2004]. AF267B was the most potent among these agonists in decreasing CSF Aβ42 and Aβ40 in rabbits [Beach et al, Curr Med Chem 3:27233, 2003]. AF267B removed vascular Aβ42 from cortex in cholinotoxin-treated rabbits [Beach et al, 2003] and decreased brain Aβ levels in hypercholesterolemic rabbits [Sparks et al, ADPD2003]. In 3x transgenic (Tg)-AD mice that recapitulate the major pathologies and cognitive deficits of AD, [Oddo et al, Neuron, 39:409, 2003], chronic AF267B treatment rescued behavioral deficits and decreased Aβ42 and tau pathologies in the cortex and hippocampus, but not in the amygdala. Notably, AChE-Is did not decrease Aß or tau pathologies in various Aß Tg mice, while nicotine even exacerbated pathological tau and had no effect on Aβ in 3xTg-AD mice [Oddo et al, PNAS, 102:3046, 2005]. Conclusions: M1 agonists are beneficial on major hallmarks of AD *via* M1 mAChR-signaling mediated by PKC, MAPK and GSK3β, respectively. PKC and GSK3β are important kinases decreased and elevated, respectively, in AD brains and are major targets in AD therapy. Unlike direct and promiscuous modulation of PKC and GSK3β, respectively, M1 mAChR-controlled downstream kinases – induced effects is a safer therapy as it mimics the physiological effects of acetylcholine on this receptor. Finally, since the etiology of AD is unknown, therapies should target all major hallmarks of AD. AF267B is the 1st reported low MW CNS-penetrable compound, with a wide safety margin, that meets this challenge and may become a major therapy in AD.

NOTES

SATURDAY, FEBRUARY 4, 2006

Main Theme **EMERGING TREATMENT OPPORTUNITIES**

Sponsored by Lundbeck

Chairpersons: **Howard Feldman, Canada**
Timo Erkinjuntti, Finland

11.00 **Future of Cholinergic Therapy in Dementia**
Ezio Giacobini, Switzerland

11.30 **Antiglutamate Treatment in Alzheimer's Disease**
Bengt Winblad, Sweden

12.00 **Pharmacoeconomics in AD drug trials**
Linus Jönsson, Sweden

12.30 **LUNCH BREAK**

Future of Cholinergic Therapy in Dementia

Ezio Giacobini
Department .of Geriatrics and Rehabilitation , University of Geneva , Medical School Geneva,Switzerland

Twenty years following 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 .The 2005 Cochrane Report from 13 randomized db controlled clinical trials , show that 6-12 mo ChEI treatment produces improvement in cognition activities , activities of daily living and behaviour. Nothing suggests that these effect are less in patients with severe or mild dementia . There is no evidence of difference in efficacy among the 3 tested ChEI . There is no evidence that treatment is NOT cost effective. New data indicate long-term (3-5 yrs) clinical effects of ChEI in certain patients. 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 etc. Most interesting is the possibility of applying ChEI therapy to mild initial dementia cases or to MCI subjects (Minimal Cognitive Impairment), however, recent results from 3 large studies show no clear efficacy. Using new ChEI ,it is possible to potentiate beta-amyloid reducing therapies such as immunization. Finally, bifunctional ChEI are being developed to add non-cholinergic to cholinergic effects. We can envision that ChEI will continue to play an important role in AD therapy for many years to come. Finally ,the possibility of rescuing cholinergic cells from degeneration is being attempted with NGF therapy directly , through gene therapy or through genetically modified stem cells .

Antiglutamate Treatment in Alzheimer's Disease

Bengt Winblad
Karolinska Institutet, Sweden

In developed countries, where elderly patients constitute an escalating proportion of the population, the prevalence of Alzheimer's disease (AD) is increasing. Treatment regimens throughout the different stages of dementia vary, with objectives broadening as the disease progresses and more symptoms become apparent. In the early stages of AD, an active patient role is encouraged and residual abilities are important. In severe dementia, as well as treating the patient, various means of reducing the burden on both the caregiver and healthcare system should be considered. The pharmacoeconomic aspects of dementia are important. The cost of managing AD is high and the financial burden increases as the disease progresses. Hospitalisation and nursing home placement account for the most significant direct costs; therapies that delay the institutionalisation of patients will result in significant cost savings.

A number of treatment strategies are currently available. Acetylcholinesterase inhibitors have been approved for the treatment of mild to moderate AD. They do not halt the inevitable progression of the disease and, as patients deteriorate, any therapeutic benefits derived from such medications are liable to decrease. The NMDA antagonist memantine has been shown to be effective in moderate and severe AD. Additionally, the results from two clinical trials studying the effects of memantine on patients suffering from mild to moderate vascular dementia have shown a significant cognitive benefit compared with placebo. Data have shown memantine to be safe and well tolerated. Memantine therefore represents a significant addition to the clinician's armamentarium for the treatment of moderate and severe AD. The treatment of nursing home patients with memantine resulted in functional, cognitive and global improvement and reduced care dependence.

Pharmacoeconomics in AD Drug Trials

Dr Linus Jönsson
Sweden

Health economic evaluation is a part of the development process for new medical technologies that is receiving increasing attention. In the presence of restrictions on available resources, not only the medical benefits of novel therapies but also cost-effectiveness will determine their usefulness and place in clinical practice. This is very relevant in the field of dementia care, where the costs of new therapies need to be weighted against potential savings in formal and informal care as well as improvements in quality of life for patients and caregivers.

There are alternative ways of generating data for cost-effectiveness analysis. Economic evaluations alongside clinical trials have apparent advantages, allowing economic evaluation to be undertaken in parallel with the evaluation of clinical efficacy. However there are also potentially serious methodological issues limiting the usefulness of this approach.

This symposium will review evidence from economic evaluations alongside clinical trials of cholinesterase inhibitors and memantine, respectively, for Alzheimer's disease, and discuss implications and methodological issues for ongoing and future studies. Focus will be on the relative merits of economic evaluation alongside clinical trials compared to alternative ways of assessing cost-effectiveness such as retrospective database analyses or modeling studies.

SATURDAY, FEBRUARY 4, 2006

Main Theme **EMERGING TREATMENT OPPORTUNITIES**

Sponsored by Janssen-Cilag

Chairpersons: **Ezio Giacobini, Switzerland**
Bengt Winblad, Sweden

13.30 **Forgetful mice: their role in drug development for Alzheimer's disease**
Karen Ashe, USA

14.15 **Stem cell therapy and other novel therapeutic approaches**
Jari Koistinaho, Finland

14.45 **COFFEE BREAK**

Forgetful mice: their role in drug development for Alzheimer's disease

Karen H. Ashe, MD, PhD
Departments of Neurology and Neuroscience, University of Minnesota Medical School
Geriatric Research Education and Clinical Center, Minneapolis VA Medical Center

Background
Understanding the molecular basis of memory loss and cognitive decline is important to developing effective treatments for Alzheimer's disease.
Methods
Our studies involve the creation of transgenic mouse models of Alzheimer's disease in order to understand how the amyloid-ß and tau proteins impair memory and cognition.
Results
The work has shown that the aggregates of amyloid-ß and tau proteins which define Alzheimer's disease neuropathologically, amyloid plaques and neurofibrillary tangles, do not cause memory deficits in mice. These investigations have led to the discovery in the brains of impaired transgenic mice of a form of the amyloid-ß protein called Aß*56 (Aß star 56) that disrupts memory in the transgenic mice and impairs memory when applied in purified form to healthy, young rats.
Conclusion
A specific amyloid-ß assembly impairs memory in plaque-forming transgenic mice. A current focus is to identify a specific form of tau, called tau* (tau star), that impairs memory in tangle-forming transgenic mice. Discovery of agents that block the formation or action of "star" proteins, including Aß* and tau*, may lead to more effective treatments for memory loss and cognitive decline in Alzheimer's disease.

SATURDAY, FEBRUARY 4, 2006

Main Theme **PREVENTION OF DEMENTIA**

Chairpersons : **Monique Breteler, The Netherlands**
Hilkka Soininen, Finland

15.15 **Searching for risk factors for Alzheimer's disease**
Lenore Launer, USA

15.45 **Hypertension and dementia**
Ingmar Skoog, Sweden

16.15 **Cholesterol and Alzheimer's disease**
Miia Kivipelto, Sweden

16.45 **Life style and cognition**
Monique Breteler, The Netherlands

17.15 **General discussion and closing remarks**

Searching for risk factors for AD

Lenore J. Launer, PhD

National Institute on Aging, Bethesda, MD.

Dementia is the most common form of neurologic disease in the elderly; Alzheimer's disease (AD) comprises approximately 65% of cases. Epidemiologic studies are designed to measure rates of, etiologic factors for, and progression of dementia. Information from epidemiologic studies provides a means to validate etiologic factors investigated in laboratory research, and can also generate hypotheses to be tested under more controlled conditions. Over the past 10 years, epidemiologic studies have given us new insights into the characteristics and progression of dementia in community dwelling individuals. There is now sufficient data to suggest that dementia may start many years before the subject meets clinical criteria for dementia and that many risk factors for vascular disease also increase the risk for AD. However, there are several 'traps' of (mis) interpretation of epidemiologic data that exist because of the nature of the dementing process, including reverse causality, selective survival, a changing external environment, individual variability in disease and aging. These issues will be discussed in the context of identifying risk factors for AD.

Hypertension and Dementia

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Hypertension has been related to the development of Alzheimer disease and vascular dementia in old age. One possible explanation for the association between hypertension and dementia is that hypertension is a risk factor for cerebral infarcts and ischemic subcortical white matter lesions. Hypertension is also related to factors such as overweight, atherosclerosis, smoking and diabetes mellitus, which all may be involved in the etiology of dementia. Hypertension may also give rise to vessel wall changes in the brain, which may lead to hypoperfusion and blood-brain barrier dysfunction, which both have been suggested to be involved in the aetiology and pathogenesis of Alzheimer's disease. Other possible explanations for the association are shared risk factors, such as disturbances in the renin-angiotensin system, psychological stress, and the formation of free oxygen radicals. Finally, it is a possibility that Alzheimer changes in middle life may induce high blood pressure. The association between blood pressure and dementia is however complex. High blood pressure preceeds dementia by decades, while blood pressure is generally lower in individuals with manifest dementia. Low blood pressure is often found in individuals with manifest dementia. Furthermore, it is debated whether high blood pressure in middle age may be more related to dementia than late-life high blood pressure. The findings of an association between hypertension and dementia may have implications for prevention and treatment. Hypertension in patients with dementia should be treated according to guidelines. Hypertensive demented patients with stroke should be treated aggressively due to an increased risk of new strokes in these patients. There is no evidence that treatment of hypertension according to guidelines may increase the risk of dementia due to lowering of cerebral blood flow. Five placebo-controlled studies on hypertension have been conducted so far with dementia or cognitive function as outcomes. Only the SYST-EUR trial showed a decreased incidence of dementia in the treatment group, although some hypertension trials showed a positive effect in subgroups of patients in post-hoc analyses. However, even if several of these studies have been large, there are a number of methodological problems that makes results so far difficult to interpret.

NOTES

POSTER SUMMARY

I

GENETIC AND LIFESTYLE DETERMINANTS OF DEMENTIA

S.Rovio MSc[1], I.Kåreholt PhD[1], T.Ngandu BM[1], B.Winblad MD, PhD[1], J.Tuomilehto MD, MpolSc, PhD[3], A.Nissinen MD, PhD[3], H.Soininen MD, PhD[2], A.Cedazo-Minguez MD, PhD[4], M.Kivipelto MD, PhD[1]

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Background: The apolipoprotein E (ApoE) ε4 allele is the most important genetic risk factor for Alzheimer's disease (AD) currently known. Evidence also links lifestyle to dementia/AD. However, the interactions between lifestyle and ApoE ε4 are unclear. **Methods:** In a population based study persons were investigated in 1972, 1977, 1982, or 1987. 1449 survivors (73%) participated in a re-examination in 1998 (mean follow-up, 21 years). 61 persons had dementia, 48 had AD.
Results: ApoE ε4 was an independent risk factor for dementia/AD adjusting for lifestyle and vascular factors (OR 2.83, 95% CI 1.61-4.97 for dementia). Alcohol drinking, smoking, and low to moderate intake of polyunsaturated fats (PUFA) at midlife were associated with increased risk of dementia/AD only among the Apoe ε4 carriers. Physical inactivity and moderate to high intake of saturated fats (SFA) were associated to increased risk of dementia/AD especially among ApoE ε4 carriers. **Conclusions:** Lifestyle factors were associated to an increased risk for dementia and AD especially among the ApoE ε4 carriers. ApoE ε4 carriers may be more vulnerable for lifestyle factors. Thus, lifestyle interventions may modify the risk of dementia, particularly among the genetically susceptible individuals.

II

SERUM CHOLESTEROL CHANGES AND COGNITION IN THE GENERAL POPULATION

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Background: Although several epidemiological studies indicate that high serum cholesterol at midlife represents a risk factor for cognitive decline/dementia, the relationship between changes in serum cholesterol levels from midlife to late life and cognition is not very clear.
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. **Results:** Cholesterol levels at midlife were significantly different between normal and cognitively impaired subjects (6.68 mmol/l and 7.17 mmol/l, respectively; $p<0.001$). This difference disappeared in late life (5.81 versus 5.9 mmol/l; $p=0.35$). However, the rate of cholesterol level decrease was significantly higher in subjects who became cognitively impaired ($p<0.001$). A faster decrease indicated lower MMSE ($r=0.39$, $r^2=0.15$, $p<0.001$) and word recall tests ($r=0.38$, $r^2=0.15$, $p<0.001$; $r=0.33$, $r^2=0.11$, $p<0.001$) scores at follow-up, even after controlling for age, gender and education.
Conclusions: Serum cholesterol levels tend to decrease over time, significantly faster in persons who become cognitively impaired later in life. This finding may explain the differences between results of studies investigating serum cholesterol and cognitive functioning in late life.

III

Fat Intake at Midlife and Cognitive Impairment Later in Life

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Background: Lifestyle and vascular factors have been linked to cognitive decline and dementia, but the role of dietary fats for cognitive impairment is less clear.
Methods: Participants of the CAIDE study (n=1409) were derived from random, population-based samples studied in midlife and re-examined in late-life (follow-up of 20.9 years). Dietary information was collected with a structured questionnaire and an interview.
Results: Abundant saturated fat (SFA) intake from milk products and spreads at midlife was associated with poorer global cognitive function and prospective memory and with an increased risk of clinical mild cognitive impairment (MCI) (OR 2.36, 95 % CI 1.17-4.74) after adjusting for demographic and vascular factors, other fats and ApoE. On the contrary, high intake of polyunsaturated fatty acids (PUFA) was associated with better semantic memory. Also frequent fish consumption was associated with better global cognitive function and semantic memory. Further, higher PUFA-SFA ratio was associated with better psychomotor speed and executive function. **Conclusions:** Our data suggests that dietary fat intake at midlife affects cognitive performance and the occurrence of MCI in late-life. Thus, dietary interventions may modify cognitive performance and the risk of MCI.

IV

DEMENTIA RISK SCORE - MODEL FOR A PRACTICAL TOOL TO PREDICT DEMENTIA RISK

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Background: Several vascular risk factors are linked to dementia. Risk scores have been developed to predict cardiovascular events, and could help also in identifying individuals at an increased risk for dementia. **Methods:** The population-based CAIDE study includes 1409 participants examined at midlife (mean age 50 years) and 20 years later. Several midlife vascular risk factors were entered into a model, and Dementia Risk Score (range 0-15) was derived based on the sum of beta-coefficients. **Results**: Occurrence of dementia during 20 years follow-up was 4.3 %. Significant predictors of dementia were age, education, hypertension, hypercholesterolemia, and obesity. Dementia Risk Score predicted dementia well (AUC 0.77; 95 % CI 0.71-0.83). The risk of dementia according to the quintiles 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 12-15. With the cut-off $\geq$9, sensitivity was 0.77, specificity 0.63, positive predictive value 0.09, and negative predictive value 0.98. **Conclusions:** Dementia Risk Score is a novel approach for developing a practical tool to predict dementia risk. It highlights the role of vascular factors in the development of dementia, and may help to identify individuals who can benefit from therapeutic interventions.

V

PHYSICAL ACTIVITY AT MIDLIFE AND THE RISK OF DEMENTIA AND ALZHEIMER'S DISEASE

S.Rovio MSc[1], I.Kåreholt PhD[1], E-L.Helkala PhD[2], M.Viitanen MD, PhD[1], B.Winblad MD, PhD[1] , J.Tuomilehto MD, MPolSc, PhD[3], H.Soininen MD, PhD[4], A.Nissinen MD, PhD[3], M.Kivipelto MD, PhD[1, 4]

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Background: Physical activity may help to decrease dementia risk, but epidemiological findings remain controversial. The aim of our study was to investigate the association between leisure time and work-related physical activity at midlife and dementia and Alzheimer's disease (AD). **Methods:** Participants were randomly selected survivors of a population based cohort previously surveyed in midlife. Altogether 1449 persons (73%) aged 65-79 years participated in the re-examination in 1998 (mean follow-up, 21 years); 117 persons had dementia and 76 had AD. **Results**: Leisure time physical activity (≥2 times/week) at midlife was protective against dementia/AD (OR 0.48; 95 % CI 0.25-0.91, OR 0.38; 95 % CI 0.17-0.85, respectively), even after adjustments for sociodemographic, vascular, and lifestyle factors, and ApoE genotype. The associations were more pronounced among the ApoE ε4 carriers. Work-related physical activity was not associated with the risk of dementia/AD.
Conclusions: Leisure time physical activity at midlife is associated with a decreased risk of dementia/AD especially among ApoEε4 carriers. Controversially, work-related physical activity at midlife may not be enough to protect against dementia/AD.

VI

INTERLEUKIN-6-174 G/C PROMOTER GENE POLYMORPHISM AND RISK OF ALZHEIMER'S DISEASE IN FINNISH POPULATION

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Background: Recently, the involvement of the inflammatory response in AD has received considerable attention. A gene coding Interleukin-6 (IL-6) is interesting since IL-6 has been reported to involve in immune functions as well as in AD. An IL-6 -174 G/C promoter polymorphism has proved to alter IL-6 transcription rates and to associate with risk of AD in some studies. We wanted to study whether the 174 G/C promoter polymorphism has involved in AD risk in aged, homogenous Finnish population. **Methods:**We examined the IL-6 polymorphism and the ApoE phenotype in a population based cohort of aged subjects (totally 616 subjects: 66 cases diagnosed with AD and 550 cognitively intact controls). Neuropsychological and clinical examination was made and cognitive functions were screened at follow-up study visits. **Results:** Frequency of G carrier genotype was higher among ApoE 4 negative AD subjects, whereas in the total study group the difference was not significant.
Conclusion: On the basis of previous reports suggesting an impact of IL-6 in AD we investigated whether the IL-6-174 G/C polymorphism is associated with LOAD in our population sample. We found a borderline association between G carrier genotype and risk of AD.

VII

CYSTATIN C AND APOLIPOPROTEIN E POLYMORPHISMS IN ALZHEIMER'S DISEASE.

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Objective: Recent studies have reported a genetic association between the 73 G/A polymorphism within the exon 1 of the Cystatin C (CST3) gene and Alzheimer's disease (AD), with conflicting results. In order to further investigate the possible involvement of CST3 and to clarify its role as risk factor for AD we performed an association study between these polymorphism and Alzheimer's disease. **Materials and methods:** We analyzed a sample of 243 Italian patients with AD consecutively gathered among outpatients at the Department of Neurology, University of Florence and 186 healthy controls. DNA was extracted by standard procedures. PC. The polymorphisms of CST3 and Apo-E genotype were determined in 429 subjects using polymerase chain reactions (PCR) and RFLP methods as previously described. **Results**: After stratification according to age, the GG frequency resulted slightly higher in younger (< 65 years) cases, but far from statistically significant. There was also no evidence of a statistical interaction between the CST3 and ApoE polymorphisms. **Discussion:** Our data from analysis of the CST3 73 G/A polymorphism in an Italian AD sample are in agreement with a major part of the negative-associations found by the other groups, suggesting that this polymorphism does not contribute to an increased risk of developing AD .
Conclusions: Our data suggest that CST3 genetic variant is not a susceptibility factor to AD, nor mitigate the effect of ApoE ε4 allele on AD risk.

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VIII

LDL RECEPTOR IS ASSOCIATED WITH ALZHEIMER DISEASE, CSF TOTAL TAU AND $A\beta_{42}$ LEVELS

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Background: The low density lipoprotein receptor (LDLr) gene locus in the chromosome 19 has been shown to be strongly associated with Alzheimer's disease (AD). Previous studies have suggested some risk haplotypes overrepresented in AD, while data are not consistent.
Methods: We genotyped 419 Finnish AD patients and 465 controls in five SNP sites and investigated whether there is correlation between genotypes and cerebrospinal fluid (CSF) AD biomarkers $A\beta_{42}$ and total tau. **Results:** Single locus association analysis showed that in women with one LDLr specific SNP increased the risk for AD. Genetic risk haplotypes were found in this study. CSF total tau and $A\beta_{42}$ concentrations showed an evidence of connection between LDL receptor gene and AD. **Conclusions:** LDL receptor gene is associated with AD risk and level of AD specific biomarkers in the brain.

IX

POLYMORPHISMS IN NEPRILYSIN GENE AFFECT THE RISK OF ALZHEIMER`S DISEASE IN FINNISH PATIENTS

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Background: Factors that influence expression and activity of the amyloid β peptide (Aβ) degrading enzyme, neprilysin (NEP) towards Aβ are potentially relevant to the etiology of Alzheimer's disease (AD). In this study we examined the genetic association of NEP gene with AD. **Method:** We genotyped 390 AD patients and 468 cognitively healthy controls originating from eastern Finland with seven intragenic SNPs. APOE allele distributions were determined for AD and control subjects. Genotypes of the study groups were compared using logistic regression analysis and haplotype frequencies of the SNPs were estimated using genotype data. **Result:** SNPs, rs989692 and rs3736187, had significantly different allelic and genotypic frequencies (p=0.01) between the AD and the control subjects. The association testing of individual haplotype distribution showed that the haplotype TTCCCCA of NEP gene were significantly overrepresented among AD cases. This haplotype encompassed the T and A allele of SNPs rs989692 and rs3736187, respectively, which were also significantly overrepresented among the AD cohort in the single locus analyses. **Conclusion:** These findings suggest that polymorphisms in the NEP gene increase the risk for AD and support a potential role for NEP in AD among Finnish population.

X

INSULIN DEGRADING ENZYME AND ALPHA-3 CATENIN POLYMORPHISMS IN ITALIAN PATIENTS WITH ALZHEIMER'S DISEASE.

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Background. Among the several attractive candidate genes for late-onset Alzheimer Disease (LOAD), located on chromosome 10q, recent observations provided evidence of an association for insulin degrading enzyme (IDE) and alpha-3 catenin (CTNNA3) genes. To investigate the role of IDE and CTNNA3 as AD-modifying genes, we performed a case-control study on four SNPs *(IDE1: rs3758505, IDE8: rs 4646958;* CTNNA3 *rs 7070570 or 4360 and rs12357560 or 4783)* **Materials and methods:** We analyzed a sample of 302 Italian patients with sporadic AD (210 LOAD, mean age at onset 72.3±4.9; 92 early-onset AD, EOAD, mean age at onset 58.33±5.5) collected at the Department of Neurology of the University of Florence and 164 sex and age matched healthy controls. **Results:** The distributions of each SNP analyzed followed the Hardy-Weinberg equilibrium in all groups. The genotype frequencies were similar in the two AD subgroups and they did not differ comparing LOAD with EOAD or with respect to controls, except for a statistically significant difference (p=0.04) observed in CTNNA3-rs12357560 for the GG genotype Haplotype distributions of the two markers for each gene, were not statistically different between the AD and control groups. **Conclusions: :** Our data suggest that IDE and CTNNA3 do not represent strong independent susceptibility factors for sporadic AD, confirming that they do not contribute directly to the disease, as previously reported.

XI

GENETIC MODIFYERS OF CADASIL?

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Background: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited systemic vascular disorder leading to subcortical vascular dementia. The defective gene is *NOTCH3* in which 137 different pathogenic mutations have been identified. Over 100 Finnish patients in 25 families share the same mutation, R133C. Despite this uniform mutational background the clinical phenotype shows great variability suggesting additional factors modifying the phenotype. We analysed intragenic *NOTCH3* polymorphisms, the *apolipoprotein E* genotype and *angiotensinogen* M235T polymorphism to clarify their possible role in the phenotypic variation.
Methods: 119 patients were included in this study. 103 carried the predominant R133C mutation and 8 had other *NOTCH3* mutations in exons 3-5. For 8 patients mutation is currently unknown, but diagnosis is confirmed by electron microscopy. Eight intragenic amino acid changing *NOTCH3* polymorphisms were tested in 14 CADASIL-patients with late onset or mild symptoms and 13 patients with early onset or severe symptoms. All 119 were genotyped for apolipoprotein E alleles (ε2, ε3, ε4) by sequencing and for angiotensinogen M235T polymorphism by restriction isotyping.
Results and conclusions: Since polymorphic *NOTCH3* amino acid substitutions were not observed, these are not the cause of phenotypic variation. Genotype data will be set against the patients´ clinical phenotype and the results will be presented.

XII

CSF Aβ42, TAU AND PHOSPHORYLATED TAU, APOE4 ALLELE AND MCI TYPE IN PROGRESSIVE MCI

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Background. The patients with mild cognitive impairment (MCI) have an elevated risk for Alzheimer's disease (AD). Especially the amnestic MCI is seen as a prodrome of AD. APOE4 allele and abnormal CSF Aβ42, Tau and phosphorylated Tau (pTau) levels are associated with elevated risk for AD. **Methods.** 60 controls and 79 MCI patients were examined at the baseline. The APOE genotyping was done by PCR and baseline CSF Aβ42, Tau and pTau were measured by ELISA. During an average of 3.5 years follow-up, 33 MCI patients developed dementia. **Results.** The CSF Aβ42 was decreased and Tau and pTau were increased in the progressive MCI patients. APOE4 allele was more frequent in the progressive MCI group than in the controls or stable MCI patients. APOE4 allele showed a dose dependent association to the Aβ42 levels in the progressive MCI patients and to all of the markers in controls.
Conclusions. The CSF Aβ42, Tau and pTau levels are predictive for progressive MCI. The APOE4 allele increases a risk for dementia in MCI patients and it also affects the CSF marker levels. Amnestic MCI and executive MCI patients are equally likely to develop dementia.
The study was supported by the Academy of Finland and the Kuopio University Hospital EVO grants.

XIII

LACUNAR INFARCTS WITH AND WITHOUT MCI: A VOXEL-BASED MORPHOMETRY AND NEUROPSYCHOLOGICAL STUDY

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Objective: Investigate what proportion of first-ever lacunar infarct (LI) patients can be classified as cognitively impaired using a neuropsychological examination on the basis of the operationalized criteria for MCI-V and to determine which of the studied variables including cognitive status (the degree of subcortical pathology as well as global and regional gray matter volumes), best discriminte patients classified as MCI-V from LI patients without cognitive impairment. **Methods:** Fourty first-ever LI patients were assessed neuropsychologically and classified into two groups according to the presence (MCI-V) or absence (Non-Cognitively Impaired-Vascular, NCI-V) of cognitive dysfunction. Subcortical vascular lesions were assessed from MRI by visual inspection. Cerebral regional gray matter volumes were compared by means of the voxel-based morphometry procedure.
Results: 55% of lacunar syndrome patients could be classified as MCI-V. LI patients classified as MCI-V performed worse than NCI-V on language, visuoconstructive, attentional, motor and premotor functions. They also had more subcortical hyperintensities mainly in the basal ganglia and thalamic regions. VBM analyses demonstrated that the MCI-V group had more atrophy in the left hemisphere including the hippocampus, parietal and temporal lobes and the cerebellum as well as in the right parahippocampal gyrus and right midle temporal region. Negative correlations between hyperintensities in subcortical regions and regional cerebral atrophy were only found in the MCI-V group. **Conclusions:** Present findings indicate that a significant proportion of LI can be classified as MCI-V using an neuropsychological evaluation. MRI differences were found between groups, the amount of subcortical damage in the basal ganglia and thalamic regions as well as the global gray matter atrophy were the main characteristics associated to the MCI-V subgroup.

XIV

MAPPING GRAY MATTER LOSS WITH VOXEL-BASED MORPHOMETRY IN MILD COGNITIVE IMPAIRED CARRIERS OF APOE ALLELE E4

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Background: Mild Cognitive Impairment (MCI) has attracted considerable interest as a potential predictor of AD, while the ApoE allele ε4 is the most consistently confirmed genetic risk factor for Alzheimer's disease (AD). **Methods:** We investigated the effect of apolipoprotein E (ApoE) on the whole brain in 51 individuals with (MCI) using voxel-based morphometry (VBM). **Results:** Between cases heterozygous for the ApoE ε4 (n=15) and those who were ApoE ε4 noncarriers (n = 28), only the right parahippocampal gyrus, with entorhinal cortex included, reached the level of statistical significance. In cases homozygous for the ε4 allele (n = 8) vs. noncarriers the greatest atrophy was located in the right amygdala followed by the right parahippocampal gyrus, the left amygdala and the left medial dorsal thalamic nucleus, similar to the atrophy found in individuals with MCI vs. controls, when the effect of ApoE polymorphism was not controlled for. **Conclusions:** The vast majority of the brain atrophy observed in individuals with MCI appears to be due to the small group of homozygous for ε4 allele, wich is of interest considering that ApoE allele ε4 was shown to be a risk factor of conversion to MCI in normal aged subjects.

XV

ALTERED CORTICAL fMRI ACTIVATION PATTERNS DURING ACTIVE AND PASSIVE CONDITIONS IN MILD COGNITIVE IMPAIRMENT

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Background: Functional magnetic resonance imaging (fMRI) is a feasible tool to investigate the early alterations in brain activation patterns in mild cognitive impairment (MCI) and Alzheimer's disease (AD). **Methods:** fMRI was performed in 21 healthy controls, 14 subjects with MCI, and 15 with mild AD. The activation paradigm comprised encoding of novel word-picture pairs and was contrasted to visual fixation. **Results:** The MCI subjects, when compared to controls, presented increased fMRI responses to novel visual stimuli (active) bilaterally in the fusiform gyrus and insula, and in frontal cortical areas. In the baseline-encoding comparison (passive), both the MCI and AD subjects showed diminished activation compared to controls in the medial parietal and posterior cingulate cortices. **Conclusions:** These fMRI results provide evidence of clear alterations in the function of cortical networks in MCI and AD during both active and resting states. The altered fMRI activation findings in areas such as the fusiform and posterior cingulate cortices, which are tightly connected to the MTL, are likely to reflect the evolving dysfunction of the MTL memory structures.

XVI

BIODISTRIBUTION AND RADIATION DOSIMETRY OF THE AMYLOID IMAGING AGENT ^{11}C-PIB IN HUMANS

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Background: Alzheimer's disease (AD) patients have shown increased uptake of the positron emission tomography (PET) amyloid imaging tracer [^{11}C]PIB in many brain regions typically affected by amyloid beta (Aβ) aggregates. To contribute to validating the use of [^{11}C]PIB as a research tool in AD, we studied the human body biodistribution and radiation dosimetry of the tracer. **Methods:** 12 source organs were investigated in 13 healthy volunteers. An i.v. injection of av. 489 MBq of [^{11}C]PIB was given and dynamic emission scans were performed up to 40 min. Region-of-interest (ROI) analysis was applied to all identifiable organs in the PET images. Time-activity values for blood were measured from arterial samples, and the urinary bladder and washout values were estimated from the excreted urine. Residence times for each organ were calculated from the time-activity curves and the MIRD-method was used to estimate the radiation exposure. **Results:** Highest absorbed doses were received by the gallbladder wall (0.042 mGy/MBq), liver (0.019), urinary bladder wall (0.017), kidneys (0.013) and upper large intestine wall (0.009). Renal clearance accounted for approximately 20% of the injected activity. The whole-body radiation exposure was 0.005 mSv/MBq. **Conclusions:** An adequate amyloid-imaging PET dose of [^{11}C]PIB results in an acceptable effective radiation dose. For example, a [^{11}C]PIB injection of 500 MBq causes an exposure of approximately 2.5 mSv. The compound is largely cleared from the body by the kidneys. These results support the use of [^{11}C]PIB as a research tool in patients and healthy volunteers.

XVII

VOXEL BASED ANALYSIS OF AMYLOID LIGAND [^{11}C]PIB UPTAKE IN ALZHEIMER'S DISEASE

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Background: Positron emission tomography (PET) studies with [^{11}C]PIB have revealed an increased tracer uptake in several brain regions in patients with Alzheimer's disease (AD). In this study our aim was to employ voxel-based analysis method to identify brain regions with statistically significant increases in [^{11}C]PIB uptake in AD as compared to controls, indicative of increased amyloid accumulation in these regions. **Methods:** Seventeen AD patients and 11 controls were studied with a 90-minute PET scan using [^{11}C]PIB as tracer. Parametric images were computed by calculating a region-to-cerebellum ratio over 60 to 90 min in each voxel. Group differences in [^{11}C]PIB uptake were then analysed with statistical parametric mapping (SPM) and automated region-of-interest (ROI) analysis. **Results:** SPM analysis showed significantly increased uptake ($p < 0.001$) in the frontal, parietal and lateral temporal cortices, as well as in the posterior cingulate and the striatum. No significant differences in uptake were found in the primary sensory and motor cortices, primary visual cortex, thalamus and the medial temporal lobe. These results were supported by automated ROI analysis, with most prominent increases in the frontal cortex ([^{11}C]PIB uptake 163% of the control mean, $p < 0.001$) and posterior cingulate (146%, $p < 0.001$) followed by the parietal (146%, $p < 0.001$) and temporal (145%, $p < 0.001$) cortices, putamen (133%, $p < 0.001$) and caudate (133%, $p = 0.004$), as well as small increases in the occipital cortex (117%, $p < 0.01$) and thalamus (115%, $p < 0.01$). **Conclusions:** The distribution of increased [^{11}C]PIB uptake in AD seen in this study is in accordance with the distribution and phases of amyloid pathology in AD, previously documented in post mortem studies.

XVIII

FAMILY CAREGIVERS' DIARIES AS A PART OF THE MULTIDISCIPLINARY INTERVENTION STUDY ALSOVA

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Background Family caregivers have the main responsibility of caring their loved ones with Alzheimer's disease. It is important to elicit the subjective viewpoints of family caregivers to capture the multidimensionality and dynamic nature of the caring process.
Methods This study is a part of ongoing intervention study ALSOVA in Finland (patients n= 221 and caregivers n=221). The family caregivers wrote unstructured diaries (n=81) for two weeks during years 2002-2004, within six months after the diagnosis of Alzheimer's disease in a family member. The second diary (n=45) was written one year after the first diary-writing period. Diaries were interpreted using the method of qualitative content analysis.
Results The diaries described the family caregivers' experiential world and chances taking place in it. In the first year diaries caregivers tried to understand what Alzheimer's disease is about. The diaries produced knowledge caregivers' motivations to care and efforts at the different stages of loved ones illness. **Conclusions** Diaries produce subjective knowledge of the experiences, emotions and meanings associated with caregiving. The family caregivers found diary writing a pleasant and therapeutic experience.

XIX

AMYLOID PLAQUES, CEREBRAL AMYLOID ANGIOPATHY AND CEREBRAL WHITE MATTER LESIONS IN AN AUTOPSY BASED STUDY

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Background: White matter lesions (WML) are frequently found in patients with dementia. Their presence is partly attributed to vascular risk factors, but other factors must play a role. Such a factor may be the presence of amyloid plaques and angiopathy, that has also been associated with the presence of WML. A previous population-based study has investigated the relation between plasma levels of amyloid β (Aβ) and WML on MRI. However, circulating levels of Aβ may not reliably reflect the actual degree of "amyloid neuropathy" ***in*** the brain.
Objective: To investigate the relation between amyloid plaques, angiopathy in brain tissue and WML on neuroimaging.
Methods: We investigated 29 consecutive non-demented patients from whom their brains came to autopsy with a recent (<3 months) cerebral CT or MRI scan.
Post-mortem biopsies were taken in a standardized way of the right frontal cortex. Tissue was fixed in formalin and embedded in paraffin. A β was demonstrated immunohistochemically on 4 µm thin sections with a monoclonal mouse anti Aβ antibody. Total Aβ staining in both plaques and arterioles was measured by an automated quantification system by two independent raters in a 5000 µm x 5000 µm area and expressed as the percentage of the total surface. WML were rated with the age related white matter changes scale (ARWMC).
The relation between amyloid plaques, angiopathy and WML (ARWMC > 0) was assessed with the Mann-Whitney U test.
Results: Mean age was 63.6 years (SD 16.0) and 41% were female. 45% had WML. The proportion of amyloid in plaques and arterioles varied from 0.0 to 1.8% and 0.0 to 0.2%, respectively. Patients with WML had significantly higher relative proportion of amyloid plaques compared to those without (0.01% (SD 0.02) vs 0.30% (SD 0.54); p=0.01). This was not found for cerebral amyloid angiopathy.
Conclusion: Patients with WML had an increased burden of amyloid plaques in their brains. This may support heterogeneity in the etiology of WML.

XX

OXIDATION OF CEREBROSPINAL FLUID PROTEINS IN AGING AND ALZHEIMER'S DISEASE

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Background: Response mechanisms to increased oxidative stress vary depending on apolipoprotein E genotype and gender. An increase in oxidative stress also plays a role in neurodegenerative disorders such as Alzheimer's disease (AD). Several oxidised proteins have been identified in plasma and brain of AD patients. **Methods:** In this study, we characterised the levels and oxidation status of the most abundant cerebrospinal fluid proteins in aging women, men, AD patients and controls. Oxidised proteins were analysed by two-dimensional gel electrophoresis, oxyblotting, mass spectrometry, and database searches. **Results and conclusions:** The levels of several proteins were decreased in AD patients as compared to controls as well as in apolipoprotein E ε4 carriers as compared to non-carriers. The oxidation status of only one identified protein, lambda chain precursor, was found to be increased in AD patients and in apolipoprotein E ε4 carriers. The levels of proteins did not generally differ between men and women. However, vitamin D-binding protein, proapolipoprotein and alpha-1-antitrypsin precursor exhibited higher extents of oxidation in men.

XXI

A LINK BETWEEN BRAIN ENERGY METABOLISM, MICROTUBULAR ACETYLATION AND TAU EXPRESSION

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Background. Disturbance in energy metabolism, as well as loss of microtubular stability and Tau pathology is implicated in many neurodegenerative disorders. NAD is important metabolic regulator. Previously we have observed that NAD-dependent tubulin deacetylase SIRT2 is highly expressed in astrocytes. It is widely accepted that in the brain astrocytes provide metabolic support to neurons in response to neuronal activity. The amount of phospho-Tau correlates with pathogenic conditions, age and animal life cycles (e.g. hibernation) - in fact, with alterations in metabolism. **Methods.** Here we studied microtubular acetylation status by using a SIRT2 overexpressing astrocytoma cell line. Primary hippocampal neurons were treated with various metabolic regulators and HDAC inhibitors. As a model for neurons under impaired metabolic conditions we used primary rat hippocampal neurons at day 13 post plating. Protein levels were examined by WB. Changes in energy metabolism were monitored by intracelular NAD as well as NAD+/NADH ratio. **Results.** In a model of nutritional stress SIRT2 overexpression can reduce microtubular acetylation. Extracellular glucose levels modify tubulin acetylation in co-cultured astrocytes and neurons. Nicotinamide, a substrate for NAD synthesis and an inhibitor of SIRT2, can dramatically increase Tau levels even under severe metabolic stress. **Conclusions.** Intact energy metabolism is a main regulator of cytoskeletal structures. Impaired metabolism drives to cytoskeletal lesions such as loss of microtubular acetylation and Tau accumulation.

XXII

LOCALIZATION OF THE STRUCTURAL AND FUNCTIONAL COMPONENTS OF THE HUMAN SIRT2 GENE PROMOTER

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Background. Disturbance of cetoskeleton is considered to be a major cause of various neurodegenerative conditions. Mammalian SIRT2 can deacetylase tubulin, which makes SIRT2 an attractive research target in neuroscience. Little is still known about regulation of SIRT2 transcription. Studying of SIRT2 promoter and functions can provide a tool for pharmacological interventions on the transcriptional level. **Methods.** Cloning of genomic fragments was performed after their PCR amplifications. The minimal promoter region was dissected by series of nested deletions. The ability of certain proteins to bind promoter DNA subfragment was studied by EMSA. DNA-affinity protein purification was employed to determine DNA-binding proteins. **Results.** Fragments of human SIRT2 promoter, varying in size from 3 kb down to 0.6 kb, were sequentially cloned into a luciferase reporter plasmid. We located minimal SIRT2 promoter on the genomic fragment 0.6 kb in size. The fragment was sufficient to drive both basal transcription and trichostatin-induced enhanced transcription of the reporter gene. We then showed which particular subfragments in promoter region are important for promoter functions and what possible DNA-binding proteins contribute to activation of SIRT2 promoter. **Conclusions.** SIRT2 transcription is significantly enhanced in responce to tubulin hyperacetylation. The SIRT2 promoter subsequences important for transcription activation were uncovered. One possible DNA-binding protein contributing to promoter regulation was shown to be PARP. The search for other regulatory proteins is underway.

XXIII

POTENTIATION OF MICROGLIAL INFLAMMATORY RESPONSE BY PROTEIN ACETYLATION

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Background: Inflammation and innate immune reactions are involved in the pathology of several neurodegenerative diseases, such as Alzheimer's disease. Inflammation is a defence reaction against diverse external and internal insults and hence is normally beneficial but, as excessive one, has deleterious effects in brain. Microglial cells are the residential macrophages in brain, and the microglial activation process itself, as well as the stimulators and inhibitors involved, have been intensively studied. Currently, we have studied whether protein acetylation status regulates the extent of microglial activation. It is known that several environmental stresses, aging and diet, which are risk factors for Alzheimer's disease, are involved in the regulation of the protein acetylation status and thus might regulate the extent of inflammatory responses. **Methods:** Several neural inflammation models, such as mouse N9 and rat primary microglia, neural co-cultures and hippocampal slice cultures were exposed to well known histone/protein deacetylase (HDAC) inhibitors and NF-kB and PI3K signalling inhibitors. **Results:** We observed that the increase in protein acetylation induced by HDAC inhibitors, such as trichostatin A (TSA), potentiated the LPS-induced inflammatory responses. NF-kB signalling inhibitors, helenalin and CAPE, inhibited the induction. TSA-induced potentiation was inhibited e.g. by PI3K inhibitor LY294002, LiCl2 and dexamethasone. **Conclusions:** It seems that environmental stress, aging and diet might regulate microglial activation via protein/histone acetylation.

XXIV

ACTIVITY OF siRNA AND DNAzymes 10-23 IN HUMAN FIBROBLASTS WITH MUTATIONS IN PRESENILINS 1 AND 2

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Background. Sequential processing of amyloid precursor protein (APP) by membrane-bound proteases, beta- and gamma-secretases, results in formation of beta-amyloid peptides (Aβ). The Aβ peptides are the main components of amyloid plaques playing a crucial role in the pathogenesis of Alzheimer disease. It has been already shown that down-regulation of beta-secretase (BACE1) (1-4) and of gamma-secretase (presenilin 1, PS-1) (5) leads to the lowering of the extra- and intracellular level of the beta-amyloid peptides Aβ40 and Aβ42. **Methods.** In the presented work we evaluated an activity of the two short interfering RNAs (siRNAs) as well as two deoxyribozymes 10-23, directed towards beta-secretase, in human fibroblasts that contain mutated presenilins 1 and 2. These two cell lines were obtained from patients with Alzheimer's disease, immortalised with EBV virus. The level of the beta-amyloid peptides released into cellular medium of 14UDS and 4NOV cell lines grown in 6-well plates was determined by an enzyme-linked immunosorbent assay using Beta amyloid ELISA kits 1-40 and 1-42 (Signet Laboratories, USA). **Results.** In PS-2 mutation-containing fibroblasts (14UDS) the level of Aβ40 was lowered to ca. 50% by siRNA-1 and DNAzyme-1 and to > 99% by DNAzyme-2. Interestingly, the latter inhibitor effectively diminished the level of Aβ40 in PS-1 mutated cells (4NOV) up to ca. 50%. However, no considerable changes in the level of Aβ42 in both tested cell lines transfected with siRNAs and deoxyribozymes were observed suggesting that this Aβ42 concentration is undetectable by ELISA test used in experiment (<15 pg).

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XXV

NOVEL METHOD FOR MEASURING $[CA^{2+}]_I$ ON ACUTE HIPPOCAMPAL SLICES WITH FLUORO-PLATE-READER

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Glutamate is a very important factor in Alzheimer's disease neuropathology. Increasing the intracellular calcium concentration ([Ca2+]i) with glutamate causes apoptosis of the neurons. We measured [Ca2+]i with Fluo3-AM fluorescent dye on acute hippocampal slices using fluoro-plate-reader with matrix well scanning function (10x10); the slices were treated with 150 microM glutamate. These slices were 0,3 mm thick, approximately 8-10 cell-layers. The fluorescent signal kinetics was different from classic calcium peak, because of the different layers' signal addition. This, *ex vivo*, method is able to screen the effect of the potential Ca^{2+} influx inhibitors against glutamate. By using other fluorescent dyes is possible to measure any cell-physiological events in hippocampal slices.

XXVI

MUTANT HUMAN EXON 1 HUNTINGTIN CAN PROTECT PC12 CELLS FROM QUINOLINIC ACID INDUCED CELL DEATH

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Background: The R6/2 mouse model of Huntington´s Disease overexpresses Exon1 of mutant human huntingtin with 142 glutamines. Medium sized spiny neurons in these mice are resistant to NMDA receptor-mediated excitotoxicity. In contrary, mice overexpressing Exon1 of human huntingtin with a shorter glutamine stretch are not resistant. The aim of this project is to find out mechanisms, which might explain the resistance of R6/2 medium spiny neurons against NMDA receptor mediated excitotoxicity. **Methods:** To confirm the resistance phenomenon in vitro, PC12 cells transfected with an inducible vector containing human Exon1 huntingtin with either 23 or 74 glutamine repeats fused to enhanced green fluorescent protein were used. Cells were treated after differentiation with different concentrations of quinolinic acid and stained afterwards for cell death. **Results:** Nuclear aggregates were only formed in PC12 cells overexpressing huntingtin with a 74 glutamine repeat as confirmed by the eGFP signal. Preliminary data suggests that cells expressing huntingtin with a 74 glutamine repeat are resistant against quinolinic acid. As expected, all cells expressing huntingtin with a 23 glutamine repeat died after treatment with quinolinic acid. **Conclusions:** Alterations of huntingtin caused by an elongated polyglutamine stretch have the potential to protect PC12 cells from quinolinic acid induced cell death. The reasons underlying this phenomenon are not yet known and have to be further investigated.

XXVII

DIVERGENT EFFECTS OF AB1-42 ON THE IONOTROPIC GLUTAMATE RECEPTOR MEDIATED RESPONSES IN CA1 NEURONS IN VIVO

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Changes in the synaptic activity are detectable early in Alzheimer's disease, long before significant cell loss manifests. Despite the fact, that Aβ1-42 interference with long term potentiation (LTP) and fEPSP is well documented, the exact mechanisms are needed to clarify. It is suggested, that disruption of LTP is in direct connection with the modulation of ionotropic glutamate receptors, NMDA, AMPA and KA by Aβ1-42.
The effects of Aβ1-42 on the ionotropic glutamate receptor-evoked neuronal firing at the CA1 hippocampal region in vivo was studied by extracellular single-unit recordings combined with microiontophoresis. Neurons were excited by repetitive ejection of either NMDA and AMPA or NMDA and KA, while Aβ1-42 was delivered iontophoretically. As previously described, the NMDA-induced firing increased. In contrast, AMPA evoked responses completely diminished either immediately, or within 30-35 min. There were also two sets of cells considering KA evoked responses: responses disappeared almost completely due to Aβ administration, or remained unchanged after Aβ1-42 ejection. A protective pentapeptide, LPYFDa protected against the NMDA and AMPA response modulatory effect of Aβ1-42. The decrease of AMPA and KA mediated responses can explain the lack of LTP and the decrease of fEPSP seen in Aβ1-42 treated animals.

XXVIII

AMYLOID-BETA 1-42 INDUCES VACUOLIZATION IN HUMAN ASTROCYTES BUT NOT IN RAT ASTROCYTES – IMPLICATION FOR TRANSGENIC ANIMAL MODELS

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Background: Effects of amyloid-beta (A-beta) on glial cells have mostly been studied using rat astrocytes although species differences have been described. Our purpose was to find out whether there are differences in astrocytic responses to A-beta between human and rat astrocytes. **Methods:** Human normal astrocytes (NHA), human CCF-STTG1 astrocytoma, rat primary astrocytes and immortalized rat astrocytes (RAIM) were exposed to human amyloid peptides and fibrils at 5 µM concentration in cell culture. **Results:** (1) Exposure to human A-beta1-42 peptide or fibrils induced cytosolic vacuoles in human astrocytes. Vacuoles appeared 9-12 h after the exposure. Rat astrocytes did not show this type of vacuolar response. Human A-beta1-16, A-beta1-28, A-beta10-20, A-beta17-21 and A-beta25-35 did not cause vacuolization in human or rat astrocytes. (2) Electron microscopic observations showed membrane-bound large vacuoles resembling the dilatations of endoplasmic reticulum. (3) Stress marker analysis showed a huge increase in ApoJ/clusterin protein expression after the exposure to A-beta1-42 in human astrocytes. This response appeared 6 h after the exposure and continued increasing up to 24 h. Other peptides did not induce this response. HSP70 and HSP90 were not affected. (4) A-beta1-42 fibrils attached strongly to human astrocytes but not as effectively to rat astrocytes. **Conclusions:** Vacuoles probably originated in endoplasmic reticulum suggesting that A-beta1-42 induces ER stress in human astrocytes but not in rat astrocytes. The observed difference between human and rat astrocytes may have implications for interpretations of transgenic animal models.

XXIX

EFFECT OF SHORT PEPTIDES ON AB(1-42) FIBRILS.

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Short peptides like Leu-Pro-Phe-Phe-Asp (LPFFD) and Leu-Pro-Phe-Tyr-Asp-amide (LPFYDa) can influence the structure and aggregation of β-amyloid peptides. Soto's pentapeptide LPFFD has been published as a β-sheet breaker (BSB). Different methods (transmission electron microscopy, atomic force microscopy, dynamic light scattering, thioflavin-T binding, diffusion ordered NMR spectroscopy, circular dichroism, FT-infrared spectroscopy) have been used for the analysis of Aβ-structure. We have found that both peptides can cause small conformational changes of Aβ, however, they can not prevent completely the formation of Aβ fibrils in 50-100 micromolar concentration using 1:1 molar ratio of Aβ and the BSB peptide.

XXX

MAPPING RISK FACTORS FOR ALZHEIMER'S DISEASE: CHOLESTEROL IS AFFECTING BRAIN GENE EXPRESSION

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Background: Alzheimer's disease (AD) is today affecting an increasing number of elderly people, with memory loss and cognitive deficits as major symptoms. The cause of the disease is not known, but several risk factors have been suggested, both genetic and environmental. Different factors combined are likely to increase or decrease the risk of developing the disease, which makes it complex to study. Some important risk factors are high cholesterol levels in mid-life and the presence of the apolipoprotein E ε4 allele (apoE4). Today it is not known by which mechanisms these risk factors work. **Method:** To investigate how hypercholesterolemia increases the risk for neurodegeneration we used brain tissue from mice that received a high cholesterol (HC) diet for nine months. We then analyzed changes in gene expression by cDNA microarray. Several candidate genes have been selected and will be investigated further on protein level. **Results:** We have found that the HC diet affect several genes with a known connection to AD, and also other genes that indicate a possible link to the pathogenesis or pathophysiology of AD. Genes involved in cholesterol metabolism, inflammation, apoptosis and oxidative stress are now investigated further. **Conclusions:** This type of study where a large amount of genes can be studied in parallel is valuable to get an overview of what mechanisms are going on in an organism. By connecting these we can get knowledge to define new mechanisms involved in risk factors for AD and to identify new targets for drug treatment.

XXXI

EFFECTS OF TESTOSTERONE AND AROMATASE ENZYME INHIBITION ON HIPPOCAMPAL LONG-TERM POTENTIATION IN ORCHIDECTOMIZED MICE

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Background. Orchidectomy (ORCH) has been reported to decrease the spine synapse density in the CA1 area of male rodent hippocampus. Conversely, the spine synapse density has been restored in ORCH rodents treated with testosterone. These effects are strikingly similar to those found in ovariectomized females treated with estradiol. Estradiol's effects on functional synaptic plasticity, such as long-term potentiation (LTP) in CA1 are well documented, whereas findings with testosterone have been less uniform. **Methods.** To address the question of whether testosterone's possible effect on hippocampal LTP is dependent on estrogen via biosynthesis from testosterone, we compared the effects of testosterone alone or combined with an aromatase inhibitor, letrozole. Male C57BL/6J mice were orchidectomized at the age of 8-10 weeks. 1 week later the mice were treated with either sesame oil or testosterone (100 microg./d, s.c., 2 d) alone or combined with letrozole (200 microg./d, s.c., 2 d). 48 hours after the last injection standard hippocampal slices were prepared. The extracellular responses to stimulation of the Schaffer collaterals were recorded in the stratum radiatum of the CA1 area and LTP was induced by 100 Hz theta burst stimulation. **Results.** Testosterone alone and combined with letrozole enhanced LTP to the same extent, whereas letrozole alone did not differ from the vehicle. **Conclusions.** These data support the idea that the effect of testosterone on hippocampal synaptic plasticity is largely independent of its conversion to estradiol.

XXXII

NITRIC OXIDE AND DOPAMINERGIC SYSTEM IN AN ANIMAL MODEL OF ALZHEIMER'S DISEASE

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Background: In the aetiology and pathogenesis of Alzheimer's disease (AD) a critical role is played by βeta-Amyloid (βA). Considerable efforts have been made to identify the cytotoxicity of βA, as well as neurochemical and behavioural sequelae associated with βA exposure. Furthermore, recent evidence suggests that nitric oxide (NO) may be involved in neuronal cell death in AD. **Methods:** In the present study, we explored the neuromodulatory role of NO on the neurochemical sequelae associated with βA exposure in rat prefrontal cortex (microdialysis technique). **Results:** The study represents our first attempt to investigate whether subchronic in vivo exposure to 7-nitro indazole (7-NI) or L-Arginine (Arg) would induce changes in the extracellular concentrations of dopamine (DA) in βA treated-rats. We measured the release of DA before, during and after βA administration by using in vivo Extracellular concentrations of DA were significantly lower in Arg-treated animals than that in vehicle-treated rats. The decrease in DA levels remained statistically significant for all detection period. 7-NI administration significantly increased DA levels with respect to the control group (P=0.041). The increase was observed for all collection time. **Conclusions:** These results suggest that dopaminergic transmission is affected by the NO production in βA-induced animal model of AD. Moreover, understanding the role of the nitrergic system in the neurochemical changes related to neuropathological features of AD might be of therapeutical importance in the treatment of this neurodegenerative disease.

XXXIII

EVALUATION OF MEMORY DEFICIT INDUCED BY BETA-AMYLOID IN A RAT MODEL

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Background: The underlying pathological cause of Alzheimer's disease has been postulated to be the toxic fibrillar form of beta-amyloid (Aβ). Our aim was to establish a rat model in which the injection of oligomeric form of the Aβ induces a memory deficit. This model will serve to test drug candidates blocking the formation of Aβ fibrils. **Methods:** Aβ1-42 solution, NMDA or physiological saline was injected bilaterally into the entorhinal cortex (EC) of adult male Wistar rats. Behavioral tests started two weeks after the operations. Short-term memory was assessed by a Y-maze spontaneous alternation task, long-term recognition memory by an object recognition test, and long-term spatial memory in a Morris water maze. **Results:** Injection of Aβ1-42 or NMDA into the EC significantly impaired the performance of rats in the object recognition test. All groups behaved similarly in the Y-maze and in the Morris-maze. **Conclusion:** Our results show that injection of Aβ1-42 into the EC deteriorates long-term recognition memory, therefore it is an appropriate model for testing drug candidates against toxic effects of oligomeric Aβ. Histological experiments are under way in order to explore the mechanisms underlying behavioral impairments.

XXXIV

MODULATION OF CEREBRAL BLOOD VOLUME AND BETA AMYLOID DEPOSITION IN AGED ALZHEIMER APP/PS1 MICE SUPPLEMENTED WITH DOCOSAHEXANOIC ACID (DHA) AND CHOLESTEROL ENRICHED DIETS

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Background. Indications exist that high cholesterol levels or low DHA levels are risk factors for Alzheimer's disease. We investigated the effect of cholesterol and DHA diets on relative cerebral blood volume (rCBV), amyloid beta (Aβ) deposition and sterol and fatty acid profiles in the brains of APP/PS1 double-transgenic AD mice. **Methods**. From 6 months of age APP/PS1 and control wild type mice were fed with a regular rodent chow, Typical Western Diet (TWD) with 1 % cholesterol or diets supplemented with DHA. rCBV in different brain areas has been determined with T2 weighted gradient echo MRI, before and directly after bolus injection of ultra small paramagnetic particles of iron oxide (USPIO). With deuterium magnetic resonance spectroscopy (MRS) techniques, rCBV of the whole brain was determined. Further, Aβ, sterol and fatty acid levels were determined in brain tissue. **Results.** Results show that a diet enriched with 1% cholesterol, increases Aβ plaque burden in the dentate gyrus of the hippocampus of 19 months old APP/PS1 mice, and in addition also tends to decrease rCBV of these TWD(t) mice. No changes in fatty acid profiles or cholesterol could be observed in brain tissue. Whereas, a DHA diet significantly increased cortex rCBV and the n3 fatty acid concentration, indicating increased membrane fluidity. **Conclusions**. Together these results suggest that changes in hemodynamics, such as rCBV, show association with amyloid deposition, indicating a more solid ground for AD as a vascular disorder. To elucidate these findings, experiments have to be repeated earlier in the course of the disease to strengthen the present results and hypothesis.

XXXV

ENHANCED ACOUSTIC STARTLE RESPONSE IN TRANSGENIC MICE CARRYING APP AND PS1 MUTATION

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Alzheimer's disease is associated with a variety of behavioral symptoms in addition to cognitive impairment, and these behavioral symptoms are usually the primary cause for institutionalization of the patients. We have used transgenic mice carrying mutated human amyloid precursor protein (APP) and presenilin-1 (PS-1) genes as a model for the disease. These animals develop amyloid plaques from 5-months of age on and show progressive impairment in their spatial learning ability. We used acoustic startle and pre-pulse inhibition as a measure to the innate reactivity of these mice in comparison to their negative littermates and APP single mutants. We tested these animals at 4, 8 and 12 months of age, and expected the reactivity to dramatically attenuate with age, as these animals had C57BL/6J background with reported age-associated hearing loss. Unexpectedly, we came across a huge individual variability in the startle responses in both genotypes and age groups. In order to clarify the reason for the individual differences we further studied acoustic startle response using different sound pressure levels for the pre-pulse and the main pulse. The results will help the interpretation of acoustic startle responses in genetically manipulated animals with C57B6 background.

XXXVI

AGE-RELATED DECLINE OF GLUTAMATE RELEASE IN APP/PS1 TRANSGENIC MICE

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Background. Glutamate toxicity is considered to be involved in pathophysiology of Alzheimer's disease (AD). One of the most distinct pathological hallmarks of AD is extracellular deposition of β- amyloid (A β) plaques in select brain regions. However, little is known about the relationships between A β accumulation and brain glutamate levels. **Methods.** A transgenic mouse line carrying human APPswe/PS1dE9 (A/P) mutation was used determine the critical age for cognitive impairment associated with AD in Morris water maze. Next, the mice were subjected to *in vivo* microdialysis. Both baseline and stimulated (KCl) release of glutamate was measured in A/P mice and their nontransgenic littermates. A parallel microdialysis was conducted in young mice for comparison. **Results.** A genotype related impairment in spatial learning was observed in A/P mice at 15 months of age ($p = 0.001$), but the difference was only marginal at 10 months ($p = 0.06$). Extracellular glutamate levels after KCl stimulation were slightly elevated in young A/P mice compared to wild type controls. In contrast, aged A/P mice failed to respond to KCl stimulation, while many control mice still responded, which resulted in a significant genotype by age interaction ($p = 0.03$). Concurrent infusion of TTX reduced extracellular glutamate levels significantly, showing the contribution of neuronal activity to the measured glutamate concentrations. **Conclusions.** Slightly increased glutamate release in young A/P transgenic mice and robust decrease in stimulated glutamate release at an older age compared to nontransgenic littermates support the idea that amyloid accumulation enhances glutamate toxicity in the brain.

XXXVII

THE ROLE OF α-SYNUCLEIN IN SYNAPTIC GLUTAMATE RELEASE

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Background. α-synuclein is the major component of Lewy bodies, which are the hallmark lesion of Parkinsons disease, dementia with Lewy bodies, and subset of patients with Alzheimer's disease pathology. The present study addresses the question whether α-synuclein has a specific role in dopamine neurotransmission or a more general role in the regulation of neurotransmitter release. **Methods**. We studied synaptic functions in vitro in three groups of mice, A30P transgenic (TG) mice carrying also the mouse endogenous α-synuclein, wild-type (WT) C57BL/6J mice, and mice of the same background with spontaneous deletion of α-synuclein (a-syn knockout, KO). We focused on mossy fiber synapses as these express the highest concentrations of A30P transgene protein in the TG mice. The recording protocol included I/O curve, paired-pulse facilitation and long-term potentiation.
Results and **Conclusions**. The data suggests that the genotypes differ in paired-pulse facilitation ratios, such that paired-pulse facilitation is decreased in both TG and KO mice compared with the WT mice. As paired-pulse facilitation is a measure of presynaptic glutamate release, these data indicate that lack or presence of abnormal α-synuclein impairs the release of glutamate. Thus the role of α-synuclein is not restricted to dopamine synapses.

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XXXVIII

THE SELECTIVE EFFECT OF AGING ON THE SUBCELLULAR DISTRIBUTION OF ESTROGEN RECEPTOR-ALPHA IN THE BASAL FOREBRAIN CHOLINERGIC NEURONS OF TRANSGENIC AND WILD-TYPE MICE

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Background. The degeneration of the basal forebrain cholinergic system plays an important role in cognitive deterioration in aging and Alzheimer's disease. Brain cholinergic neurons and their projections are affected by changes in the circulating levels of estrogens which exert their effects mainly through the estrogen receptors. In this study, we have investigated the effect of aging, estrogen status and transgenic genotype on the number of cholinergic neurons and the estrogen receptor alpha (ERα) content in the medial septum-vertical (MSVDB) and horizontal limb of the diagonal band of Broca (HDB) and nucleus basalis magnocellularis (NBM). **Methods.** Six- and 12-month-old female double transgenic mice carrying mutated human amyloid precursor protein and presenilin-1, and their nontransgenic littermate controls, which had been sham-operated or ovariectomized at the age of 3 months were used in this study. Brain sections were double immunostained for choline acetyltransferase (ChAT) and ERα and used for stereological cell counting. **Results.** We found that the number of ChAT-immunoreactive (ir) neurons containing nuclear ERα-ir in the MSVDB was significantly lower in 12- than 6-month-old mice. However, there were no similar changes observed in the HDB and NBM. The age of the mice, the transgenic genotype or ovariectomy had no effect on the total number of ChAT-ir neurons, or on the number and percentage of all ChAT-ir neurons which contained ERα in all analyzed regions. **Conclusion.** These results indicate that aging selectively modifies the translocation of ERαs from the nucleus to the cytoplasm in the basal forebrain cholinergic nuclei.

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INTERACTION BETWEEN DOPAMINERGIC AND CHOLINERGIC NEURONS AT THE LEVEL OF THE PREFRONTAL CORTEX ON LEARNING AND MEMORY

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Background: Alzheimer's disease (AD) and Parkinson's disease (PD) are characterized by a loss of cholinergic neurons and dopaminergic neurons, respectively. Although, these clinical syndromes have originally been described as two distinct diseases, there is an overlap in clinical and pathological features in these patients. **Methods:** To study a possible interaction between dopamine and acetylcholine neurons, we have lesioned the dopaminergic neurons in the ventral tegmental area (VTA) and the cholinergic neurons in the nucleus basalis magnocellularis (NBM) with 6-OHDA and 192 IgG-saporin, respectively. Five weeks after surgery, the animals were assessed for deficits in learning and memory in the Morris water maze test. **Results:** A significant increase in latency to find the platform was found in VTA lesion alone and NBM+VTA lesion groups, but not in NBM lesion alone group. In addition, we also looked at activity levels under basal conditions and after an injection with a low dose of apomorphine. We saw an increase in activity in the VTA leison alone and the VTA+NBM lesioned animals. No significant difference was found in the skilled paw use test in the staircase between all the groups. **Conclusion:** Our findings suggest that the dopamine neurons in the VTA have an important function in learning. However more research is needed

to further elucidate whether or not an interaction between dopamine and acetylcholine neurons exist at the level of the prefrontal cortex.

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IMPAIRED OLFACTORY DISCRIMINATION IN TRANSGENIC MICE EXPRESSING HUMAN WILD TYPE ALPHA-SYNUCLEIN

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Background: In Parkinson's disease and other neurodegenerative diseases alpha-synuclein (ASY) aggregates to form insoluble intracellular fibrous Lewy inclusions. ASY gene duplication, triplication, and mutations are also linked to familial forms of Parkinson's disease. We have developed a transgenic mouse under ASY promoter overexpressing wild type human ASY. These mice show high expression in immunopositive cells for human ASY mainly in hippocampus, amygdala and piriform cortex. The brainstem, including dopaminergic cells in s.nigra have little expression. **Aim:** To test the idea that overexpression of human ASY may lead to formation of pathological ASY aggregates resulting in neural dysfunction, we conducted a large behavioral test battery on these mice, focusing on the known behavioral correlated of affected brain areas. **Results:** hASY tg mice did not differ from their littermate controls in exploratory activity in a new environment or in the rotarod that assesses motor coordination and balance. They learning of spatial nagivation in Morris water maze and fear conditioning were also similar to control mice. However, hASY tg mice were impaired in an odor discrimination task with four odor pairs, where only one odor of the pair was associated with food reward. **Conclusion:** Olfactory problems have been reported in Parkinson patients independent of dopaminergic dysfunction. The hASY mouse may model the underlying pathology, which we propose to be a direct cause by accumulation of ASY in the olfactory cortical areas and amygdala.

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