UNIVERSITY OF KUOPIO
Department of Neuroscience and Neurology

The Second Kuopio Alzheimer Symposium

Proceedings of the Symposium

March 13.-15. January 2001

Kuopio, Finland

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Tuesday, March 13, 2001

Opening ceremonies

Professor Hilkka Soininen, Department of Neurology, University of Kuopio

Rector Petteri Paronen, University of Kuopio

Pathogenesis I: Chairmen Konrad Beyreuther and Colin Masters

Mechanisms of Degeneration in Alzheimer's Disease - Towards a Rational Therapy
Colin Masters, Australia

A Proteolytic Function of Presenilins
Harald Steiner, Germany

Apolipoprotein E Receptors in Brain Development and Neurodegeneration
Uwe Beffert, USA

Activation of Developmental Programmes in Alzheimer's Disease
Thomas Arendt, Germany

Prevention of Alzheimer's Disease - Experiments and Strategies
Konrad Beyreuther, Germany

Pathogenesis II: Chairmen Khalid Iqbal and Eckhard Mandelkow

The Why, What and How of Alzheimer Neurofibrillary Degeneration
Khalid Iqbal, USA

Tau Protein in Alzheimer's Disease: Phosphorylation and Role in Intracellular Trafficking
Eva Mandelkow, Germany

Mechanism of Tau Aggregation into Paired Helical Filaments and Role of Phosphorylation
Eckhard Mandelkow, Germany

Neuronal Model System for Alzheimer's Disease Neurofibrillary Degeneration
Inge Grundke-Iqbal, USA

The Poster Session

Wednesday, March 14, 2001

Can We Identify Early Alzheimer's Disease?
Martin Rossor, UK

Early Pathology: Chairpersons Irina Alafuzoff and Dennis Dickson

Neuropathology in Aging
Daron G. Davis DG, USA

Neuropathology of Early Alzheimer's Disease
Dennis Dickson, USA

The Biochemical Frontier Between Normal Aging and Alzheimer's Disease
Nicolas Sergeant, France

Pathological Correlates of Dementia in a Community Based Population
Irina Alafuzoff, Finland

Neuroimaging: Chairmen Timo Erkinjuntti and Philip Scheltens

Neuroimaging and the early diagnosis of AD
Mony de Leon, USA

Hippocampal Atrophy: Comparison between Visual Rating and Volumetry
Lars-Olof Wahlund, Sweden

Could MRI Be Used to Measure Progression in AD?
Nick Fox, UK

Functional MRI in Alzheimer's Disease
Philip Scheltens, The Netherlands

PET Activation Studies in MCI and AD Patients
Agneta Nordberg, Sweden

Cognitive Aspects: Chairmen Tuomo Hänninen and Steven Ferris

Cognition in Normal Aging
Yaakov Stern, USA

Item-Specific Measurement of Memory Impairment
Herman Buschke, USA

Concept of Mild Cognitive Impairment
Steven Ferris, USA

Prognosis of Mild Cognitive Impairment
Ronald Petersen, USA

Thursday, March 15, 2001

Therapy I: Chairmen Martin Farlow and Bengt Winblad

The Evolution of Alzheimer's Disease: Candidate Genes
Caleb Finch, USA

Fingerprints of Pharmacogenomics as Indexes of Onset and Progression of Alzheimer's Disease Dementia
Giulio Pasinetti, USA

The Next Generation of AD Therapeutics: The Future is Now
Kevin Felsenstein, USA

Pharmacoeconomic Studies in AD
Bengt Winblad, Sweden

Therapy II: Chairmen Ezio Giacobini and Caleb Finch

Treatment of Alzheimer's Disease Year 2001
Ezio Giacobini, Switzerland

M1 Agonists from Treatment Towards Disease Modifying Agents in Alzheimer's Disease: Concepts and Perspectives
Abraham Fisher, Israel

Donepezil in Mild to moderate Alzheimer's Disease: Results of A One-year Double-blind, Randomized Trial
Bengt Winblad, Sweden

Galantamine: Clinical Implications for Nicotinic Receptor Modulation
Luc Truyen, USA

Disease Progression and Response to Therapy in Alzheimer's Disease: Are There Predictors?
Martin Farlow, USA

Transgenic Models: Chairmen Claudio Cuello and Heikki Tanila

Animal Models for Alzheimer's Disease: An Overview
Claudio Cuello, Canada

Neuropathology and Behavioral Changes in APP-transgenic Rats
Paul Chapman, UK

Learning and Synaptic Plasticity in APP/C-100 Transgenic Animals
Matthew Shapiro, USA

Cells and Mice Carrying Presenilin Mutations: Tools to Unleash AD Pathology
Takeshi Tabira, Japan

Does Amyloid Accumulation Relate To Behavioral Changes in APP+PS-1 Transgenic Mice?
Heikki Tanila, Finland

Jubilee Symposium of the Finnish Neurological Society: Chairpersons Jouko Isojärvi and Hilkka Soininen

History of Neurology In Finland
Matti Haltia, Finland

Mechanism-Based Therapeutic Approaches in Alzheimer's Disease: Lessons from Molecular Neurobiology
Sangram S. Sisodia, USA

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Pathogenesis I:
Chairmen Konrad Beyreuther and Colin Masters

Mechanisms of Degeneration in Alzheimer's Disease - Towards a Rational Therapy

Colin L Masters[*1] and Konrad Beyreuther[2]

[*1]Department of Pathology, The University of Melbourne, and the Mental Health Research Institute of Victoria, Parkville, Victoria 3052, Australia. [2]Center for Molecular Biology, The University of Heidelberg, D-69120, Germany.

The amyloid theory of Alzheimer's disease presents us with several distinct rational therapeutic strategies. These include:

1. Inhibitors of β- and γ-secretase.
Preclinical and early phase clinical trials are taking place. The big unknown is the therapeutic window available for by-passing any adverse effect on Notch signalling or on any other critical pathway running in parallel with APP/Aβ processing.

2. Promoting the dissolution and clearance of Aβ amyloid.
Methods which either prevent the aggregation of Aβ when it is released from a lipid environment or allow the insoluble aggregates of Aβ to be mobilized are coming to fruition. Metal ions (such as Cu^{++} or Zu^{++}) may promote aggregation, and chelators or compounds directed to the metal binding sites on Aβ are proving of interest. Enchancing the macrophage/microglial system through active or passive immunisation with Aβ has proven to be effective in experimental Aβ amyloid models.

3. Targeting the mechanism underlying Aβ toxicity.
Aβ appears to induce neurodegeneration through mechanisms that involve metal-mediated oxyradical formation or an interaction between oligomeric Aβ species and the cell membrane. These mechanisms allow for assay development for screening of compounds which may abrogate the toxic properties of Aβ.

Alone or in combination with other anti-amyloid strategies, the above approaches are about to provide the crucial test of the validity of the amyloid theory of Alzheimer's disease.

A Proteolytic Function of Presenilins

Harald Steiner and Christian Haass

Adolf Butenandt-Institute, Dept. of Biochemistry, Laboratory for Alzheimer's and Parkinson's Disease Research, Ludwig-Maximilians-University, 80336 Munich, Germany

Most of the mutations associated with familial Alzheimer's disease (FAD) have been identified in the two presenilin (PS) genes, particularly the PS1 gene. Like the mutations identified within the APP gene, mutations in PS1 and PS2 cause the increased generation of Aβ42. PS1 has been shown to be functionally involved in βAPP processing and in Notch signaling, a key process in cellular differentation. A gene knock out of PS1 in mice leads to an embryonic lethal phenoptype similar to that of mice lacking Notch. In addition, absence of PS1 results in reduced γ-secretase cleavage and leads to an accumulation of βAPP C-terminal fragments, decreased Aβ production and reduced formation of the Notch intracellular cytoplasmic domain (NICD), a key molecule involved in Notch

mediated signal transduction. In PS1/PS2 double knockout cells total inhibition of Aβ and NICD production is observed. Recent evidence suggests that both presenilins, PS1 and PS2, may be identical with the elusive γ-secretase and exhibit properties of an unusual aspartyl protease. Mutagenesis of either of two highly conserved intramembraneous aspartate residues at position 257 or 385 of PS1 results in a lack of PS endoproteolysis and leads to an accumulation of βAPP C-terminal fragments with concomitant reduced Aβ production. In addition, the aspartate mutants of PSs have been shown to block NICD production and consequently Notch signaling. Moreover, aspartyl protease transition state analogue inhibitors that block γ-secretase can be directly cross-linked to PSs. Finally, PSs share considerable sequence homology around the critical active site aspartate at position 385 with recently described polytopic bacterial aspartyl proteases. Mutagenesis studies revealed that a glycine residue at position 384, directly adjacent to the critical aspartate, is part of the presenilin active site where it constitutes a highly critical residue for PS function. Whereas a rather subtle substitution (G384A) occurs as a FAD mutation and shows the highest Aβ42/Aβtotal ratio reported for PS1 mutations so far, other (artificial) mutants at this position lead to reduced function in both βAPP and Notch endoproteolysis.

Apolipoprotein E Receptors in Brain Development and Neurodegeneration

Uwe Beffert, Michael Gotthardt, Marion Trommsdorff, Thomas Hiesberger, Joachim Herz

Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, TX 75390-9046, USA

Apolipoprotein E (ApoE) is a component of plasma lipoproteins. It mediates their transport through the circulation and their binding to specific receptors on the surface of numerous cell types. The common ε-4 isoform of ApoE (ApoE-4) was found to be genetically associated with late-onset Alzheimer disease (AD). However, the molecular mechanisms by which ApoE-4 affects the onset of AD have remained unknown. Increasing evidence has since accumulated that links ApoE not only to neurodegeneration, but also to the ability of the brain to recover after injury. ApoE genotype possibly also influences the performance of complex memory tasks and cognitive association. ApoE binds to a specific class of cell surface receptors, the low-density-lipoprotein (LDL) receptor gene family. Several members of this ancient family of endocytic receptors are abundantly expressed in the brain, in particular on the surface of the neurons. This gene family arose very early during evolution, and long before the appearance of

ApoE, suggesting that these multifunctional receptors may play critical roles in the brain that are unrelated to lipid metabolism and that could be affected by ApoE. Signaling by neuronal receptors is universally recognized as an important factor for the survival of neurons. We have therefore searched for mechanisms by which LDL receptor family members might transmit signals to neurons. A picture is now emerging that suggests that LDL receptor family members may function as co-receptors in diverse signaling pathways during neuronal development, and likely also in the adult brain.

Activation of Developmental Programmes in Alzheimer's Disease

Thomas Arendt

Paul Flechsig Institute for Brain Research, Department Neuroanatomy, University of Leipzig, Germany

Degeneration in AD primarily occurs in a subset of neurons that in the adult brain retains a high degree of structural plasticity and in these neurons is associated with the activation of mitogenic pathways and a cell cycle re-entry. Brain areas affected by AD pathology are those structures involved in the regulation of "higher brain functions" that become increasingly predominant as the evolutionary process of encephalization progresses. The functions these areas subserve require a life-long adaptive reorganization of neuronal connectivity that allows for the aquisition of new epigenetic information. With the increasing need during evolution to organize brain structures of increasing complexity, these processes of dynamic stabilization and de-stabilization become more and more important but might also provide the basis for an increasing rate of failure.

In the present paper, evidence is summarized that it is the labile state of differentiation of a subset of neurons in the adult brain that allows for ongoing morphoregulatory processes after development is completed but at the same time renders these neurons particularly vulnerable. The delicate balance between G_0-arrest and G_1-entry might be prone to a variety of potential disturbances during the lifetime of an individual. Morphodysregulation in AD, accompanied by an activation of intracellular mitogenic signalling might, thus, be a slowly progressing dysfunction that eventually overides the differentiation control and results in dedifferentiation, a condition in conflict with the otherwise 'mature' background of the nervous system. Cell-cycle and differentiation control might thus provide the link between structural brain self-organization and neurodegeneration that both are unique to human.

Prevention of Alzheimer's Disease - Experiments and Strategies

Konrad Beyreuther and Colin L. Masters°

ZMBH, University Heidelberg, Germany and °Department of Pathology, The University Melbourne, Australia

To understand and prevent synaptic loss and neurodegeneration in Alzheimer's disease (AD) we have analyzed the physiological function of APP and its Aβ domain. Studies in transgenic Drosophila melanogaster and primary mammalian neurons suggest that APP may modulate synaptic plasticity. Deletion analyses showed that the axonal transport of APP is dependent on the Aβ domain suggesting that the Aβ sequence serves a function as axonal sorting signal of APP. In AD, excess intracellular and extracellular Aβ may therefore convert Aβ's physiological function to a pathogenic one by inhibiting the axonal transport of proteins using the same transport machinery as APP thus leading to synaptic dysfunction and neuronal death. Because both the apoE ε4 allele and stroke may be associated with higher cholesterol levels in neurons and higher risk of developing AD, we studied the influence of cholesterol on neuronal Aβ generation in vivo and in vitro. By lowering cholesterol levels with statins (HMG-CoA reductase inhibitors), the formation of Aβ becomes drastically reduced suggesting that both the physiological and pathogenic regulation of axonal transport by Aβ appears to be controlled by cholesterol. This implies a link between brain cholesterol, APP transport, Aβ production and the risk of developing Alzheimer's disease. The intriguing relationship between neuronal cholesterol levels and Aβ production opens new and immediately accessible vistas for the treatment and prevention of Alzheimer's disease.

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The Why, What and How of Alzheimer Neurofibrillary Degeneration

K. Iqbal, A. Alonso, M. Bennecib, C.-X. Gong, N. Haque, S. Khatoon, A. Sengupta, J.-Z. Wang and I. Grundke-Iqbal

New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, New York 10314, U.S.A.; E-mail: kiqbal@mail.con2.com

Independent of the etiology, i.e. genetic or non-genetic, neurofibrillary degeneration is pivotally involved in the pathogenesis of Alzheimer disease (AD) and related tauopathies. The discovery of the cosegregation of mutations in the tau gene with the disease in inherited cases of frontotemporal dementia with Parkinsonism-linked to

chromosome 17 (FTDP-17) have confirmed that tau abnormalities alone can be sufficient and can be a primary cause of neurodegeneration and dementia. In AD and all other tauopathies tau accumulates only in the abnormally hyperphosphorylated state. The level of tau in AD brain is as much as in a normal age-matched brain but the total level of tau in the former is 4-8 fold increased. This increase in tau levels is in the form of the abnormally hyperphosphorylated tau. Although a major pool of abnormally hyperphosphorylated tau is seen as polymerized into PHF, as much as 40% of the total abnormal tau is present in the cytosol. Unlike normal tau which stimulates assembly and stabilizes microtubules, the cytosolic abnormal tau (AD P-tau) inhibits assembly and disassembles microtubules. The AD P-tau on one hand competes with tubulin/microtubules in associating with normal tau, MAP1 and MAP2. On the other hand the sequestration of normal tau but not of MAP1 or MAP2 results in the self association of the AD P-tau into tangles of PHF and straight filaments (SF). Dephosphorylation but not Deglycosylation of AD P-tau inhibits its self assembly into PHF/SF. Thus, it appears that the abnormal hyperphosphorylation of tau is cytotoxic and the inhibition of this pathological hyperphosphorylation is a promising therapeutic target for AD and related tauopathies.

Studies were supported in part by the New York State Office of Mental Retardation and Developmental Disabilities and NIH grants AG05892, AG08076, NS18105 and TW00507.

Tau Protein and Alzheimer's Disease: Phosphorylation and Role in Intracellular Traffic

E.-M. Mandelkow, K. Stamer, J. Biernat, B. Trinczek, E. Mandelkow.

Max-Planck-Unit for Struct Mol. Biol., Hamburg, Germany.

One of the hallmarks of Alzheimer's disease is the aggregation and hyperphosphorylation of tau protein. We are studying the question of how the abnormal modifications are related to one another and how they affect the physiological functions of the neuron, such as axonal transport. We have identified a number of phosphorylation sites, including some that affect tau's interactions with microtubules (e.g. S214 during mitosis). Phosphorylation affects the tau-microtubule interaction to different extents. The most potent effect is observed with the kinase MARK (4 isoforms) which phosphorylates Ser 262 of tau and similar KXGS motifs in MAP2 or MAP4. Overexpression of MARK in cells leads to hyperphosphorylation of MAPs on KXGS motifs and to the disassembly of microtubules, resulting in morphological changes and cell death (1). On the other hand, the phosphorylation at the KXGS motifs in tau is important for tau-

induced process formation in cell models (2). This points to a role of MARK in the development and maintenance of cell polarity. In Alzheimer's disease, neuronal polarity is lost, and tau which is normally mostly axonal becomes accumulated in the somatodendritic compartment. We therefore studied the effect of tau on intracellular transport and found that tau inhibits the kinesin-dependent transport of vesicles and organelles in the plus-direction of microtubules (3). As a result, minus-end directed transport by dynein predominates. We have now tracked single vesicles and organelles in live cells and find that tau does not change the speed of motors once attached to a microtubule, but increases the probability of slipping off the track (shorter run lengths) and decreases the probability of attachment (4). The net effect is to cause clustering of mitochondria, peroxisomes, and intermediate filament protein near the cell center, retraction of the endoplasmatic reticulum, and slowdown of exocytosis. While tau inhibits organelle transport, the kinase MARK has the opposite effect. It facilitates organelle transport by removing tau from the microtubule tracks. This illustrates the interplay between motors, MAPs, and kinases in the local regulation of traffic. In the case of neuronal cells, mitochondria, peroxisomes, and intermediate filaments largely disappear from the cell processes, leading to energy deprivation and vulnerability of the cells.

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Pathway of Tau Aggregation Into Alzheimer Paired Helical Filaments

E. Mandelkow, M. von Bergen, A. Schneider, S. Barghorn, J. Biernat, E.-M. Mandelkow.

Max-Planck-Unit Struct. Mol. Biol., Hamburg, Germany.

Alzheimer's disease and related tauopathies show the accumulation of tau protein into paired helical filaments (PHF) and the higher order structures of neurofibrillary tangles or neuropil threads. We are studying the pathway of aggregation in an effort to understand the molecular interactions and their determinants. (a) As a first step, the dimerization of tau by oxidation and formation of disulfide bridges greatly enhances aggregation so that tau dimer can be regarded as effective building blocks of PHFs. (b) Assembly is further enhanced by the presence of polyanionic cofactors (heparin, poly- Glu,

RNA, or other acidic peptides). (c) Once these conditions are met, tau undergoes a nucleation phase involving oligomers of 4-7 tau dimers (1). The nucleation barrier can be overcome by external seeds such as PHFs from Alzheimer disease brains. (d) After nucleation, PHFs must elongate and later coalesce with other PHFs into tangles. Phosphorylation at key residues (e.g. Ser262, Ser214) detaches tau from microtubules, thus enhancing the pool of tau molecules. It was often assumed that phosphorylation also promotes the aggregation of tau into paired helical filaments. Our recent results (2) show, however, that the opposite is the case. The same phosphorylation sites that are most potent in dissociating tau from microtubules are also most potent in inhibiting PHF assembly (e.g. S262, S214). The other sites (mostly in Ser- Pro motifs) show a similar tendency but much less pronounced. Once PHFs are formed, a fraction of the sites can still be phosphorylated in the polymer and are therefore accessible to kinases. Another unresolved question is how tau forms apparently regular fibers (the PHFs) with an "amyloid" character (in terms of dye binding such as thioflavin S), even though the tau molecule shows a mostly random coil structure in solution. This can be explained in part by the influence of a short motif, 306-VQIVYK-311 at the beginning of the third repeat, which tends to form beta structure and thus provides a basis for the self-assembly of the protein (3). - Finally we have studied the effect of tau mutations observed in frontotemporal dementias (FTDP-17). Although they lie mostly in the microtubule-binding domain they have only a weak influence on tau-microtubule interactions. By contrast, they show an enhanced tendency for PHF aggregation in the presence of polyanions; this is particularly evident for the mutations P301L and delK280 (4).

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Neuronal Model System for Alzheimer Neurofibrillary Degeneration

Inge Grundke-Iqbal and Khalid Iqbal

New york State institute for Basic Research in Developmental Disabilities; 1050 Forest Hill Road, Staten Island, New York 10314, U.S.A.; E-mail: igiqbal@excite.com

Microtubule associated protein tau is a phospho protein, the biological activity of which is regulated by its degree of phosphorylation. In AD brain the tau is abnormally

hyperphosphorylated. It is present both in the cytosol and as well as aggregated into paired helical filaments (PHF) as intraneuronal neurofibrillary tangles. In vitro studies show that the cytosolic abnormally hyperphosphorylated tau, the AD P-tau, which is most likely a precursor of PHF, sequesters normal tau, MAP1 and MAP2 and inhibits the assembly and causes disruption of microtubules. In contrast the PHF/neurofibrillary tangles are apparently inert polymers of the abnormal tau. They might represent a mechanism by which the affected neurons inactivate the AD P-tau. Thus, it is critical to understand the processes involved in the abnormal hyperphosphorylation of tau. Towards this objective we have found (i) that inhibition of the activities of protein phosphatases (PP)-2A and PP-1 by okadaic acid or calyculin A in SY5Y human neuroblastoma cells in culture leads to the hyperphosphorylation of tau at the M4, PHF-1 and Ser-422 sites. Like AD P-tau, tau isolated from these cells does not promote but inhibits the in vitro assembly of microtubules. The treated cells also undergo degeneration which can be partially reversed by the microtubule stabilizers taxol and Sabeluzole; (ii) in adult rat brain slices kept metabolically active in oxygenated artificial CSF, the inhibition of PP-2A activity by okadaic acid for 3 hours results in the abnormal hyperphosphorylation of tau at the Tau-1, 12E8, PHF-1 and Ser-422 sites and its accumulation in the pyramidal neurons of the cornu ammonis and neocortical neurons; the tau isolated from the treated brain slices is also compromised in binding to microtubules and promoting the assembly of microtubules in vitro; and (iii) the treatment of neural progenitor cells isolated and propagated from adult rat hippocampus with FGF2 leads to hyperphosphorylation of tau at the Tau-1 site by promoting the overexpression of tau and GSK-3. These studies suggest that more than one molecular mechanisms might contribute to the abnormal hyperphosphorylation in the Alzheimer brain.

Supported in part by funds from the New York State Office of Mental Retardation and Developmental Disabilities and NIH grants NS18105 and AG08076.

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Poster Session

Pathogenesis and animal models

1. Characterisation of novel γ-secretase inhibitors blocking the endoproteolysis of Presenilin 1.

Jonathan D.J. Wrigley, David Williams, Alan Nadin, Geneviéve Evin, Colin L. Masters, Timothy Harrison, José L. Castro, Mark S. Shearman & Dirk Beher.

Merck Sharp & Dohme Research Laboratories, Terlings Park, Harlow, Essex CM20 2QR, UK; Department of Pathology, University of Melbourne, Australia

Background. Intramembranous cleavage of the β-amyloid precursor protein by γ-secretase is the final processing event generating amyloid-β peptides, which are believed to be the causative agent for Alzheimer's disease. Recently, a close linkage between presenilins and γ-secretase has been established as a result of genetic and biochemical studies. Here we report that novel inhibitors of γ-secretase which physically interact with presenilin 1 (PS1) directly inhibit its endoproteolytic processing.

Methods. Human SH-SY5Y neuroblastoma cells were treated with novel γ-secretase inhibitors for 16 h and the effects on PS1 processing analysed by immunoblotting of solubilised membrane fractions.

Results. We describe a specific inhibition of PS1 endoproteolysis as defined by the pharmacological means of dose-dependency, time-course, and structure-activity

relationship, leading to an increase in full-length polypeptide. Furthermore, a unique compound was discovered which has the ability to produce a "pharmacological knockout" of PS1 fragments.

Conclusions. These data strengthen the intimate linkage between γ-secretase and PS1 as certain compounds developed with the aim to inhibit g-secretase block PS1 endoproteolysis. The discovery of a remarkable compound which was able to induce a "pharmacological knockout" of PS1 fragments should provide a valuable tool to study the biology of presenilins.

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2. Levels of cdk5 and p35 are reduced in neuronal apoptosis

Petri Kerokoski, Jukka Puoliväli, Maaria Roschier, Tiina Suuronen, Antero Salminen, Hilkka Soininen, and Tuula Pirttilä

Dept. of Neuroscience and Neurology, University of Kuopio, Kuopio, Finland; Dept. of Neurology, Kuopio University Hospital, Kuopio, Finland

Background. Cyclin-dependent kinase 5 (cdk5) is believed to be involved in the phosphorylation of tau protein. Recent studies have revealed altered protein levels of cdk5, and its neuron-specific activator p35 during neuronal cell death by necrosis.

Methods. We studied rat hippocampal neuronal cultures subjected to pro-apoptotic treatments, and measured the protein levels of cdk5 and p35, and tau phosphorylation using immunoblotting. In vitro kinase assay was used in determination of cdk5 activity.

Results. We observed that in cells treated with etoposide, cyclosporin A, 4-hydroxynonenal (HNE), or okadaic acid, there was an early reduction in the protein levels of p35, and later also in cdk5 with all treatments except etoposide. The level of p25, a calpain cleavage product of p35 suggested to have increased ability to activate cdk5, was reduced paralleling the amount of p35. The changes in the p35 and p25 protein levels coincided with decreases in cdk5 activity and tau phosphorylation after treatment with HNE and etoposide. However, the relationship between the p35 and p25 levels and cdk5 activity was complex.

Conclusions. We conclude that neuronal apoptosis is accompanied with a decrease in the levels of p35, p25 and cdk5, and tau phosphorylation. These changes may reinforce the neuronal damage.

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3. Increased vulnerability to focal brain ischemia in APP751 overexpressing mice

M. Koistinaho, M. Kettunen, L. Higgins, R. Kauppinen and J. Koistinaho

A.I. Virtanen Institute for Molecular Sciences, University of Kuopio, Kuopio, Finland; Scios Inc., Sunnyvale, CA, USA

Background and Methods. We studied the role of the amyloid precursor protein (APP) in ischemic brain damage by permanently occluding the middle cerebral artery (MCA) in 8 and 20-month-old mice overexpressing the 751-amino acid isoform of human APP. Pedigrees originating from two different founder mice (F10 and F15, expressing same amount of APP751) were compared with age-matched wild type JU mice. Infarct volumes were quantified from T2-weighted images obtained by magnetic resonance imaging (MRI) using a 4.7 T vertical magnet. Gadodiamide bolus tracking perfusion MRI was performed before and after MCA occlusion. Inflammatory responses were evaluated histochemically using F4/80 antibody, a microglia marker, and a dually phosphorylated epitope-specific antibody for activated form of p38 MAPK.

Results. At 8 months of age, both transgenic pedigrees had 34% larger infarcts than the control mice ($p<0.002$). At 20 months, the infarcts were 35% larger ($p<0.0001$) in F10 pedigree and 41% larger in F15 pedigree ($p<0.0001$) when compared with age-matched controls. Perfusion parameters showed no difference in cortical perfusion between the animal groups. F4/80 and p38 MAPK immunostainings in the periinfarct regions were stronger in APP transgenic than in control mice.

Conclusions. APP751 overexpression increases the vulnerability to ischemic brain injury in both young adult and aged mice by a mechanism, which may involve inflammation.

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4. Amyloid β_{1-42} accumulation does not affect regenerative sprouting in APP/PS1 mutant mice

I. Kadish and Th. van Groen

University of Kuopio, Dept. of Neuroscience and Neurology, P.O. Box 1627, FIN 70211 Kuopio, Finland.

Background. It has been demonstrated by many studies in rats that, following entorhinal cortex ablations, the dentate gyrus shows a sprouting response of non-lesioned axons. We hypothesized that this response would be altered in transgenic mice expressing two AD mutations, i.e., PS1 (A246E mutation)

and APP_{swe}, when they show severe AD pathology, i.e., at 15 month of age. The $A\beta_{1-42}$ levels at this age were 40 µg/g for the AD animals.

Methods. We lesioned the entorhinal cortex in these mice, young double mutant mice, and in age-matched control animals. The entorhinal cortex was unilaterally lesioned by injections of ibotenic acid; four weeks later the animals were sacrificed and transcardially perfused. The brains were cut and stained, the most consistent changes were present in the material stained for synaptophysin, a protein that marks presynaptic terminals.

Results. Following the lesions the ipsilateral hippocampus demonstrates sprouting, i.e., an increased expression of synaptophysin was present in the outer molecular layer of the dentate gyrus. The young control, and the young AD mice displayed a robust sprouting response to the lesion, but surprisingly, neither the age-matched control or the AD mice showed any diminishment in the sprouting response compared to the young animals.

Conclusion. The presence of the AD mutations causing high levels of Aβ in these mice did not change the response of the brain to lesions compared to normal age-matched control mice.

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5. Increased Aβ accumulation in the Hippocampus of APP/PS1 mice correlates with impaired water maze performance

T. Heikkinen, M. Heikkilä, J. Wang, J. Puoliväli, T. Van Groen, H. Tanila.

Department of Neuroscience and Neurology, University of Kuopio, Finland.

Background. Various mutations in the genes encoding the amyloid precursor protein (APP) and presenilin 1 (PS1) genes lead to increased beta amyloid (Aβ) accumulation and development of amyloid plaques in the brain and lead to familial Alzheimer's disease (AD). Several transgenic mouse lines carrying these mutations are used to study the role of Aβ and amyloid plaques in cognitive functions.

Methods. We investigated the performance of 11 months old APP/PS1 double transgenic male mice in two cognitive tasks, the Morris water maze and a position discrimination T-maze. We also examined the relationship between hippocampal Aβ levels, number of amyloid plaques and the performance in the behavioral tests. The APP/PS1 mice have elevated brain Aβ levels and develop amyloid plaques at the age of 9 months.

Results. In this study we show that 11 months old APP/PS1 male mice are impaired in the Morris water maze but not in the T-maze compared to wild type mice. The impaired water maze performance of the APP/PS1 mice correlates with the increased levels of hippocampal Aβ.

Conclusions. These results show that the APP/PS1 mice are impaired in a hippocampal memory task and this impairment is related to the elevated levels of hippocampal Aβ. These results suggest that the overexpression of hippocampal Ab is implicated in the impairment of certain cognitive functions.

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6. The Electrophysiological Changes In APP/PS1 Double And Single Mutant Transgenic Mice

Jun Wang, Sami Ikonen, Heikki Tanila

Department of Neuroscience and Neurology, University of Kuopio and Department of Neurology, Kuopio University Hospital. P.O.Box 1627, FIN-70211 Kuopio, Finland

The mutations of certain familiar forms of Alzheimer's disease (AD) involve three genes: amyloid precursor protein (APP), presenilin 1 (PS1) and 2 (PS2). In order to find electrophysiological characteristics of these mutations we used transgenic mice carrying human APP_{swe} and PS1-A264E mutations to record EEG and auditory evoked potentials (EP). In APP/PS1 double mutant mice there were significant differences in cortical theta ($P<0.01$) and beta ($P<0.01$) power in EEG and in cortical P35 latency ($P<0.05$) when compared with wild-type mice (mice age was from 7 months to 13 months). Also cortical P35 latency increased significantly during age in both groups ($P<0.01$). But there were no differences in hippocampal theta ($P>0.05$) and hippocampal frequency of maximum power ($P>0.05$). For further study we recorded cortical EEG and P35 latency in APP, PS1 single mutant mice comparing APP/PS1 double mutant and wild-type mice when mice age was 8 months. The results showed that there were differences between the groups in cortical theta ($P<0.05$) and beta ($P<0.01$) and in cortical P35 latency ($P<0.001$). APP single mutant mice were different from PS1 ($P<0.05$) and wild-type mice ($P=0.05$) but no difference from APP/PS1 mice ($P>0.05$) in cortical theta power. In cortical P35 latency APP mice also showed difference from wild-type mice ($P<0.05$) but no difference from APP/PS1 mice ($P>0.05$). These results suggest that APP/PS1 double mutant mice have quite different electrophysiological features and that it is mainly the APP mutation that induces the changes in EEG and P35 latency. These findings could be useful indicators for early clinical diagnosis of certain familiar AD.

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7. Altered auditory gating in Alzheimer's disease and in transgenic mice with APP and PS1 mutations

K. Gurevicius, J. Wang, S. Ikonen, A. Pääkkönen, Ina M. Tarkka, H.Tanila

Dept. Neuroscience and Neurology, Univ. Kuopio; Dept. Clinical Neurophysiology, Kuopio University Hospital, Finland

Background. Habituation of evoked P50 response to repeated auditory stimuli is well documented in humans and mice, and is thought to reflect normal function of interneurons. Loss of this habituation (auditory gating) is characteristic of schizophrenic patients, and has been associated with a mutation in the gene coding for α7-nicotine receptor subunit. We wanted to determine whether the same electrophysiological deficits can be demonstrated in Alzheimer patients and transgenic mice.

Methods. The study comprised of 22 patients with mild Alzheimer's disease and 25 aged-matched controls in human studies, and 17 mice expressing human APP_{swe} + PS1 mutations and 21 wild type control mice. The mice were followed up from 7 to 13 months of age, and the ERPs were recorded monthly. Paired clicks (10-20 ms, isi 500 ms) were presented every 10 s to mice and a train of 4 stimuli every 12 s to humans. Evoked responses were recorded from 19 scalp electrodes in humans and between frontal and parietal screws or between two deep hippocampal electrodes in mice.

Results. Alzheimer patients showed attenuated auditory gating of P50 response. Also doubly transgenic mice had weaker auditory gating of the corresponding N30-40 than wild type mice. Despite a dramatic age-related increase in the brain amyloid load, the transgenic mice did not show any further impairment in the auditory gating with age.

Conclusions. Similar electrophysiological characteristics AD patients and transgenic mice suggest a common underlying pathological mechanism. However, impaired auditory gating cannot be accounted for by amyloid accumulation in plaques, but may be associated with direct antagonism of α7-nicotine receptors by $Aβ_{1-42}$.

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8. Age dependent impairment in spatial learning of APP+PS1 mice

M. Heikkilä, T. Heikkinen, J. Wang, J. Puoliväli, H. Tanila.

Department of Neuroscience and Neurology, University of Kuopio, Finland.

Background. Mutations in the genes encoding amyloid precursor protein (APP) and presenilin 1 (PS1) lead to increased beta amyloid (Aβ) accumulation and development of amyloid plaques in the brain in the familial form of Alzheimer´s disease (AD). Several transgenic mouse lines carrying these mutations have been shown to be cognitively impaired especially in spatial learning.

Methods. In this study, we investigated the effect of aging on cognitive functions in APP/PS1 double transgenic male mice by using the Morris water maze and the position discrimination task in a T-maze. Transgenic and control mice were tested at the age of 4 and 11 months. Previously, it has been shown that these mice have elevated Aβ levels in the brain and develop amyloid plaques starting at age of 9 months. Early in the process, the amyloid plaques are most common in the hippocampus and the subiculum.

Results. At the age of 11 months, the APP/PS1 mice were clearly impaired in the Morris water maze compared to the wild type mice. However, the task performance of the mouse lines did not differ at age of 4 months. By contrast, APP/PS1 were as good as the wild type mice at either age in the position discrimination task which is not sensitive to hippocampal damage.

Conclusions. The task specific and age dependent impairment in spatial learning in the APP/PS1 mice corresponds to the progressive amyloid pathology that is most severe in the hippocampus. Therefore, these data suggest that amyloid accumulation in the hippocampus is closely associated with the cognitive impairment in these mice.

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9. Intraamygdala injection of physostigmine improves spatial learning and memory in Alzheimer's disease in rats

Heidary A. , Sarkaki A. , Zahedi A.A.

Dept. of physiology, Ahwaz University of Medical Sciences, P.O.Box: 61355-189, Ahwaz-IRAN, Fax:0098-611-361544 or 332036 E-mail: sarkaki-a@aums.ac.ir

Background. The role of central cholinergic system in learning and memory processes has been investigated in Alzheimer's disease (AD). Many findings showed some degrees impairment of spatial learning and memory in patients with nucleus basalis magnocellularis (NBM) degeneration. It seems that cholinergic projections of NBM to amygdala may be involved in learning and memory.

Method. In this work we have evaluated the effect of intraamygdala injection of physostigmine, an

acethylcholinsterase inhibitor via implanted bilateral guide cannula on spatial learning and memory deficits following bilateral NBM electrolytic lesion(1mA,30sec. passed through a 0.18 mm diameter teflon coated stainless steel electrode) in male N-MARI rats(160-220gr). Acquisition and recall of active avoidance task was studied in the equal 3-arms Y-maze with light and electrical shock(successive 30 trials,one session daily alone).

Results. Results showed that administration of physostigmine (2.5 and 5 g/µl) produced significant improvement of both acquisition and recall(retrieval) of spatial task. Lower and higher doses of physostigmine (1.25 and 10 µg/µl respectively) produced weaker effects on this task.

Conclusion. These findings support that cholinergic projections of NBM to amygdala are important in this task, and intraamygdala injection of physostigmine prolongs acethylcholine effect by prevents cholinsterase action and can improve this ability.

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10. The Effects of Long Term Treatment With Metrifonate on Amyloid Pathology, Cholinergic Activity and Cognitive Function in APP and PS1 Doubly Transgenic Mice

L. Liu, S. Ikonen, J. Puoliväli, T. Heikkinen, T. Tapiola, S. Iivonen, T. van Groen and H. Tanila

Dept. of Neuroscience and Neurology, University of Kuopio, Finland

Background. Cholinesterase inhibitors (CEIs) have been shown to increase the release of the secreted form of amyloid precursor protein (sAPP) from superfused brain cortical slices of rat *in vitro*, and thus may preclude the formation of the amyloidogenic Aβ.

Methods. To investigate whether CEI could decrease Aβ production and slow down the accumulation of Aβ *in vivo*, we chronically administered metrifonate, a second-generation CEI, to 7-month-old doubly transgenic APP and PS1 mice and their litter mate controls for 7 months. Behavioral studies, including open field test, T-maze and water maze, were conducted after 6 months treatment with metrifonate and the mice were sacrificed at the age of 14 months. The levels of sAPP and Aβ were analyzed from hippocampi using Western blot and ELISA respectively. ChAT activity of hippocampus and parietal cortex was measured and the histology of the mouse brains was also studied.

Results. We found that although the long term treatment with metrifonate increased the ratio of Aβ 42/40, meanwhile it also

increased both Aβ 42 and 40 levels in the hippocampus of the mouse brain without increasing the expression of APP. A statistically different response of ChAT activity to chronic treatment with metrifonate was found between transgenic mice and wild type controls. Cognitive deficits of transgenic mice at this age were not detected in this study, but the locomotor activity and the swimming speed of both metrifonate treated groups were reduced. Moreover, a downregulation of the density of cholinergic fibers was observed in the mouse with AChE histochemical staining.

Conclusion. Our results indicate that metrifonate can not inhibit the overproduction of Aβ in the brains of APP and PS1 doubly transgenic mice.

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11. Aging, estrogen, and place cells

Wilson IA, Ikonen S, and Tanila H.

Dept. Neuroscience and Neurology, University of Kuopio, 70211 Kuopio, Finland.

Background. Alzheimer's disease ravages declarative memory of the hippocampus, which can be modeled by the so-called place cell property of hippocampal neurons. We therefore used rat hippocampal place cells in order to examine the workings of spatial memory in relation to two current issues of Alzheimer's, aging and ovariectomy. Aging is known to cause spatial memory deficits in rats, while estrogen-level has been shown to modulate hippocampal physiology.

Methods. In the first experiment we recorded place cells from 9 aged Long-Evans males (27 months old) and 6 young males (7 months old); in the second experiment we recorded from 7 ovariectomized, aged Wistar females (20 months old), 6 ovariectomized-plus-estradiol-injected, aged females, and 7 sham-operated, aged females. These five groups of animals were all recorded in two environments, alternating between a familiar one and a novel one for a total of 5 times, each recording taking 7 minutes.

Results. Rats from all groups had almost equal numbers of stable / unstable fields in the familiar environment; instead, the most discriminating parameter was the response to the change in environment. Place cells of the young male rats generally exhibited stable, new fields in the new environment, while aged male rats, particularly those who had performed poorly on the spatial water maze task, had place fields which remained rigid despite changes in the surrounding environment. Of the female, aged rats, a sizable portion from all groups showed this rigidity as well.

Conclusions. Aged female rats had place cell characteristics which resembled those of aged male rats, and thus estrogen manipulations neither accelerated nor allayed the spatial memory deterioration of aging.

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12. Effects of Estrogen on NMDA Receptor Mediated Plasticity in the Mouse Hippocampus.

I. Siverina, J. Puoliväli, R. Pussinen, H. Tanila.

Dept. Neuroscience and Neurology, Univ. Kuopio, Finland

Backgroung. Estrogen has a neuroprotective effect and estrogen replacement therapy delays the onset of Alzheimer's disease. Studies have also linked estrogen with improved performance of spatial learning and memory in humans and rodents. Ovariectomy attenuates NMDA-mediated neurotransmission, which can be reversed by ERT in a mice model. To test the interaction between estrogen and NMDA receptors, we compared the dose-response curves for a competitive NMDA antagonist in ovariectomized (OVX) mice with or without ERT.

Methods. Female ovariectomized C57Bl/6J mice were used in both behavioral (12 months old) and electrophysiological (7 months old) studies. Half of the mice were given ERT in the form of a subcutaneous pellet. The mice received either (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) at 0.5, 2.0, or 5.0 mg/kg or saline intraperitoneally during behavioral studies, and direct infusion of drug (CPP, 5.0, 10.0 uM) to the incubation fluid during slice studies. Spatial learning and memory was studied in the water maze (WM) and field potentials (EPSP) in hippocampal CA1 area in vitro.

Results. CPP impaired finding of the hidden platform (WM) dose-dependently. During the last day of testing, the impairing effect of CPP was more pronounced in the OVX mice than in the OVX+ ERT mice. Also in the probe trial with the platform removed, OVX+ERT mice receiving CPP at 2 or 5 mg/kg spent more time in the former platform location than the OVX mice. The initial induction of LTP without CPP did not differ between the OVX and OVX+ERT groups. However, CPP at 5 µM blocked LTP in the OVX but robust LTP was still observed in the OVX+ERT mice. We found a significant difference in the EPSP amplitude and slope between the groups at 15 and 30 minutes after LTP induction.

Conclusion. The data is consistent with the hypothesis that estrogen affects the number of NMDA receptors in the hippocampus, because a higher dose of CPP was needed in the ERT group than in the control group to depress learning and LTP.

13. Selegiline combined with enriched-environment housing attenuates spatial learning deficit after focal cerebral ischemia in rats

Jukka Jolkkonen, Kirsi Puurunen, Jouni Sirviö, Antti Haapalinna, Juhani Sivenius

Department of Neuroscience and Neurology, University of Kuopio, Kuopio; Orion Corporation, Orion Pharma, Turku; Department of Neurology, University Hospital of Kuopio; and Brain Research and Rehabilitation Center Neuron, Kuopio, Finland

Background. Selegiline is an irreversible monoamine oxidase B inhibitor that is suggested to have neuronal rescuing properties. The present study investigated whether selegiline facilitates behavioral recovery after focal cerebral ischemia in rats.

Methods. The right middle cerebral artery of rats was occluded for 120 min using the intraluminal filament method. Selegiline (0.5 mg/kg, SC) was administered 30 min before rats housed in enriched-environment had their daily exercise in a labyrinth containing traversable objects, providing both sensorimotor stimulation and spatial exercise. Selegiline was given once a day, beginning on the second day after induction of ischemia and continuing for 30 days. A limb placing test, a foot-slip test and a modified version of Montoya's staircase test and a water-maze were used for evaluation of behavioral deficits.

Results. The selegeline-treated ischemic rats housed in enriched-environment had significantly shorter escape latencies ($P<0.001$) and path lengths ($P<0.005$) than did the ischemic control group. The rats housed in an enriched-environment that received selegiline were more likely to make attempts to reach food pellets with the affected ($P<0.05$) and nonaffected ($P<0.01$) forelimbs. There was no significant improvement in sensorimotor tasks when selegiline treatment was combined with enriched-environment housing. Selegiline treatment without the enriched-environment was not beneficial in any of the behavioral tests.

Conclusions. The present study showed that selegiline treatment combined with training and housing in an enriched-environment diminished a spatial learning disability of ischemic rats.

14. In vivo regulation dopamine and noradrenaline release in the medial prefrontal cortex by alpha$_{2A}$-adrenergic receptors

Ihalainen, J.A. and Tanila H

Dept. Neuroscience and Neurology, University of Kuopio, 70211 Kuopio, Finland

Background. Alpha2-adrenoceptors (α2-ARs) regulate many central nervous system functions, mainly by inhibiting neuronal firing and release of various neurotransmitters. The distribution of α_{2A}-ARs in the frontal cortex and other brain structures suggests that they may have important role in the regulation many cognitive functions. In this study, in vivo release of dopamine (DA) and noradrenaline (NA) were determined in α_{2A}-AR KO and control (WT) mice in the medial prefrontal cortex (mPFC).

Methods. One concentric microdialysis probe was implanted into the right mPFC in adult, female, α_{2A}-AR KO (n=5) and WT (n=5) mice. The effect of two concentrations (10^{-9} - 10^{-8} M) of locally injected α2-agonist, dexmedetomidine (Dex) and mild stress, handling, were studied in DA and NA release in the mPFC. The dialysate was analyzed on-line using HPLC with electrochemical detection.

Results. Dex dose-dependently decreased DA and NA releases in WT but to a lesser extent in KO mice. In KO mice DA concentrations remained at a higher level after handling-induced stress than in WT mice. Dex treatment had no effect on DA and NA release in KO mice after handling but in WT mice NA release was significantly lowered.

Discussion. These results reveal that α_{2A}-ARs have an important role in regulating NA and DA release in the mPFC. Therefore, α_{2A}-AR selective drugs may alleviate cognitive deficits associated with stress-induced release of NA and DA in the prefrontal cortex.

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15. Prolyl oligopeptidase does not correlate with β-amyloid accumulation in Alzheimer patients or in transgenic mice

K.S.M. Laitinen, T. van Groen, H. Tanila, J. Venäläinen, A. Koivisto, H. Soininen, P. T. Männistö, I. Alafuzoff

Dept of Pharmacology and Toxicology, Dept of Neuroscience and Neurology, Dept of Neurology and Dept of Pathology, University and University Hospital of Kuopio, Finland

Background. Prolyl oligopeptidase (POP) is a cytosolic endopeptidase involved in the degradation of several proline containing neuropeptides implicated in learning and memory processes. Several studies have suggested that POP may participate in the pathogenesis of Alzheimer's disease (AD).

This study evaluated the activity of POP in human and animal tissue by investigating postmortem brain samples from AD patients as well as transgenic mice with excessive deposits of β-amyloid (Aβ).

Methods. The investigated patients fulfilled the histopathological CERAD criteria for AD, and the transgenic mice co-expressed familial AD-linked human presenilin 1 (A246E) and amyloid precursor protein (APP_{swe}). The brain tissues were homogenized and POP activity was assayed fluorometrically from cytosolic fraction with suc-Gly-Pro-AMC as substrate.

Results. In AD cases, the POP activity correlated significantly with the premortem estimated mini mental state examination scores, senile/neuritic plaque score, neurofibrillary tangle count but not with Aβ load. The transgenic mice have enormously elevated levels of cortical $Aβ_{1-42}$, however there were no differences in their POP activity compared to wild type mice.

Conclusions. Our results indicate that POP does not participate in Aβ-related etiology of AD. The low POP activity seems to be associated with neuronal damage, seen as neurofibrillary degeneration in AD.

Pathology

16. Cajal-Retzius cells in Alzheimer's disease and normal aging

Riedel A, Miettinen R, Mikkonen M, Alafuzoff I, Soininen H and Arendt Th.

Paul Flechsig Institute for Brain Research, Department of Neuroanatomy, Leipzig University; Department of Neuroscience and Neurology; and Department of Pathology, University of Kuopio and Kuopio University Hospital.

Background. Neurodegeneration in Alzheimer´s disease (AD) is associated with aberrancies in neuronal plasticity and repair. Furthermore, a variety of molecular developmental mechanisms is re-activated. Cajal-Retzius cells (CRc), playing a critical role in signaling and trophic functions during embryonic development, might also be involved in neuronal repair in the mature nervous system. Thus, the question arose whether these cells undergo quantitative or qualitative changes in AD.

Methods. CRc of the entorhinal cortex / hippocampal

formation in AD and normal elderly were immunostained using anti-Reelin monoclonal mouse antibody 142 (deBergeyck et al. 1998). Several cytochemical markers were applied in double labelling experiments to further characterize CRc.

Results. Reelin-immunoreactive CRc were highly abundant in the entorhinal cortex of both AD cases and normal elderly displaying a high degree of intra-individual morphological variability. However, no major difference in number was observed between the groups. Neither was there any apparent association of CRc or other Reelin-immunoreactive structures with plaques or tangles.

Conclusions. CRc apparently do not degenerate in AD. Moreover, they seem to preserve the ability to undergo changes in soma shape, dendritic orientation and even laminar distribution both in AD and elderly controls. Whether CRc are specifically involved in neuronal remodeling during aging and neurodegeneration remains to be investigated.

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17. Loss of cholinergic neurons of septum pellucidum in Alzheimer's disease: a Golgi and electron microscope study

S.J.Baloyannis, V.Costa

1st Department of Neurology, Aristotelian University, Thessaloniki, Greece

Background. Morphological and morphometric alterations have been described in the nucleus basalis in Alzheimer's disease, resulting in progressive cholinergic deficit, which is associated with the decline of the mental faculties of the patients.In the present study we tried to proceed to morphological and morphometric estimation of the neuronal networks of the septum pellucidum, which is also one of the main cholinergic structures of the brain.

Methods. This morphological study is based on examination of twelve brains obtained at autopsy 30 min. after death. Samples from the septum pellucidum were excised and immediately immersed in Sotelo's fixing solution and they were processed for electron microscope. The brains, which were processed for the silver impregnation techniques, were remained for two weeks in formalin.Then the septum pellucidum was excised and immersed in potassium dichromate (7g potassium dichromate in 300 ml of water) for ten days. Then the specimens were immersed in 1% silver nitrate for ten days, according to rapid Golgi method. The morphological and morphometric study was carried into effect in a Zeiss axiolab photomicroscope.The results were correlated with relevant morphological and morphometric analysis of the neuronal

population of the septum pellucidum of normal brains of the same age with the patients.

Results. The morphological analysis, revealed a marked change of the cytoarchitecture of the septum pellucidum of the patients suffered from Alzheimer's disease. Loss of neurons and astrocytic proliferation were also very prominent phenomena in the brains of Alzheimer's patients. The septum pellucidum is characterized normally by the presence of numerous small round, elongated or triangular neurons arrainged in round homocentric networks. In the brains of Alzheimer's patients the homocentric neuronal networks were disrupted and disarrainged and large number of neurons were replaced by astrocytes. Numerous synaptic alterations concerning the dendritic spines of the small round neurons and the triangular ones were seen in electron microscope. Some of the synapses, which remained still intact, contained limited number of round synaptic vesicles and elongated, morphologically alterated, mitochondria.The morphometric analysis revealed an average loss of neurons of the septum pellucidum approaching to 70%.

Conclusions. The above described morphological and morphometric observations, plead obviously in favour of substantial neuronal loss and synaptic alterations in the septum pellucidum of the brains of patients suffered from Alzheimer's disease, a fact which eventually increases the cholinergic deficit.

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18. Neuronal plasticity in Alzheimer's disease: Calretinin-immunoreactive neurons express polysialylated neural cell adhesion molecule in the hippocampus

Giedrius Kalesnykas, Mia Mikkonen, Irina Alafuzoff, Hilkka Soininen and Riitta Miettinen

Department of Neuroscience and Neurology, Department of Pathology, University and University Hospital of Kuopio, Finland

Background. Recently, we found an increased number of polysialylated neural cell adhesion molecule (PSA-NCAM)-immunoreactive infragranular cells in the dentate gyrus of patients having Alzheimer's disease (AD). PSA-NCAM is a polysialylated form of cell surface glycoprotein that is highly expressed during neural development. In adults, it is prominent in situations where remodeling and repair is required. Furthermore, colocalization studies have shown that it is coexpressed with a thymidine analog, BrdU, which is a marker for newly generated neurons. In another study, we further found that neurons containing calretinin (CR) were well preserved in AD cases. CR is a calcium binding protein and in the dentate gyrus, it is expressed in interneurons many of which have similar morphological features as those expressing PSA-

NCAM. The aim of this study was to investigate whether CR and PSA-NCAM are colocalized.

Materials and methods. Hippocampal sections from both control and AD cases were double immunostained for CR and PSA-NCAM using fluorescent-labelled antibodies. Confocal laser scanning microscope was used for the analyses of colocalization.

Results. The colocalization analyses showed that about one-fourth of CR-immunoreactive neurons express PSA-NCAM. The neurons, which were positive for both of these markers, were mainly located in the hilus of the dentate gyrus and they were multipolar or bipolar in shape. Some scattered double-labelled neurons were also found in the outer molecular layer of dentate gyrus and in CA3 area of hippocampus. These neurons were mainly large and multipolar.

Conclusions. The results suggest that a subpopulation of CR containing neurons take part in plastic processes in the human hippocampus.

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19. α-Synuclein pathology in Eastern Finland: A review of 774 cases

Laura Parkkinen, Hilkka Soininen, Irina Alafuzoff

Department of Neuroscience and Neurology, Department of Neurology, Department of Pathology, Kuopio University and Kuopio University Hospital, Kuopio, Finland

Lewy bodies (LBs) and dystrophic neurites (DNs) have been considered as a common substrate for dementia, but also a frequent finding in the unimpaired elderly population. The primary component of this pathology involves α-synuclein. We wanted to establish the prevalence of α-synuclein pathology in the Eastern Finnish population. We also wished to see whether differences could be detected in α-synuclein pathology in relation to age, gender or AD pathology.

We examined the α-synuclein pathology adapting immunohistochemistry in a well-characterized autopsy material. Our study included patients from a clinical study of dementia of Alzheimer's type, subjects from a prospective longitudinal clinical study of aging, a cohort of consecutive clinical autopsy cases collected for one year and a sample of forensic autopsy cases.

None of the cases lacking LBs or pale bodies in the substantia nigra on haematoxylin and eosin (H&E) staining displayed any α-synuclein positive inclusions. The extent of α-synuclein

pathology was, however, more prevalent than the extent of LBs in H&E. Overall, α-synuclein pathology was found in 109 (14%) out of the total 774 subjects over 40 years of age. In the demented group (n=209), 23% showed LB formation, whereas in the non-demented group (n=565), 11% had α-synuclein positive structures. According to further subdivision by study groups α-synuclein immunoreactivity varied from 8% to 27%.

These results indicate that the prevalence of α-synuclein pathology clearly depends on the selection of material. Age at death, gender or AD pathology did not seem to influence the extent of α-synuclein pathology.

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20. Oxidative modification of proteins in Alzheimer's disease brain

Minna A. Korolainen, Gundars Goldsteins, Jari, Koistinaho, Irina Alafuzoff and Tuula Pirttilä

Department of Neuroscience and Neurology, A.I.Virtanen Institute for Molecular Sciences, University of Kuopio, P.O.Box 1627, FIN-70211 Kuopio

Background. Oxidative stress appears to play an important role in Alzheimer's disease (AD) pathogenesis. Oxidative modification of neuronal proteins has been demonstrated to take place in the AD brain. Thus, characterisation of major targets of protein oxidation in AD is important for diagnostic purposes and developing new therapies.

Methods. Frontal cortex tissue of ten patients with histopathologically verified AD and nine controls were used in present study. In order to analyse protein oxidation by detection of protein bound carbonyls, protein extracts were derivatised with 2,4-dinitrophenylhydrazine (DNPH) and then analysed by 2D gel electrophoresis followed by an anti-dinitrophenyl (DNP) immunoblotting.

Results. 2D image analysis revealed differences in protein oxidation in both cytosolic and Triton-X-100 soluble fractions of brain samples. Assessment of the degree of oxidation showed changes in several distinct carbonylated proteins in AD samples compared with controls. The most prominent differences were found in the alkaline region (pI=8.0-9.0) at Mw=35-50kDa.

Conclusions. Up to 150 oxidized proteins were detected in AD and control brains. The extent of oxidation of particular proteins differed between AD and controls. Further identification of these proteins has been undertaken.

This study has been supported by the Academy of Finland and TEKES.

21. Neuropathological characterization of variant Alzheimer's disease with spastic paraparesis

Auli Verkkoniemi, Hannu Kalimo, Anders Paetau, Mirja Somer, Takeshi Iwatsubo, John Hardy, Matti Haltia

Departments of Pathology, University of Helsinki and Helsinki University Central Hospital (AV, AP, MH), Department of Pathology, Turku University Hospital (HK), The Family Federation of Finland (MS), Department of Neuropathology and Neuroscience, Graduate School of Pharmaceutical Sciences, University of Tokyo (TI), Neurogenetics Laboratory, Mayo Clinic Jacksonville (JH)

Background. Variant Alzheimer's disease (varAD) is clinically characterized by presenile dementia and spastic paraparesis. In the original Finnish family varAD was due to a deletion encompassing exon 9 of presenilin 1 gene. Twenty-two patients were identified in four successive generations.

Methods. The brains and spinal cords of five patients were fixed in 4% phosphatebuffered formaldehyde. Representative tissue samples were studied using methods of histology, immunocytochemistry , confocal microscopy and electron microscopy.

Results. The primary and association cortices of these affected individuals and their hippocampi showed a profusion of eosinophilic, roundish structures with distinct borders. These "cotton wool" plaques were immunoreactive for Aβ42/43. However they were devoid of congophilic cores, and fibrillar amyloid could not be identified within them by electron microscopy. CWPs were particularly numerous in the medial motor cortex representing the lower extremities. Degeneration of the lateral corticospinal tracts was observed at the level of medulla oblongata and the spinal cord. In addition, variable numbers of diffuse and cored plaques were found in the cerebral cortex. The cerebellar cortex contained nonnneuritic cored amyloid plaques but no CWPs.

Conclusions. These neuropathological features distinguish varAD from most other forms of early or late onset AD.

22. Screening for presenilin-1 mutations in Finnish early onset Alzheimer's Disease patients

S. Iivonen, M. Hiltunen, A. Mannermaa, S. Helisalmi, M. Pirskanen, I. Alafuzoff, M. Lehtovirta, A.M. Koivisto, S. Tuisku and H. Soininen

Department of Neurology; Department of Clinical Genetics; Department of Pathology; University Hospital and University of Kuopio, P.O. Box 1777, FIN-70211 Kuopio, Finland; and Kokkola Central Hospital, Finland

Background. Mutations in the presenilin-1 (PSEN-1) gene are known to cause familial early onset Alzheimer's disease (AD). Highly penetrant PSEN-1 mutations are more frequent cause of AD than mutations in two other early onset AD genes, PSEN-2 and amloid precursor protein.

Methods. Studies were performed using haplotyping, direct PCR sequencing and RFLP analysis.

Results. We identified a novel 4.6-kb genomic deletion in PSEN-1 in an early onset AD family (mean onset age 43 years), which leads to an inframe exclusion of exon 9 (δ9) from the mRNA transcript. The clinical and neuropathological features of patients in this family resembled those of the typical AD. We also found a causative missense mutation P264L in exon 8 in an early onset AD family (mean onset age 54 years). In addition, E318G substitution was found in AD patients and controls indicating that this substitution is noncausative for AD. However, the allele frequency of the E318G variant was significantly increased in both familial (P = 0.005; OR 7.6 CI 2.2-25.7) and sporadic (P = 0.03; OR 3.1 CI 1.1-8.2) AD groups when compared to the control group.

Conclusions. We found two causative mutations (δ9, P264L) in the early onset AD families and a possible risk factor for AD (E318G) in the PSEN-1 gene.

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23. APP locus and late-onset Alzheimer's disease in a very elderly Finnish population

Liisa Myllykangas, Tuomo Polvikoski, Matti Haltia, Sari Rastas, Auli Verkkoniemi, Pentti J. Tienari, Raimo Sulkava

Department of Pathology, University of Helsinki, Finland; Department of Neurology, Helsinki University Central Hospital, Finland; ans Division of Geriatrics, Department of Public Health and General Practice and Medicine, University of Kuopio, Finland

Background. Mismetabolism of amyloid precursor protein (APP) has been demonstrated to play an important role in the pathogenesis of early-onset Alzheimer's disease (AD). Mutations in the APP gene on chromosome 21q21 have been identified in early-onset AD families, and APP gene dosage appears to contribute to early-onset AD in chromosome 21 trisomy. It remains unestablished whether variation in the APP gene is involved in late-onset AD as well. Although direct analyses of the coding or the promoter sequences of APP have not detected disease-predisposing polymorphisms, it is possible

that there is variation outside these regions that may affect APP gene expression.

Methods. We performed a thorough genetic analysis on the impact of the APP locus on late-onset AD. In total 9 markers, 4 single nucleotide polymorphisms (SNPs) and 5 repeat markers located within or close to the APP gene were analyzed in a population-based sample (Vantaa 85+ Study). The genotype distributions of neuropathologically verified AD cases (n=124) and controls (n=76) were compared.

Results. No evidence for allelic association between the APP markers and AD was found. Stratification according to the APOE ε4-allele carrier status did not change the results.

Conclusions. Our results do not support the hypothesis that allelic variation at or near the APP locus would confer susceptibility to AD in this very elderly Finnish population.

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24. Dipeptidyl carboxypeptidase 1, estrogen receptor alpha and interleukin 1 alpha polymorphisms in Finnish late onset Alzheimer's disease

Mia Pirskanen, Mikko Hiltunen, Susan Iivonen, Arto Mannermaa, Seppo Helisalmi, Maarit Lehtovirta, Anne Maria Koivisto, Markku Laakso and Hilkka Soininen

Department of Neuroscience and Neurology, University Hospital and University of Kuopio, Kuopio, Finland. Chromosome and DNA Laboratory, University Hospital of Kuopio, P.O.B 1777, FIN-70211 Kuopio, Finland; Chromosome and DNA Laboratory, University Hospital of Kuopio, P.O.B 1777, FIN-70211 Kuopio, Finland; Department of Neuroscience and Neurology, University Hospital and University of Kuopio, P.O.B 1777, FIN-70211 Kuopio, Finland; Department of Internal Medicine, University Hospital and University of Kuopio, P.O. B 1777, FIN-70211 Kuopio, Finland

Background. In addition to the apolipoprotein E (APOE) gene, other polymorphic genes are also likely to operate as risk factors in late onset AD (LOAD). We studied the role of the polymorphism in three different genes implicated in LOAD; the insertion/deletion polymorphism of dipeptidyl carboxypeptidase 1 (ACE), the *PvuII* and *XbaI* polymorphisms of estrogen receptor alpha (ESR1) and the C-to-T transition polymorphism at -889 of the interleukin 1 alpha (IL1A).

Methods. ACE, ESR1 and IL1A polymorphisms were genotyped from 86 LOAD patients and 200 representative control subjects.

Results. No association was found between the LOAD and control ACE (χ^2, p = 0.81), ESR1 *PvuII* (χ^2, p = 0.85), ESR1 *XbaI* (χ^2, p = 0.78) or IL1A (χ^2, p = 0.80) genotype.

Conclusions. Our data suggest no association between the studied ACE, ESR1 and IL1A polymorphisms and LOAD.

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25. Midlife vascular risk factors and late-life mild cognitive impairment. A longitudinal, population-based study.

Kivipelto M, Helkala E-L, Hänninen T, Laakso M, Hallikainen M, Alhainen K, Soininen H, Tuomilehto J, Nissinen A.

Department of Neuroscience and Neurology, and Department of Public Health and General Practice, University of Kuopio; Department of Neurology, Kuopio University Hospital, Kuopio; North Karelia Central Hospital, Joensuu; and National Public Health Institute, Helsinki, Finland

Background. Mild cognitive impairment (MCI) has been considered as a predictor of Alzheimer's disease (AD). Vascular risk factors may be important in the development of cognitive impairment and AD. However, the role of vascular risk factors for MCI, as well as the prevalence of MCI, remains still virtually unknown. The aim of this study was to evaluate the impact of midlife elevated serum cholesterol levels and blood pressure (BP) on the subsequent development of MCI, and to investigate the prevalence of MCI in elderly Finnish population applying the MCI criteria devised by the Mayo Clinic AD Research Center.

Methods. Subjects were derived from random, population-based samples previously studied in one of the surveys carried out in 1972, 1977, 1982 and 1987. After an average follow-up of 21 years, altogether 1449 (73%) subjects aged 65-79 years participated in the re-examination in 1998.

Results. A total of 82 subjects, 6.1% of the population, met the criteria for MCI. Midlife elevated serum cholesterol level (>= 6.5 mmol/l) was a significant risk factor for MCI (OR 1.9, 95% CI 1.2-3.0, $p<0.001$ adjusted for age and BMI) with the effect of systolic BP approaching significance ($p=0.07$).

Conclusion. Our data point to a role for midlife vascular risk factors on the development of MCI in late-life, and raise also the question whether active treatment of hypercholesterolemia, and possibly also hypertension at midlife may postpone or prevent the development of cognitive impairment.

26. APOE ε4 is associated with weight loss in women with AD: a population-based study

M. Vanhanen, M. Kivipelto, K. Koivisto, J. Kuusisto, L. Mykkänen, E-L. Helkala, T. Hänninen, K. Kervinen, Y.A. Kesäniemi, M.P. Laakso, H. Soininen, M. Laakso

Departments of Neurology (Vanhanen, Kivipelto, Koivisto, Hänninen, M.P.

Laakso, Soininen), Medicine (Kuusisto, Mykkänen, M. Laakso), and Community Health and General Practice (Helkala) of the Kuopio University Hospital and the University of Kuopio, Kuopio, and from the Department of Internal Medicine and Biocenter Oulu (Kervinen, Kesäniemi) of the University of Oulu, Oulu, Finland

Background. Our objective was to investigate whether the apolipoprotein E (APOE) ε4 allele is associated with weight loss in patients with Alzheimer's disease (AD) or in nondemented elderly subjects: Weight loss has been considered as a typical feature of AD. APOE ε4 is a risk factor for AD and was recently proposed to be associated with weight loss in elderly women. It is not known whether APOE ε4 is associated with weight loss in AD or in the general population.

Methods. Weight and BMI measurements at an average interval of 3.5 years and APOE phenotype determination were performed in an elderly population (n=980), including 46 AD patients and 911 control subjects at the end of the follow-up.

Results. On average, AD-patients with the ε4 allele lost weight 1.9±4.0 kg (BMI 0.8±1.8 kg/m^2) whereas ε4 noncarriers gained 1.2±3.8 kg (BMI 0.4±1.5 kg/m^2) (both $p<0.05$), after controlling for diabetes and exercise. However, when men and women were analyzed separately, weight loss was observed only in those AD women with the ε4 allele. Clinically significant weight loss, defined as loss of =5% of body weight, occurred more frequently in both AD-patients (30% versus 6%, $p<0.05$) and controls (28% versus 18%, $p<0.001$) carrying the ε4 allele.

Conclusions. The APOE ε4 allele may contribute to the unexplained weight loss in AD, especially in women.

Cognition

27. Associative encoding activates the perirhinal cortex: An fMRI study

Maija Pihlajamäki, Heikki Tanila, Tuomo Hänninen, Mervi Könönen, Mia Mikkonen, Ville Jalkanen, Kaarina Partanen, Hannu J. Aronen, and Hilkka Soininen

Department of Neuroscience and Neurology, University of Kuopio; Department of Clinical Radiology, Kuopio University Hospital; Department of Neurology, Kuopio University Hospital, and Department of Radiology, Helsinki University Central Hospital

Background. It is well established in non-human primates that the hippocampus and the ento- and perirhinal cortices are

necessary for declarative memory encoding. In humans, the neuropathologic and neuropsychologic changes in early Alzheimer's disease (AD) further support a role for the rhinal cortex in long-term memory processes. Little is known, however, about the function of the rhinal cortex in humans *in vivo*. Therefore, functional magnetic resonance imaging (fMRI) was used to examine the participation of the rhinal cortex in visual associative encoding.

Methods. A total of 12 subjects (6 males, mean age = 25) participated in this study. fMR-imaging was performed on a Siemens Vision 1.5 T scanner (gradient-echo EPI sequence; 128 sets of 16 5-mm axial slices). Image processing was performed using MEDx software.

Results. The most consistent MTL activation ($p < 0.00001$) was found in the posterior perirhinal cortex, in the medial bank of the collateral sulcus. The hippocampus, posterior parahippocampal gyrus, and the temporo-parietal association cortices were also activated.

Conclusions. To our knowledge, this is the first fMRI study reporting activation in this medial part of the perirhinal cortex, the site of the early neuropathologic changes in AD.

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28. Scopolamine modulates pre-attentive auditory processing in elderly subjects: an MEG study

Eero Pekkonen, Iiro. P Jääskeläinen, Seppo Kaakkola, Juha Huttunen, Jyrki Ahveninen

Department of Neurology, Helsinki University Central Hospital, Finland; Cognitive Brain Research Unit, Department of Psychology, University of Helsinki, Finland; BioMag Laboratory, Medical Engineering Centre, Helsinki University Central Hospital, Finland

Background. Pre-attentive auditory processing leading to stimulus detection can be studied objectively with event-related potential (ERP) components termed P50 and N100, which are generated in the auditory cortex. Our previous studies with magnetoencephalography (MEG) have shown that magnetic P50m and N100m responses are delayed, and that N100m delay significantly correlated with the impaired language functions in patients with Alzheimer's disease (AD).

Methods. Here we studied whether the pre-attentive auditory processing, as measured with P50m and N100m, is modulated by the cholinergic activity in healthy elderly subjects. Monaurally presented auditory responses to the frequent sounds were recorded with a whole-head 122-channel magnetometer from 9 elderly subjects after intravenous infusion of either scopolamine or glycopyrrolate using a randomised, double-

blind design. Scopolamine is a centrally acting acetylcholine muscarinic receptor antagonist, whereas a control drug glycopyrrolate has only peripheral effects resembling that of scopolamine.

Results. Scopolamine significantly delayed P50m and N100m, decreased N100m and increased P50m response.

Conclusion. Our results indicate that the cholinergic system modulates differently magnetic ERP components underlying pre-attentive auditory processing as shown by auditory P50m and N100m responses. MEG could be useful in measuring cholinergic dysfunction in patients with AD.

References. Pekkonen E et al. Impaired preconscious auditory processing and cognitive function in Alzheimer's disease. Clinical Neurophysiology 1999, 110(11): 1942-1947.

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29. Action verbal fluency in Alzheimer's Disease

Seija Pekkala

Department of Phonetics, University of Helsinki, Finland

Background. Alzheimer's disease (AD) is characterized by a specific dysfunction of semantic memory, either due to impaired retrieval or degradation of semantic information. In the category fluency task with nouns, the AD patients' ability to produce category-related words, to organize semantic information into clusters of subcategory items, and to shift mental set is deteriorated (Binetti et al. 1995; Troyer et al. 1997).

Methods. In order to study the integrity and search of verb categories, the semantic fluency task (60 s) was performed by 20 mildly and 20 moderately demented AD patients and 30 normal controls (NC). The semantic categories of the verbs were to cook, to do sports, to build and to clean. The total number of correct words, the number of clusters and switches and the number and type of errors were recorded.

Results. Compared to the performance of NC group, an increase in errors and a decrease in correct responses contributed to the performance of both AD groups. The AD patients produced more perseverations and intrusions and showed less clustering and switching within all verb categories. The performance was more affected in the group of moderately demented AD patients.

Conclusions. The AD participants' performance indicated impaired organization and search of verbs. The semantic

fluency task proved to be suitable for studying verbs. However, differences between nouns and verbs should be kept in mind when analyzing the semantic representation of category related verbs.

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30. Language symbols, Cognitive Impairment and Cognitive Intervention in the Alzheimer's Disease

Henry S. R. Kao

Department of Psychology, University of Hong Kong

All human writing systems share certain visual spatial properties as the basis of cognitive processing, especially in the case of reading and writing. These written scripts vary in the extent and complexity of such visual properties, which affect the efficient operation of cognitive functions. These properties include square, closure and symmetry. Recent experiments have demonstrated that active processing of such variations in Chinese and English scripts account for differential cognitive facilitation in reading as well as in writing. Script forms rich in these geometric properties resulted in greater cognitive activation than those poor in such properties. These findings formed the basis of an innovative approach in the intervention of cognitive impairment in the Alzheimer's Disease.

Our recent research made use of the training of Chinese calligraphic handwriting (CCH) as an example of this approach for the treatment of cognitive deficiencies of the AD patients. The training of CCH has shown significant improvement in the patient's short-term memory, pictorial memory, orientation, as well as motor control abilities (Kao, et. al., 2000, Alzheimers Reports). This presentation will focus on the theoretical foundation of this CCH intervention as well as a treatment protocol on AD cognitive impairment.

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31. Estimation of frontal lobe dysfunction in patients with Alzheimer's disease by Frontal Assessment Battery (FAB)

Agnieszka Gorzkowska, Stanislaw Ochudlo, Grzegorz Opala

Katedra i Klinika Neurologii Wieku Podeszlego, Department of Late Age Neurology, Silesian Medical University, Medyków Street 14, 40-752 Katowice, Poland; e-mail: neurowp@infomed.slam.katowice.pl , fax: +4832-2046164

Objective. The aim of this study was to assess frontal lobe dysfunction in patients with AD by Frontal Assessment Battery (FAB).

Background. The functional changes in the frontal lobe in

Azheimer's disease are noted. The changes are manifested by an impairment of cognitive functions associated with this cerebral region.

Design/Methods. The FAB is short and fast test to assess frontal lobe dysfunction. It includes six subtests (conceptualization, verbal fluency, motor series, conflicting instruction and go-no go, prehension behavior). Originally, authors (A.Dubois et al, 1998) used the FAB to assess frontal lobe dysfunction in patients with various degree of the disturbances confirmed by Positron Emission Tomography (PET). A group of twenty one patients (11 women and 10 men) aged 51-82 with probable Alzheimer's disease diagnosed according to NINCDS-ADRDA was studied. Mini-Mental State Examination (MMSE) score in our group was 19. Using SPECT (Single Photon Emission Computed Tomography) it was shown, that 14 of 21 patients had decreased frontal 99mTc-HMPAO up-take.

Results. The FAB scores were 7,7. The patients with MMSE of 24 to 29 points, showed features of frontal lobe dysfunction (FAB=11,6; from 10 to 13 points). Intensification of these disturbances increased with a cognitive functions' decline (MMSE< 24, FAB=5,3; from 2 to11 points). FAB presents progress of changes, which result from process of progressive cerebral damages - also his frontal region.

Conclusions. Our preliminary results suggest that the FAB is sensitive score to assess frontal lobe dysfunction among the patients with Alzheimer's disease with and without of frontal up-take decrease in SPECT.

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32. Characteristics of Two Telephone Screens for Cognitive Impairment

Tarja Järvenpää, Juha O. Rinne, Ismo Räihä, Markku Koskenvuo, Minna Löppönen, Susanna Hinkka, Jaakko Kaprio

University of Turku; Turku City Hospital; University of Oulu; Finland

Background. Telephone instruments designed to detect cognitive impairment can facilitate follow-up in longitudinal studies and can be used for screening populations in epidemiological studies. In this study, we evaluated the feasibility of two telephone screens for cognitive impairment, a self-report interview referred to as the TELE and the Telephone Interview for Cognitive Status (TICS).

Methods. We studied 56 subjects, 30 patients with a clinical diagnosis of mild to moderate Alzheimer's disease (AD) and 26 age-matched healthy controls, using the TELE and the TICS instruments.

Results. The sensitivity and specificity of the TELE to differentiate AD patients from healthy controls was 90.0 % and 88.5 %, and of the TICS, 86.7 % and 88.5 %, respectively. When receiver operator characteristic (ROC) curves were constructed, the area under the curve (AUC) for the TELE was 96.0 % (SE 2.4 %), and for the TICS 90.3 % (SE 4.2 %). In the patient group, the Pearson's correlation between the TELE and the Mini-Mental State Examination (MMSE) was 0.78, $p < 0.0001$, and between the TICS and the MMSE 0.77, $p < 0.0001$. The correlation between the TELE and the Sum of the Boxes of the Clinical Dementia Rating scale (CDR-SB) was - 0.71, $p < 0.0001$, and between the TICS and the CDR-SB - 0.75, $p < 0.0001$.

Conclusions. These results indicate that both screens are sensitive and specific instruments for differentiating AD patients from healthy controls and have a strong correlation with face-to-face measures of cognitive function.

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33. Definition of cognitive impairment in a nondemented elderly population

Susanna Tuomainen, Tuomo Hänninen, Matti Vanhanen, Merja Hallikainen, and Hilkka Soininen

Department of Neuroscience and Neurology, University of Kuopio; Department of Neurology, Kuopio University Hospital, Kuopio

Objectives. To compare two different neuropsychological criteria for the characterization of cognitive impairment.

Material and methods. We evaluated cognitive function in a population- based sample of 806 subjects (aged 60-76 years). Cognitive impairment was defined either as a) impaired delayed memory performance or b) general cognitive deficit (MMSE score). Cut off score of one SD below the mean in each age- and education-specific group was used.

Results. The overlap between subjects identified by these two sets of criteria was only 21.8%. The subjects fulfilling both criteria had more health related problems and used more frequently medication than cognitively normal subjects. The subjects identified as impaired by the memory criteria had more health related problems than the subjects identified by MMSE criteria.

Conclusion. There was only a relatively minor overlap between subjects identified as cognitively impaired by these two definitions based either on delayed memory score or MMSE. This discrepancy will presumably result in different predictive value for the conversion to dementia. Thus, the harmonization of the criteria for cognitive impairment falling

short of dementia is of crucial importance in studies searching for prognostic factors of cognitive decline in mild cognitive impairment.

Diagnosis

34. Structural scanning in dementia evaluation: Impact on diagnosis and treatment.

Steen G. Hasselbalch, Leif Sørensen, Carsten Gyldensted, Hans Brændgaard, Ann-Sofie Teller, Gunhild Waldemar.

Memory Disorders Research Unit, The Neuroscience Center, Rigshospitalet, Copenhagen University Hospital, and Departments of Neuroradiology, and Neurology, Århus University Hospital, Denmark.

Background. CT or MRI in patients refered for memory problems are used to rule out potentially reversible conditions (subdural hematomas, NPH, or tumors), and to verify other possible etiologies, such as cerebrovascular disease. The aim of this study was to evaluate the added value of structural scanning in diagnosis and treatment of cognitive dysfunction.

Methods. From two out patient memory clinics, the records of 336 consecutive scanned patients (303 CT, 17 MRI, 16 CT+MRI) refered for memory complaints were reviewed, and patients were grouped in one of several diagnostic groups using international diagnostic criteria without knowledge of scan results. Treatment was assigned to these groups according to well established clinical guidelines. Following the addition of scan results, changes in diagnosis and treatment were recorded.

Results. Structural scanning changed the diagnosis in 37% of the patients, 11 patients had one or more potentially reversible pathologies, and in 116 patients diagnosis changed because of presence/absence of cerebrovascular disease in patients not suspected/suspected of vascular pathology. In 33% of the patients, scanning changed treatment primarily because of addition of thromboprophylactic medication.

Conclusions. In one third of patients refered to memory clinics for dementia evaluation, structural scanning changed diagnosis and treatment, primarily because of visualization of cerebrovascular disease in patients with no clinical symptoms of vascular pathology.

35. Brain acetylcholinesterase activity in Alzheimer's

Disease measured with PET

Juha O. Rinne, Valtteri Kaasinen, Kjell Någren, Anne Roivainen, Meixiang Yu

Turku PET Centre, University of Turku, Turku, Finland

Background. Cholinergic deficiency is the most pronounced and consistently reported biochemical abnormality in Alzheimer's disease (AD). The current drugs for AD are inhibitors of acetylcholinesterase (AChE).

Methods. We investigated brain AChE activity with positron emission tomography (PET) in nine patients with AD and four healthy controls. Carbon-11 labelled acetylcholine analogue MP4A was used as a tracer. About 550 MBq (15 mCi) was injected intravenously and a 60 min dynamic PET study was performed. Concentrations of MP4A and its metabolites were determined from arterial blood samples. The regions of interest (ROIs) were drawn on the MRI images and copied into PET images. The regional uptake of MP4A was evaluated by calculating regional brain retention indices (min^{-1}) from the retention index images. After initial PET scan, AD patients were started either with donepezil (four patients, dose 10mg daily) or rivastigmine (five patients, dose 4.5 mg twice a day) medication and were rescanned after three months of treatment.

Results. The reduction in AChE activity ranged from 41 to 48 % for donepezil and 41 to 45 % for rivastigmine in the temporal cortex, frontal cortex and thalamus. In healthy controls a nonsignificant 3-4% intrasubject variability was seen in the test-retest analysis.

Conclusions. The results show that carbon-11 labelled MP4A can be used to evaluate brain AChE activity, and that this activity is significantly reduced by current AD drugs. In the future, mapping of AChE activity with PET may provides a tool for the assessment of possible regional selectivity, dose dependence etc. of cholinesterase inhibitors in AD.

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36. Amyloid Precursor Protein (APP) in Platelets: a Biochemical Marker for an Early Diagnosis of Alzheimer Disease

Barbara Borroni, Francesca Colciaghi, Paola Corsini, Luca Rozzini, Flaminio Cattabeni, Monica Di Luca, Alessandro Padovani

Clinica Neurologica, Università degli Studi di Brescia; Istituto di Farmacologia, Università degli Studi di Brescia

Objective. To evaluate accuracy of platelet APP forms' ratio

(APPr) for the early identification of Alzheimer Disease (AD). Background. Three major APP forms with molecular weight of 130, 110, 106 kd are present in human platelets. It has been demonstrated that the ratio between 130 kd and 106- to 110 kd APP forms is markedly reduced in Alzheimer disease.

Methods. The study was performed on 42 mild AD patients (mAD), 30 patients with mild cognitive impairment (MCI), and 55 aged-matched controls subjects (CTRL). Platelets were subjected to Western Blot analysis, using monoclonal antibody 22C11.

Results. Compared to CTRL (mean ± SD= 0.89 ± 0.3), mean APPr was decreased in mAD (mean ± SD = 0.44 ± 0.15; $p<0.0001$) and in MCI (mean ± SD = 0.63 ± 0.37; $p<0.001$). A cut-off level of 0.59 resulted in sensitivity of 88.1% and specificity of 87.5% for mAD and CTRL. With regard to MCI, 19/30 (63.3%) subjects showed an APPr below the cut-off (see table for normality curves in the three groups).

Conclusion. Levels of APPr has a high sensitivity and specificity to differentiate AD from normal aging and other dementias. Findings on MCI suggests that platelet APPr may be useful for the early identification of AD.

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37. Increased CSF levels of neurofilament protein in individuals with white matter changes

M Sjögren, M Blomberg,, K Blennow, and A Wallin

Institute of Clinical Neuroscience, Göteborg University, Sweden; Neurotec Institute,Huddinge Hospital, Sweden; The Research Council, Sweden; Correspondence: Magnus Sjögren, Inst Clinical Neuroscience, Sahlgrenska universitetssjukhuset / Mölndal, SE 431 80 Mölndal, Sweden, Phone: +46-31-3432382, E-mail: magnus.sjogren@medfak.gu.se

Background. White matter changes on CT or MRI of the brain are common in normal aging with an increasing frequency in Alzheimer's disease (AD) and subcortical vascular dementia (SVD). The corresponding neurochemical changes in the Cerebrospinal fluid (CSF) are largely unknown.

Methods. CSF levels of tau, β-amyloid42 (Aβ42) and the light neurofilament protein (NFL) were investigated in 37 patients with AD, 18 patients with SVD and 20 controls. Specially designed ELISAs were used for the quantification of CSF-tau, CSF-Aβ42 and CSF-NFL. The Blennow/Wallin scale was used to semi-quantify WMC.

Results. Individuals with extensive WMC had increased CSF-NFL ($p=0.003$), but no changes were found for CSF-tau or CSF-Aβ42. The results were corrected for age, gender and

degree of cognitive impairment. In the group with mild or no WMC, significant correlations were found between the mini-mental state examination score (MMSE) and CSF-tau ($R=-0.59$, $p<0.001$), CSF-NFL ($R=-0.72$, $p<0.001$), and CSF-Aβ42 ($R=0.44$, $p<0.001$).

Conclusions. Since NFL is mainly found in large myelinated axons, WMC may be associated with axonal degeneration especially of this type of neurons.

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38. Clinical and neuropathological findings in patients with possible idiopathic normal-pressure hydrocephalus; A prospective study of 38 patients.

Bech RA, Høgh P, Juhler M, Gjerris F, Nissen A-M, Waldemar G.

Departments of Neurology and Neurosurgery and the Memory Disorders Research Unit, The Neuroscience Center, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark.

Background. In patients suspected of idiopathic normal-pressure hydrocephalus (INPH) it is speculated, whether cerebral parenchymal changes induced by degenerative or arteriosclerotic disorders are involved in the pathophysiology.

Methods. Thirty-eight consecutive patients, referred with a clinicoradiological suspicion of INPH, underwent a clinical evaluation and frontal cerebral biopsy. Special emphasis was put on the clinical and/or neuropathological presence of cerebrovascular disease (CVD) and Alzheimer's disease (AD). **Results.** Twenty-nine patients fulfilled rigorous clinical, radiological and hydrodynamic shunt criteria and underwent shunt operation, independently of the cerebral biopsy findings or clinically diagnosed concomitant disorders. Only 33 percent of the shunted patients improved. Pathological changes were described in over half of the biopsies, with Alzheimer changes ($n = 9$) and vascular changes ($n = 9$) being the most frequent findings. Of the evaluated patients over half had clinical concurrent CVD, and half fulfilled the NINCS-ADRDA criteria for possible AD. No correlation was found between the cerebral parenchymal findings and the clinical diagnoses of AD and CVD, respectively. The presence of pathological biopsy changes did not preclude a clinical improvement after shunt operation, but the clinicoradiological diagnosis of CVD was associated with a poorer outcome. **Conclusions.** The heterogeneous clinical and cerebral biopsy findings support the perception of INPH as a multietiological clinical entity.

Therapy

39. No Change in behavioural and psychiatric symptoms on withdrawal from Thioridazine: Preliminary Report from a Placebo Controlled Disontinuation Trial in Dementia

Alan Thomas, John O'Brien, Alan Swann, Clive Ballard

Institute for the Health of the Elderly, Newcastle University, UK

Background. There is increasing concern about adverse effects of thioridazine, especially in the elderly with dementia yet it remains a widely prescribed drug.

Methods. A placebo controlled, double blind discontinuation trial of thioridazine on patients with dementia residing in residential or nursing homes, with stable behaviour problems who had been receiving thioridazine for at least 3 months. 18 were randomly allocated to thioridazine and 18 to placebo for 3 months. Behavioural and Psychiatric symptoms were evaluated with the Neuropsychiatric Inventory at baseline and monthly thereafter.

Results. There were no significant differences in any of the sample characteristics (placebo v thioridazine: age 81.0 v 80.1, female gender 72% v 56%, MMSE score 5.9 v 3.8), but the placebo treated group had significantly higher baseline NPI scores (17.5 v 10.7 t=2.5 p=0.02). Fourteen patients withdrew from the study, 12 (33%) because of worsening behavioural problems (6 in each group) and 2 died (1 in each group). Thirty-five of the patients completed at least 1 follow-up assessment. Patients receiving placebo had a significantly greater improvement in NPI score than those continuing to receive thioridazine (-4.2 v +2 t=2.1 p=0.04).

Conclusions. Two-thirds of the patients were able to complete 3 months of placebo treatment without clinically significant worsening and they showed no evidence of any significant deterioration of behavioural symptoms, paradoxically there was even some evidence to indicate improvement. We recommend a carefully monitored trial of treatment discontinuation may be an appropriate option for dementia patients with stable behavioural problems.

We would like to thank Research into Ageing for funding this study.

Pathology

40. The new consensus recommendations for the

postmortem diagnosis of Alzheimer's disease: how practical are they?

Tuomo Polvikoski and Matti Haltia

Department of Pathology, University of Helsinki and Helsinki University Central Hospital, Helsinki

Background: The new consensus recommendations for the postmortem diagnosis of Alzheimer's disease provide an estimate of the likelihood that Alzheimer's disease pathological changes underlie dementia, by using various combinations of the CERAD score of neocortical neuritic plaques and the stage of neurofibrillary pathology according to Braak and Braak

- high likelihood: neuritic plaque score "frequent " and stages V or VI of the neurofibrillary pathology
- intermediate likelihood: neuritic plaque score "moderate " and stages III or IV
- low likelihood: neuritic plaque score "infrequent " and stages I or II

To test how practical the consensus recommendations are, we have analyzed the CERAD scores and Braak stages of postmortem brains derived from a population-based cohort of individuals aged 85 years or more.

Methods: 227 formaldehyde-fixed brains were available for the study. The neuritic plaque score was analyzed according to the CERAD protocol, and the stage of neurofibrillary pathology according to Braak and Braak.

Results: The neurofibrillary stage was I or more in 220 brains. When the neuritic plaque scores of these 220 brains were analyzed, only 41 % of the neuritic plaque scores and the stages of neurofibrillary pathology matched as suggested according to the consensus recommendations.

Conclusions: The consensus recommendations seem to be too rigid, because it was possible to estimate the likelihood of Alzheimer's disease according to the consensus recommendations in less than half of the cases.

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Early Pathology:
Chairpersons Irina Alafuzoff and Dennis Dickson

Neuropathology in Aging

D.G. Davis, F.A.Schmitt, D.R. Wekstein, W.R. Markesbery

Sanders-Brown Center on Aging, Departments of Pathology and Laboratory Medicine, Neurology and Physiology, University of Kentucky Medical Center, Lexington, KY. USA

The Sanders-Brown Center on Aging conducts a longitudinal study on aging and Alzheimer's disease which now spans more than a decade. The Center follows a large number of cognitively normal volunteer subjects who have consented for autopsy. The neuropathologic autopsy findings described are from a study of 89 elderly, well-educated, prospectively followed cognitively normal volunteer subjects. These subjects had annual mental status testing and most had neurologic and physical examinations every two years. Some subjects had been followed for up to ten years. The major findings of this study

are: Only 15 of 89 (17%) had no or few neuropathologic alterations. Thirty-eight percent of these subjects met The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) diagnostic criteria for Alzheimer's disease (AD) (9 possible, 17 probable, 8 definite) and 29% met National Institute on Aging-Regan Institute (NIA-RI) guidelines (2 low, 18 intermediate, 6 high). Three subjects had abundant neurofibrillary pathology without senile plaques limited to medial temporal lobe structures, sufficient for the diagnosis of hippocampal-only AD. These three subjects did not meet either CERAD or NIA-RI diagnostic guidelines. Cerebral infarction was common - 34 (38%) had grossly visible infarcts and 61 (68%) had microinfarcts. Fifty-eight percent of the microinfarcts were remote and 19% were acute. The four most common sites of microinfarcts were cerebellum, occiptal lobe, neostriatum and hippocampus. Hippocampal sclerosis was present in two subjects. Vascular amyloid deposition was present in 73 of 89 subjects (82%) in meningeal vessels and 51 of 89 (57%) in parenchymal vessels, or in both in 51 of 89 (57%). Argyrophilic grains were present in 22 subjects (24%) in the entorhinal cortex, hippocampal CA1 or the amygdala, or a combination of these regions. These 22 subjects had rare microinfarcts, occasional Lewy bodies, AD changes with or without concomitant severe vascular disease, and some had no other neuropathologic alterations. Using the Lewy Body Disease Consensus Diagnostic Guidelines, 2 subjects had the brainstem form, 1 had the limbic form, and 3 had the neocortical form. Overall, our study demonstrates that the brains of a large percentage of aged, cognitively normal, well-educated subjects contain a wide variety of neuropathologic alterations. The study also gives evidence to support that individuals display a large CNS cognitive functional reserve in spite of harboring a significant neuropathologic burden.

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Neuropathology of Early Alzheimer's Disease

D. Dickson, E. Grober, H. Crystal, R. Lipton, J. Parisi, C. Jack and R. Petersen

Mayo Clinic, Jacksonville, FL & Rochester, MN and Albert Einstein College of Medicine, Bronx, NY, USA. Dennis W. Dickson, MD: Department of Pathology, Neuropathology Laboratory, Mayo Clinic 4500 San Pablo Road, Jacksonville, FL 32224; (904) 953-7137 phone, (904) 953-7117 fax, dickson.dennis@mayo.edu

Defining the pathology of early Alzheimer's disease (AD) is challenging since brain studies are made at a single point in time (i.e. death) in a species (i.e. humans) notable for pathologic heterogeneity. The pathology of advanced AD, but not the transition from normal aging to AD, is known. Advanced AD is characterized by widespread amyloid

deposition and neurofibrillary degeneration in limbic and neocortical areas. Clinicopathologic studies show good correlations between cognitive deficits and neurofibrillary degeneration, and the stereotypic pattern of neurofibrillary degeneration has lead to staging schemes, such as that of Braak and Braak. While stages V-VI are associated with dementia and stages I-II are not, the clinical presentations of stages III-IV are less predictable. In the Bronx Aging Study we found correlations between early memory impairments and stage III, while mental status changes were not found until stage IV and beyond. Unfortunately, there is no way of studying the rate of progression through Braak stages; it can only be estimated from cross-sectional studies. A problem with assigning a diagnosis of "early AD" to cases that do not meet pathologic criteria for AD is that there is no way of knowing if the pathology observed would necessarily have progressed and at what rate. Biomarkers are needed that accurately reflect brain pathology at various stages. Hippocampal atrophy on MRI has been proposed as such a biomarker. Analyses of hippocampal pathology to antemortem MRI hippocampal volumes demonstrated good correlations between Braak stage and hippocampal atrophy when other processes were excluded. Unfortunately, other non-AD dementias (e.g., hippocampal sclerosis) had hippocampal atrophy, and hippocampal atrophy was less in dementia with Lewy bodies, which can be difficult to clinically differentiate from AD. Finally, the pathology of six subjects enrolled in Mayo Alzheimer Research Center who died with mild cognitive impairment, a possible clinical correlate of early AD, revealed pathologic heterogeneity (three had AD; one had tangle only; two had argyrophilic grains). Further studies are needed to better define the pathology and clinical means to accurately diagnose early AD.

The Biochemical Frontier Between Alzheimer's Disease and Aging

Nicolas Sergeant and André Delacourte

INSERM U422, Lille, France

Two types of cortical brain lesions characterize Alzheimer's disease (AD): extracellular amyloid deposits and neurofibrillary degeneration (NFD). Amyloid deposits are made of aggregated amyloid-β peptides. NFD consists of intraneuronal accumulation of fibrillar structures made of pathological tau proteins (Tau 60, 64, 69 and 74), named PHF-tau. Similar lesions are found, in moderate quantities, during "normal" cerebral aging, suggesting a continuum between "normal" aging and AD. But is "normal" cerebral aging related or distinct from AD? Arguments come from our biochemical study of the spatiotemporal distribution of brain lesions in 130 subjects followed prospectively, including 60 non-demented aged cases.

PHF-tau were always observed in the hippocampal area of non-demented patients older than 75 years, sometimes with no trace of amyloid deposits. The pathway of tau pathology in other cortical brain regions was stereotyped, sequential and hierarchical. We have defined 10 stages of tau pathology progression, that correspond to 10 brain areas successively affected: Tau pathology always starts in the hippocampal formation (stages 1 to 3) followed by the anterior, inferior, mid-temporal cortex (stages 4 to 6), then the polymodal association cortical areas of the superior temporal, parietal and frontal cortex (stages 7 and 8), and finishing in the primary motor and visual cortices (stages 9 and 10). Before stage 6, the disease often remains asymptomatic whereas clinical symptoms of AD are well correlated with the progression of tau pathology in the polymodal association cortical areas. Because amyloid deposits are a prerequisite event in AD, we conclude that there is a biochemical frontier between 'normal' cerebral aging and AD. The Criteria to Establish a (post-mortem) Biochemical Diagnosis of AD (CEBDAD) are the followings: for patients aged over 75 years, tau pathology up to stage 3 and no amyloid plaques corresponds to "normal" or more precisely to "usual" aging. Amyloid deposits associated with a tau pathology up to stage 6 corresponds to infra clinical AD while stages 7 to 10 with both lesions correspond to clinical AD.

Pathological Correlates of Dementia in a Community Based Population

Irina Alafuzoff

Department of Neurology, University of Kuopio, Finland

We have carried out a neuropathological study of an elderly Finnish population. Two separate population samples were investigated. The first sample included individuals, born between 1912 and 1921, who were in 1991 drawn randomly from the Kuopio population registry to become members of an aging study. The sample from population registry included 1192 subjects and since 1991, 71 necropsies have been carried out. The second sample included those who had died within the medical service area of Kuopio University Hospital and an autopsy including neuropathological investigation had been carried out. The second, autopsy series of 1998 included 269 subjects within a region of 250 000 inhabitants and 2000 deceased in 1998.

The first sample included 22 males and 49 females, mean age at death was 80 years, ranging from 71 to 87 years. 31% were clinically estimated being demented, with MMSE mean value of 13, ranging from 0 to 22. The second sample included 152 males and 117 females, mean age at death was 69 years, ranging from 16 to 93 years. 12% were clinically estimated

being demented. The number of necropsy cases with age at death exceeding 71 years (age at death comparable to the first sample) was 134 individuals. In 17% subjects clinical signs of dementia were noted.

The severity of Alzheimer- type pathology, vascular lesions and other pathologies were assessed. In the first sample, Alzheimer-type pathology was seen in 74%, vascular lesions in 53% and Lewy inclusions in 14% of the subjects. b-amyloid aggregates estimated using immunohistochemistry were seen in 77% of the cases. In 51% of subjects mixed pathology was seen. When divided into clinical groups Alzheimer-type pathology was seen in 57%, vascular lesions in 47%, Lewy inclusions in 10% and b-amyloid aggregates in 71% of non-demented compared to 78%, 59%, 23% and 79% of demented individuals.

In the second sample of 134 subjects, age at death comparable to the first sample, Alzheimer-type pathology was seen in 52%, vascular lesions in 43%, Lewy inclusions in 14% and b-amyloid aggregates in 60 % of the subjects. Mixed pathologies were seen in 57% of the material. When divided into clinical groups Alzheimer-type pathology was seen in 49%, vascular lesions in 37%, Lewy inclusions in 13% and b-amyloid aggregates in 57% of non-demented compared to 67%, 67%, 19% and 70% of the demented individuals.

Alzheimer type and vascular pathology were common features in both investigated samples and concomitant lesions were frequently seen. Results were significantly influenced by the sampling procedure. The number of lesions was generally higher in demented, however there were several non-demented individuals with numerous lesions lacking clinical symptoms. There were no clear thresholds of pathological features that predicted the clinical dementia status in the investigated material.

UNIVERSITY OF KUOPIO
Department of Neuroscience and Neurology

The Second Kuopio Alzheimer Symposium

Proceedings of the Symposium

March 13.-15. January 2001

Kuopio, Finland

Neuroimaging:
Chairmen Timo Erkinjuntti and Philip Scheltens

Neuroimaging and the early diagnosis of AD
Mony de Leon, USA

Hippocampal Atrophy: Comparison between Visual Rating and Volumetry
Lars-Olof Wahlund, Sweden

Could MRI Be Used to Measure Progression in AD?
Nick Fox, UK

Functional MRI in Alzheimer's Disease

Philip Scheltens, The Netherlands

PET Activation Studies in MCI and AD Patients

Agneta Nordberg, Sweden

In Vivo Hippocampal Formation Pathology: An Early Marker for Staging Alzheimer's Disease

M. J. de Leon, A. Convit, S. DeSanti, and M. Bobinski.

Center for Brain Health, Neuroimaging Laboratory, New York University School of Medicine, 560 First Ave. New York, N.Y. 10016. mony.deleon@med.nyu.edu

Post mortem examinations consistently document hippocampal formation neurofibrillary degeneration in Alzheimer's disease (AD). AD pathology in the entorhincal cortex (EC) and hippocampus is also found in patients with mild cognitive

impairments (MCI) and is often restricted to the EC in cognitively normal elderly. This evidence suggests that EC changes occur early in the course of AD and precede hippocampal and neocortical neuronal changes.

Objective: To identify in vivo patterns of brain change that predict cognitive decline by studying normal elderly and MCI patients longitudinally (3 years) using in vivo MRI and 18FDG-PPET.

Methods: In a longitudinal study of declining and non-declining normal and MCI patient groups, we estimated the volume and rate of glucose metabolism of the entorhinal cortex, hippocampus, and temporal lobe neocortex. Post mortem materials were used to validate the in vivo anatomical sampling techniques.

Results: In vivo patterns of atrophy and metabolic reduction are consistent with the neuropathology and extend these observations. Among normal elderly, baseline changes in the entorhinal cortex precede and predict hippocampal volume and metabolism losses and the decline in memory performance characteristic of MCI patients. MCI patients that clinically deteriorate to AD, show at baseline damage to the hippocampal formation and at follow-up additional temporal lobe neocortical changes.

Conclusions: These observations suggest the in vivo detection of brain changes in both normal elderly and MCI patients that predict decline. Moreover, these data support the staging of longitudinal AD-related brain and behavior changes that mark the progression of the illness.

Visual Rating and Volumetry of the Medial Temporal Lobe on Magnetic Resonance Imaging in Dementia

Wahlund Lars-Olof, Julin Per, Johansson Sven-Erik and Scheltens Philip

Department of Clinical Neuroscience, Neurotec, Karolinska Institute, Huddinge University Hospital, Department of Neurology, Academisch Ziekenhuis Vrije Universiteit, Amsterdam, The Netherlands, Department of Statistics, Stockholm University, Stockholm, Sweden.

Objectives - Atrophy of medial temporal lobe structures such as hippocampus and entorhinal cortex on MRI may distinguish Alzheimer's disease patients (AD) form healthy controls. However, the diagnostic value of visual inspection and volumetry of medial temporal lobe atrophy (MTA) on MRI in a clinical setting is insufficiently known.

Methods - MTA from 143 subjects was visually rated from

hard copies, using a 0-4 rating scale and a comparison was made with the volumes (ccm) of the medial temporal lobe as estimated with volumetry, using a stereological method. All subjects were recruited in an unselected way in a clinical setting in the center for memory impairments at the Huddinge University Hospital. AD patients (n=41), patients with other dementias (OD) (Vascular Dementia , Fronto-Temporal Dementia and Unspecified Dementia)(n=36) as well as non-demented subjects (n=66) were included. MTA and volumetry were evaluated as a diagnostic tool by performing logistic regression analysis including age, sex and MMSE score and calculating the sensitivity and specificity and percentage correct classification.

Results - Visual and volumetric analysis yielded statistical significant differences between AD patients and non-demented subjects, as well as between the OD and non-demented subjects. Combining MMSE scores and visually rated MTA ratings yielded a sensitivity of 95% for AD, 85% for OD. Non-demented subjects were identified with a specificity of 96% . Volumetry did not have an added value over the MMSE score alone.

Conclusions - Visual rating of MTA is a clinically useful method for differentiating AD from controls and is both quicker and more accurate than volumetry.

Could MRI be used to measure progression in AD?

Nick Fox

Dementia Research Group, The Institute of Neurology, UCL, Queen Square, London, UK

The advent of symptomatic treatments for Alzheimer's disease (AD) has led to increased interest in the use of MRI in trials. These include improving clinical diagnosis in AD and related disorders, providing inclusion and exclusion criteria and identifying at-risk groups (e.g. MCI) for preclinical studies. However, it is the modification of disease progression, the next and crucial challenge for drug development that has created a new role for MRI. Surrogate markers of disease progression are now needed. Markers of progression are sought in the hope that they may provide cost-effective ways of identifying therapies that slow the disease. Such markers would also be important in improving our understanding of the disease and in providing prognostic information.

An ideal surrogate marker would be simple, non-invasive and well-tolerated by patients; it should have biological face-validity; it should be both sensitive to disease progression and specific to the disease process. In addition, in order to be used

in large trials, the technique must be robust and easily applicable in a range of centres. Such measures do not exist. Indeed it is likely that no one measure would meet all these requirements. One potential candidate marker however is rate of cerebral atrophy derived from serial imaging. Since the early 1980s, serial imaging studies (mainly CT) have shown that rates of cerebral atrophy are increased in AD relative to normal ageing. When subjects are rescanned after an interval of more than one year, rates of ventricular enlargement are significantly greater (x10) in AD than in normal controls and regional rates of change, e.g. in the minimum thickness of the medial temporal lobe, have also been shown to be increased. Surprisingly there have been very few longitudinal MRI studies in AD. The hippocampus has however been measured from serial MRI and hippocampal atrophy rates has been shown to be increased (~4%/year) compared to age matched elderly controls (~1.6%/y). Hippocampal and other reggional measurements usually involve manual outlining of the particular region of interest and there is a critical dependence on the reproducibility of identifying anatomical structures. The also involve a priori decisions about which areas to measure. Furthermore, unless carefully controlled for, variations in scan acquisition can make large differences to the rates of change measured - easily sufficient to obscure or to mimic the changes due to AD.

Registration of serial MRI may offer a useful alternative method of measuring atrophy from serial MRI. Once scans are accurately registered, the atrophy is calculated directly from subtraction images in a semi-automated and reproducible manner. The volumetric acquisition required is rapid (7-10 minutes) and available on conventional 1.5T scanners. The analysis method is unbiased and independent of a priori decisions about regions of interest; it is automated, avoiding laborious manual measurements which have significant inter- and intra-observer errors. The technique can adjust for scaling errors introduced by the scanner and scan-rescan reproducibility is high: 0.2% of brain volume. Using this technique we have found rates of atrophy in AD to be approximately 2.4% (±1.1) per year , significantly higher than control rates of 0.4±0.4%/y. Based on these figures, to have 90% power to detect a drug effect equivalent to a 20% reduction in the rate of atrophy, under 200 patients would be needed per treatment arm of a one year placebo controlled trial, allowing for 10% patient drop out rate plus 10% unusable scan pairs. Rates of cerebral loss derived in this way are also sensitive markers of early disease. Furthermore these rates of atrophy correlate with cognitive decline.

In summary rates of global and regional atrophy measured from serial MRI appear to have potential in measuring progression in AD - registration-derived measures of atrophy may prove useful because of their potential for robust automation. These measures should remain complementary to clinical markers but

may help in distinguishing true progression effects from pure symptomatic benefit in AD and related disorders.

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Functional MRI in Alzheimer's Disease During Memory Encoding

Ph. Scheltens, S.A.R.B. Rombouts, M.P. Witter, F. Barkhof

Graduate School for Neurosciences Amsterdam, Research Institute Neurosciences, Vrije Universiteit, Amsterdam, The Netherlands.

We applied functional magnetic resonance imaging (fMRI) with a learning task in healthy elderly subjects and patients with Alzheimer's disease (AD) to study brain activation during memory performance. The purpose was 1) to determine the feasibility of fMRI during a learning task in healthy elderly subjects and patients with AD and 2) to test our hypothesis that brain activation is decreased in the medial temporal lobe (MTL) memory system in AD patients compared to controls.

In 12 mild to moderate AD patients and 10 elderly control subjects, activation of the MTL memory system was studied. We used two learning tasks that required encoding of new information into memory and applied a random effects data analysis. After the fMRI experiment, subjects were tested for recognition of the encoded objects.

In elderly control subjects, activation during memory encoding was observed in medial and lateral temporal lobe structures (fusiform, parietal and occipital parts and the hippocampal

formation), and frontal cortex, as reported previously in young controls. Focusing on the MTL, we found that activation was significantly decreased in AD patients compared to controls in the left parahippocampal gyrus and hippocampus bilaterally during the first encoding task, but not during the second ($p < 0.05$, uncorrected).

fMRI with a learning task appeared feasible in elderly subjects and AD patients. The measured functional signal decrease in MTL areas warrant further exploration of the (early) diagnostic utility of fMRI in AD and other dementias.

PET Activation Studies in MCI and AD Patients

Nordberg, A, Almkvist O, Jelic V, Nilsson A, Wall A, Långström B

NEUROTEC, Karolinska Institutet and Uppsala PET Centre, Sweden

With the present conservative approach of the clinical criteria for Alzheimer´s disease (AD) the diagnosis is very often given when the clinical symptoms of the disease is quite clear. Successful drug treatment initiated in the early course of the AD disease will prompt the need for an early presymptomatic diagnosis.

The data so far suggest that identification of early functional changes in brain can be obtained by imaging techniques such as positron emission tomography (PET) even at a presymptomatic stage of the disease. In MCI undergoing repeated PET studies during two years, we observed that 26 percent of the MCI patients converted to AD and that the deficits in cortical glucose metabolism predicted clinical outcome in 93 percent of the cases. Detection of neuroreceptor impairments including the nicotinic receptors might be an earlier marker for disease processes than glucose metabolism and cerebral blood flow. Since there is an indirect correspondence between early behavioural impairment in attention and early neuropathological changes in AD, functional brain studies performed during attentional task will provide a further insight into the early dynamic disturbances in AD brain function.

We have observed that MCI and AD patients have demonstrated task-related changes in activation during sustained attention associated with a brain network including the frontal and parietal brain regions.

UNIVERSITY OF KUOPIO
Department of Neuroscience and Neurology

The Second Kuopio Alzheimer Symposium

Proceedings of the Symposium

March 13.-15. January 2001

Kuopio, Finland

Cognitive Aspects:
Chairmen Tuomo Hänninen and Steven Ferris

Cognition in Normal Aging

Yaakov Stern, PhD

Sergievsky Center and Taub Institute Columbia University College of Physicians and Surgeons New York, NY USA

In considering cognition in normal aging, there are several basic questions that must be addressed. The most basic is, should we expect declines in cognitive function in normal aging, or is any decline associated with an abnormal or pathologic process. A related question is whether pathologic age-related cognitive decline is necessarily related to Alzheimer's disease vs another pathologic entity. We have addressed these questions using both epidemiologic and neuroimaging approaches. Our findings to date suggest that there are cognitive changes that normally occur in aging. Further, we may be able to separate pathologic cognitive decline into two forms, one consistent and the other inconsistent with subclinical Alzheimer's disease.

Item-Specific Measurement of Memory Impairment in Alzheimer's disease

Herman Buschke

Department of Neurology; Albert Einstein College of Medicine; New York, USA

Detection and diagnosis of Alzheimer's disease depend on accurate memory measurement. Memory is commonly measured by counting the number of items retrieved by free recall. Such unit-weighted counting assumes that all items have equal value as measures of memory. However, serial position curves characteristically show primacy and recency effects which indicate that all items do not have equivalent value, suggesting that counting may not provide optimum measurement of memory performance, and that item-specific weighting might improve measurement of memory and detection of memory impairment. In Alzheimer's disease (AD) there is a characteristic loss of primacy that is not measured by unit-weighted counting, suggesting that detection of AD might be improved by item-specific weighting. Retention-weighting of free recall by aged with and without AD increased the effect size, and increased the area under the ROC curve, improving discrimination by increasing specificity without reducing sensitivity. Item-specific weighted memory measurement may improve detection of memory impairment and Alzheimer's disease.

Concept of Mild Cognitive Impairment

Steven H. Ferris, Ph.D.

Silberstein Aging and Dementia Research Center, NYU School of Medicine, New York, N.Y. 10016

Since Alzheimer's disease (AD) is a neurodegenerative disorder with a slow, gradual progression and an insidious onset of cognitive impairment and other clinical symptoms, clear borders between normal, age-related cognitive decline and the symptoms of very early AD have been difficult to define. This difficulty is compounded by the qualitative similarities between the very mild cognitive decline associated with brain aging and the pattern of impairment seen in early AD. It has been recognized since the 1980s that there is a gray zone of "mild cognitive impairment" (MCI) that is worse than expected due to aging but less severe than in patients who receive a diagnosis of AD based on current criteria. Longitudinal studies have shown that individuals with MCI are at high risk for progressing to a dementia diagnosis (10-15% per year), with AD as the major

subtype. As identified at dementia research centers using established AD diagnostic criteria, MCI generally represents prodromal AD. Furthermore, treatments for AD that may slow disease progression (e.g., antioxidants, anti-inflammatory agents such as COX 2 inhibitors, neuroprotective agents and anti-amyloid compounds) are now under clinical study. Consequently, the evaluation of such treatments in trial designs involving MCI cases has become an important scientific bridge between traditional AD trials and primary prevention trials. This presentation will (1) review the historical development of MCI as a target for treatment; (2) discuss some issues in establishing operational diagnostic criteria for "MCI of the AD type" (e.g., is it just memory impairment and is there subtle decline in complex functioning?); (3) present some recent research on methods for identifying those patients with MCI who are at high risk for progressing to AD, and (4) summarize current MCI clinical trials assessing new treatments that may slow disease progression.

Prognosis of Mild Cognitive Impairment

Ronald C. Petersen, Ph.D., M.D.

Mayo Clinic; Rochester, Minnesota, USA

Mild cognitive impairment is becoming recognized as an increasingly important topic of study in the field of aging and dementia. Mild cognitive impairment refers to the clinical state whereby a person is mildly cognitively impaired (usually with a memory deficit) but is not demented. These persons are aware of their cognitive difficulties and have evidence for an objective memory impairment on neuropsychological testing. However, their general cognitive functions are preserved, and their activities are daily living are intact. Importantly, these persons are not demented. The validity of the concept of mild cognitive impairment is derived from studies of the longitudinal outcome of these subjects. Several studies from the literature on mild cognitive impairment will be reviewed with respect to longitudinal outcome. Predictors of rate of progression will also be reviewed. Evidence exists indicating that in some studies, apolipoprotein E-4 carrier status, features of memory performance and atrophic hippocampi on MRI are predictive of a more rapid progression. In general, most studies indicate that persons with a mild cognitive impairment progress to dementia or Alzheimer's disease at a rate of approximately 10-15 percent per year which is in contrast to age-matched control subjects in the normal population who become demented at a rate of 1-2 percent per year. Mild cognitive impairment is an increasingly important concept to study since it may represent a target for therapeutic intervention.

UNIVERSITY OF KUOPIO
Department of Neuroscience and Neurology

The Second Kuopio Alzheimer Symposium

Proceedings of the Symposium

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Kuopio, Finland

Therapy I:
Chairmen Martin Farlow and Bengt Winblad

The Evolution of Alzheimer's Disease: Candidate Genes
Caleb Finch, USA

Fingerprints of Pharmacogenomics as Indexes of Onset and Progression of Alzheimer's Disease Dementia
Giulio Pasinetti, USA

The Next Generation of AD Therapeutics: The Future is Now
Kevin Felsenstein, USA

Pharmacoeconomic Studies in AD
Bengt Winblad, Sweden

The Evolution of Alzheimer's Disease and ApoE

Caleb Finch, USA

Alzheimer disease (AD)-like neuropathology increases progressively during aging in all primates, and, in some species, is concurrent with reproductive decline in females and cognitive impairments. The schedule of AD during aging may have evolved in early humans in relation to the apolipoprotein E (apoE) allele system, which is not found in other primates, and to the increasing duration of postnatal care. The delay of independence and the increasing length of maturation required that the schedule of AD-like neurodegeneration be slowed, otherwise parental caregivers would already have become impaired. It is hypothesized that the uniquely human apoE e3 allele from the e4 of primate ancestors evolved during human evolution in relation to the rapid increases of brain size and the emergence of grandmothering. The evolution of menopause is also considered in relation to the protective effect of estrogen

on AD.

This presentation is based on the article by CE Finch and RM Sapolsky: "The evolution of Alzheimer disease, the reproductive schedule, and apoE isoforms." Neurobiol Aging 20: 407-428 (1999).

Fingerprints of Pharmacogenomics as Indexes of Onset and Progression of Alzheimer's Disease Dementia

Giulio Maria Pasinetti

Neuroinflammation Research Center, Department of Psychiatry, Mount Sinai School of Medicine, New York, NY 10128; E-mail: gp2@doc.mssm.edu

The elucidation of the changes in gene expression in the brain of cases at high risk for Alzheimer's disease (AD) dementia is critical to the design of appropriate pharmacological strategies to slow the clinical progression of the disease. For example, recent studies in our labs using postmortem brain from AD cases that died at various clinical stages of AD dementia, found that the expression of cyclooxygenase (COX)-2, an enzyme involved in inflammation but also in neuronal functions, is elevated in neurons of AD cases characterized by mild dementia (Ho et al., Arch. Neurol., 58:1-6, 2001). Because therapeutic trials of potential disease-modifying regimens with COX inhibitors select patients at one or more stages of clinical disease, this observation become immediately relevant to the design of anti-inflammatory drug trials. Based on this evidence, using cDNA and protein microarrays we continued exploring the variation in gene expression in the brain during early AD. We found that the expression of genes involved in synaptic plasticity and neurotrasmitter release is selectively altered in the brain of cases characterized by questionable AD dementia, even before the cases reach criteria for AD diagnosis. Most interestingly, the changes appear to precede the altered expression of COX-2, and other molecular indexes of neurodegeneration at later dementia stages (Ho et al., Neurosci Lett., 298:191-194, 2001). The studies provide a novel experimental framework for strategies leading to the improved diagnosis, treatment and the eventual prevention of AD.

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Aβ Inhibition: The Beginning of The End or The End of The Beginning?

Kevin M. Felsenstein, Ph.D.

Principal Scientist, Neuroscience Drug Discovery, Bristol-Myers Squibb

Pharmaceutical Research Institute, 5 Research Parkway, Wallingford CT 06492, tel: 203-677-7671 fax: 203-677-7569 , email: kevin.felsenstein@bms.com

Discovery efforts targeting the generation of Aβ may result in slowing, stopping, or preventing Alzheimer's Disease. Examination of such compounds revealed significant decreases in Ab concentration in brains of animals after single dose administration. Clinical candidates have been identified forming the foundation of the next generation of therapeutics. Challenges include documenting pharmacodynamic effects and defining surrogate markers of efficacy. Regulatory approval will likely require quantitative measures on cognitive and behavioral function, in addition to effects detected using in vivo brain imaging. The therapeutic challenge will be when and how to treat patients. If the clinical trials are not up to the challenge then many of the potential disease modifying approaches may ultimately fall short. We will examine the process involving the characterization of these compounds and the implications on the treatment of AD.

UNIVERSITY OF KUOPIO
Department of Neuroscience and Neurology

The Second Kuopio Alzheimer Symposium

Proceedings of the Symposium

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Kuopio, Finland

Therapy II:
Chairmen Ezio Giacobini and Caleb Finch

Treatment of Alzheimer's disease year 2001

Ezio Giacobini

Department of Geriatrics, School of Medicine, Geneva Switzerland

Beta amyloid -reducing therapies. Genetic analysis has paved the way for our understanding of the basic biology of beta-amyloid (beta-A) relation to AD. Two new therapeutic approaches aim to test the validity of the hypothesis that that beta-A is intimately if not causatively associated to AD and therefore prevention or reduction of amyloidosis would

ameliorate the clinical manifestations of the disease. Following the identification of the three secretases involved in beta-A production, the effect of selectively inhibiting beta secretase activity and reducing beta-42 production is currently tested in the clinic.The second alternative, immunization by passive transfer of anti-beta-A antibodies is presently in clinical phase II (Schenk et al. 1999).

Long-term stabilization with cholinesterase inhibitors. The reports of a long term (2 years or longer) cognitive stabilization in a percentage of ChEI treated patients support the basic finding that some reversible or irreversible AChEI promote the non-amyloidogenic route of APP (amyloid precursor protein) (Mori et al, 1996). It has been demonstrated that this effect on APP metabolism is due to stimulation of PKC (protein kinase C) activity and enhancement of alpha-secretase activity (Pakaski et al. 2000, Racchi et al. 2000).

Modulation of cholesterol intake. Hypercholesterolemia accellerates amyloid pathology (Refolo et al. 2000) and reduction of cholesterol intake slows plaque development in transgenic mouse models (Duff et al 2000).The validity of this hypothesis needs to be tested in the patient.

Estrogen and anti-inflammatory therapies. Inspite of negative clinical results reported during the year 2000, these two approaches still remain valid hypotheses to be tested with new products and different protocols.

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M1 Muscarinic Agonists from Treatment Towards Disease-Modifying Agents in Alzheimer's Disease: Concepts and Perspectives

A. Fisher, Z. Pittel, R. Haring, R. Brandeis, N. Bar-Ner, D.G. Walker, P.E. Potter, H. Sonego, I. Marcovitch, N. Natan and T.G. Beach

Israel Institute for Biological Research, POBox 19, 74100 Ness-Ziona, Israel; Sun Health Research Institute, POBox 1278, Sun City, AZ, USA; email: fisher_a@netvision.net.il

M1 muscarinic agonists may represent, in principle, a more rational treatment of Alzheimer's Disease (AD) then the recently FDA-approved acetylcholinesterase inhibitors. However some of the tested muscarinic agonists failed in clinical trials in AD patients. Failure of some muscarinic agonists that a priori major had major clinical limitations cannot be used against this strategy as these cannot be considered M1 selective. The history teaches us that sometimes the 1st, even when it failed paved the way to more effective 2nd generation therapeutics with similar mechanism, providing there is a sensible hypothesis that can support such a strategy. In this context, three major hallmarks in AD, a cholinergic hypofunction, β-amyloid (Aβ) and hyperphosphorylated tau protein are apparently linked. Subtoxic concentrations of Ab impair the coupling of M1 muscarinic receptors (mAChR) with G proteins, yet it is unclear if this effect relates to Ab-induced cell death. In fact, it can be speculated that Ab-induced M1 mAChR-G protein uncoupling may represent an unexplored physiological role for Ab (e.g. a negative feedback to a prolonged activation of M1 mAChR). However, in AD such uncoupling may lead to a chronic impaired signaling, a decrease of trophic and non-amyloidogenic amyloid precursor protein (APPs) and generation of more Ab, aggravating further the cholinergic deficiency. M1 muscarinic agonists from the AF series [AF102B, AF150(S) & AF267B] may attenuate such vicious cycles, providing a rational therapeutic strategy in AD. Aβ- and oxidative stress-induced apoptosis is mediated by reactive oxidative species and this effect is attenuated both by antioxidants and these M1 agonists. The attenuation of Aβ- and oxidative stress-induced apoptosis via M1 mAChR activation by muscarinic agonists is a novel finding. This adds further support to the future use of AF102B, AF150(S) and AF267B as disease modifying agents in AD. In addition to these effects, these M1 agonists, inter alia, elevate APPs levels; show neurotrophic-like effects (in vitro); decrease tau protein hyperphosphorylation (in vitro in cell cultures and in vivo in apolipoprotein E-deficient mice); and restore cognitive impairments with an excellent safety margin in several animal models for AD [review Fisher Jap J Pharmacol 84: 101, 2000]. In rabbits, with Aβ sequence identical to the human Aβ, both AF267B > AF150(S) decreased significantly CSF Aβ(1-42 & 1-40), while AF102B reduced Aβ(1-40). Finally AF102B decreased significantly CSF Aβ(total) in AD patients [Nitsch et al. Ann Neurol, 48: 913, 2000]. In summary, our M1 agonists have beneficial effects on three major hallmarks of AD (cognition, Ab and tau). This could represent the unique clinical value of such drugs, since no other compounds were reported yet with such combined effects. The use of M1 agonists in AD, alone or in drug combinations, may also be expanded for other neurological, neuropsychiatric and

autoimmune diseases with a cholinergic deficiency. Remarkably, Cevimeline (AF102B) was approved by the FDA on Jan 2000 for treatment of dry mouth in Sjogren's syndrome, a widespread autoimmune disease that affects exocrine glands and sometimes impairs cognition. Cevimeline is now marketed in the USA for this indication, being the 1st M1 muscarinic agonist approved by this Agency.

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Galantamine: Clinical Implications for Nicotinic Receptor Modulation

Luc Truyen, MD PhD

Janssen Research Foundation, Titusville NJ, USA

Galantamine is a novel therapeutic agent for dementia with a dual mode of action - it modulates pre- and postsynaptic nicotinic receptors in addition to inhibiting acetylcholinesterase activity. This would further amplify cholinergic neuro-transmission but also glutamatergic and serotonergic neurotransmission. As such it is the only approved therapeutic agent in AD having these effects which might contribute to the observed consistent and relevant efficacy.

In randomized, double-blind, placebo-controlled studies of up to 6 months' duration, galantamine has provided a broad spectrum of benefits in patients with AD and produced clinically meaningful outcomes, including improved global functioning, maintained cognitive function, preserved functional abilities, and delayed emergence of behavioural symptoms. Patients who completed one of these 6-month studies were eligible to enter a 6-month, open-extension study, in which cognitive and functional abilities were maintained at or above baseline levels for at least 12 months. These results indicate that galantamine slows the progression of symptoms in AD, thereby helping patients to maintain independent living and improve caregiver burden.

Disease Progression and Response to Therapy in Alzheimer's Disease: Are There Predictors?

Martin R. Farlow, M.D.

Professor, Department of Neurology; Indiana University School of Medicine, Indianapolis, IN 46202

A number of factors have been suggested to predict rates of

disease progression and response to cholinesterase inhibition therapy in AD including age, gender, disease stage, genotype and drug dosage. Recent studies of APOE genotype have not demonstrated clear associations between genotype and either underlying rate of disease progression or response to therapy. Most recent large cholinesterase inhibitor trials have demonstrated significant dose-response effects, with the patients in higher dose groups or who are titrated to higher dosages achieving the best responses. Even at maximum tolerated dosages in several of these studies only 25 to 40% of the patients achieve a clinically significant improvement in their cognitive deficits as measured by neuropsychological testing, suggesting it may be possible to define a responder group. However, in these studies patients achieved similar levels of response in other symptomatic domains such as activities of daily living and behavior, with the responses in these other domains occurring independently in individual patients. It appears that 75 to 80 % of patients in these studies will achieve a significant response in at least one domain, making it difficult to justify limiting therapy by health care systems or 3rd party payers to a smaller number of patients who may be responders by one criteria such as MMSE.

It has been suggested that the beneficial effects of cholinesterase inhibition therapy may decrease as dementia progresses. However, patients with moderate stage AD, who untreated progressed more rapidly than mild stage patients, have been reportedly demonstrated in the large pivotal trials of available drugs to have a larger response than mild stage patients. These results are consistent with recently autopsy studies by Ken Davis et al., which suggest that cholinergic deficits become much more prominent as AD progresses to the more moderate and severe stages.

A recent analysis used placebo data from the double-blind placebo controlled portion of a large cholinesterase inhibitor trial to divide the initially placebo-treated patients into slowly and rapidly progressive groups. In a following open label phase of the study, where all patients were treated with the drug, patients who were labeled as rapidly progressive had the largest response to therapy, and indeed this methodology appears to be the best single predictor of who will respond to cholinesterase inhibitor therapy.

UNIVERSITY OF KUOPIO
Department of Neuroscience and Neurology

The Second Kuopio Alzheimer Symposium

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Transgenic Models:
Chairmen Claudio Cuello and Heikki Tanila

Animal Models in Alzheimer's Research. A Brief Overview

Claudio Cuello

Department of Pharmacology & Therapeutics, McGill University, Montreal, QC, Canada

This presentation will provide a brief overview of some of the main accomplishments and future challenges in developing suitable transgenic models of Alzheimer's CNS pathology. The main phenotypic features resulting from the transgenic expression of diverse AD-related genes will be discussed as well as early attempts to generate rat transgenic models.

Neuropathology and Behavioral Changes in APP-Transgenic Rats

Paul Chapman

Cardiff School of Biosciences, Cardiff University, Cardiff, UK

Transgenic rodents have begun to provide invaluable insights into the means by which specific pathologies can produce the symptoms of Alzheimer's disease (AD). The most successful and widely studied models involve transgenic expression of mutated amyloid precursor protein (APP) in mice. While these models have demonstrated that some very important forms of pathology can be reproduced in mice by overexpressing APP, they have also raised questions about some of the behavioral and pathological correlates that appear to be absent or difficult to measure. We have therefore developed a rat model of AD to complement the mouse models. Our preliminary evidence suggests that an APP overexpressing rat may prove to be a useful AD model. In the meantime, the Tg2576 mouse continues to demonstrate its value in both determining basic mechanisms and in testing therapeutic strategies.

Tg2576 mice, carrying the human APP695 with the Swedish mutation under the PrP promoter, demonstrate age-dependent deficits in learning several standard rodent tasks, including spatial reference memory in the water maze and episodic-like memory in the T-maze. The decline in performance begins at an age when formic acid soluble β-amyloid is beginning to accumulate and also correlates with changes in synaptic physiology, including decreased synaptic plasticity and enhanced excitability. Although research into the transgenic rats is still preliminary, they do produce formic acid soluble β-amyloid by 20 months of age. A broad range of behavioral and electrophysiological examinations that are ideally suited to rats is ongoing.

Learning and Synaptic Plasticity in APP/C-104 Transgenic Animals

Matthew Shapiro & Josephine Nalbantoglu

McGill University, Montreal, Québec H3A-1B1 Canada, Mount Sinai School of Medicine, New York, NY 10029 USA.

Amyloid deposits are one of the defining features of Alzheimer's disease, yet the causal role of amyloid in the disease process is unclear. To test if amyloid over-expression altered behavior and brain function, transgenic B6/C3 mice

were produced with the C-104 fragment of the human APP gene under transcriptional control of the human neurofilament (NF-L) promoter. In 1994, transgenic mice 10 and 18 months of age were compared to either nontransgenic littermates, mice with only the NF-L promotor, or age and strain-matched controls. The mice were tested in the Morris water maze, synaptic plasticity was assessed in hippocampal slices, and beta-amyloid, gliosis, and reactive astrocytes were assessed in the hippocampus and cortex. Transgenic mice were impaired in spatial, but not cued learning in the water maze, had transient LTP but normal LTD, and had more extracellular amyloid deposits, gliosis and reduced cell density compared to control mice. In 2000, we tested the progeny of the same line of mice in the Morris water maze. The mice were tested in the same tests as before, as well as a delayed-matching to location task. Although the mice showed a trend toward the same spatial learning impairment as the original cohort, learning and memory was for the most part spared. Histological and physiological measures have not yet been assessed in mice of this generation.

Cells and Mice Carrying Presenilin Mutations: Tools to Unleash AD Pathology

Takeshi Tabira

Department of Demyelinating Disease and Aging, National Institute of Neuroscience, Kodaira, Tokyo 187-8502, Japan

Presenilin 1 (PS1) and 2 (PS2) are genes for familial Alzheimer's disease (AD). Their mutations cause elevation of Aβx-42 production and secretion. Our PS1 transgenic mice showed accelerated neuronal loss in the cerebral cortex and hippocampus of aged mutant mice. In mutant mice, dark neurons were significantly increased and some of the dark neurons showed positive staining for apoptosis, and neurons with intraneuronal Aβx-42 accumulation were significantly increased.

We examined intraneuronal Aβx-42 deposits in AD brains and found that such neurons were significantly more frequent in isolated AD and PS1 mutant familial AD cases. Examination of AD brain with cotton wool type senile plaques also revealed intraneuronal accumulation of Aβx-42 without showing core formation, inflammatory changes and dystrophic neurites. Thus, we hypothesized that accumulation of Aβx-42 in neurons is an early and important event in Alzheimer pathogenesis.

Neuroblastoma cells were transfected with wild and mutant type PS2 genes using adenovirus vector. Both PS2 transfectants showed accelerated apoptosis more significantly in mutants.

Relationship between apoptosis and intracellular Aβx-42 is under study.

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Does Amyloid Accumulation Relate to Behavioral Changes in APP+PS1 Transgenic Mice?

Heikki Tanila

Dept. Neuroscience and Neurology, Univ. Kuopio, Finland

Transgenic (tg) mice carrying mutated forms of human APP gene have greatly advanced our understanding of the role of beta amyloid in the pathogenesis of Alzheimer's disease. Not only do the mice develop amyloid plaques in the same brain areas as AD patients, but also express memory problems. However, in the majority of reports, the cognitive deficits in tg mice are present already at a young age and also in mice with no plaque depositions.

To assess the relationship between amyloid accumulation and cognitive deficit in tg mice, we compared the hippocampal amyloid pathology and spatial learning in tg mice of 4, 11, and 17 months of age. The mice were doubly mutant, carrying human APPswe mutation combined with PS1 A246E mutation (Borchelt, Neuron, 1997). The first plaques in these mice appear at 8-9 months of age, and are largely restricted to the hippocampus and subiculum in the early phase of the disease. These mice show age-dependent impairment in the water maze task that is testing the hippocampal functioning. Importantly, the tg mice do not differ from wt mice in their water maze performance at 4 months of age but a clearly genotype effect can be seen at 11 months. In contrast, the tg mice do not differ from their wt controls at any age in a habit learning task that is independent of hippocampal integrity. In our preliminary observations, the total A beta 1-42 levels and plaques counts in the hippocampus correlate with impaired water maze performance.

These data support the idea that accumulation of A beta 1-42 in the neural tissue is causally linked to neuronal dysfunction and

cognitive impairment.

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History of Neurology in Finland

Matti Haltia

Department of Pathology, University of Helsinki, Helsinki, Finland

Academic medicine has been taught in Finland since the establishment of the Royal Academy inTurku in 1640, but medical studies gained popularity first in the 18th century. Johan Haartman (1725-1787), a pupil of Linnaeus, founded an academic teaching hospital in 1759, and wrote an extensive treatise on cerebral apoplexy.

However, independent research in neurosciences began only after the transfer of the university to Helsinki in 1828, with studies in comparative neuroanatomy by Evert J. Bonsdorff, a pupil of Anders Retzius. Bonsdorff's disciple, Otto E.A. Hjelt, since 1857 professor of pathological anatomy in Helsinki, wrote his professorial thesis on "The regeneration of peripheral nerves after excision". This experimental neuropathological study was proposed and supervised by his teacher and life-long friend, Rudolf Virchow, Father of modern cell biology and pathology. Following the example of Virchow at la Charité in Berlin, Hjelt managed to attach a small clinical ward to his new Department of Pathology in Helsinki. This clinical unit at the

Helsinki General Hospital was destined to become the cradle of Finnish neurology when Hjelt was succeeded by Ernst A. Homén (1851-1926).

After graduating from the University of Helsinki in 1879 Homén studied in Leipzig with Julius Cohnheim, the pioneer of experimental pathology, and with the neuropathologist Carl Friedländer in Berlin. Homén's stays in Paris in 1882-1883 and again in 1886 were, however, of the most decisive importance for his future career. At the Paris University and Collège de France he carried out experimental studies of secondary spinal tract degeneration under the guidance of Vulpian and Ranvier. At Hopital de la Salpetrière he enjoyed the clinical teaching of Jean Martin Charcot, since 1881 the first professor of neurology in the world, and was also in touch with Louis Pasteur. Appointed professor of pathological anatomy at the University of Helsinki in 1886, Homén immediately started an intense activity in the fields of experimental and clinical neuropathology. Simultaneously, he transformed the clinical ward attached to his department into a specialized clinical unit devoted to the study and treatment of diseases of the nervous system and musculature. He investigated the effects of the newly discovered bacteria and their toxins on the nervous system and the effects of axonal transsection on neuronal perikarya. However, his best known contribution is his original clinical and pathological description of hepatolenticular degeneration.

Homén trained a series of gifted pupils, including Christian Sibelius (1869-1922), the first full professor of psychiatry, and Jarl Hagelstam (1860-1935), the holder of the first personal professorship in neurology at the University of Helsinki. Important early work was also performed in sensory and neurophysiology by Robert Tigerstedt (1853-1923) and the Nobel laureate Ragnar Granit, both professors of physiology at the University of Helsinki. The clinical neurological activity initiated by Homén and his school continued and expanded gradually within the frame of internal medicine and neuropsychiatry. Finally, in 1961, an independent chair and department of neurology were established at the University of Helsinki and, later, at the four other medical schools of Finland, followed by chairs in clinical neurophysiology, pediatric neurology, and neuropathology. This academic diversification was paralleled by a rapid expansion of the neurological services offered to the public all over the country, a development which has continued to the present day.