



Abstracts Involving Washington University Investigators

International Conference on Alzheimer's Disease & Related Disorders

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Booklet prepared by:
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1 st WU Author	Abstract (Presenting Author in Bold Type)
Brendza P2-063	<p><i>In Vivo</i> Dynamics of Amyloid Associated Neuritic Dystrophy Before and After Anti-Aβ Immunotherapy</p> <p>Topic: Animal and Cellular Models - Animal Models, Transgenic</p> <p>Robert P. Brendza¹, Brian J. Bacskai², Kelly A. Simmons¹, Jesse M. Skoch², William E. Klunk³, Chester A. Mathis³, Kelly R. Bales⁴, Steven M. Paul⁴, David M. Holtzman¹, ¹Washington University School of Medicine, Saint Louis, MO, USA; ²Mass General Hospital, Charlestown, MA, USA; ³University of Pittsburgh, Pittsburgh, PA, USA; ⁴Eli-Lilly, Inc., Indianapolis, IN, USA. Contact e-mail: brendzab@neuro.wustl.edu</p> <p>Presentation Number: P2-063</p> <p>Keyword: transgenic model, amyloid, neuropathology</p> <p>Background: Neuritic plaques are one of the defining features of Alzheimer's disease (AD) pathology. These structures are composed of extracellular accumulations of amyloid-β peptide (Aβ) and other plaque-associated proteins surrounded by large, swollen degenerating axons and dendrites (dystrophic neurites) and activated glial cells. Dystrophic neurites are thought to disrupt neuronal function, but whether damage to affected neurites is static, dynamic or reversible is unknown.</p> <p>Objective(s): We are studying the dynamics of neurite-amyloid interactions and characterizing the properties of amyloid toxicity <i>in vivo</i>, using a transgenic mouse model of AD. We are using our system to investigate whether dystrophic neurites undergo dynamic changes over time and if reducing both soluble and insoluble Aβ will promote the rapid recovery of amyloid-associated neuritic dystrophy.</p> <p>Methods: We are analyzing neuritic plaques in the brains of living PDAPP; Thy-1:YFP transgenic mice, a transgenic mouse model that develops AD-like pathology and also stably expresses yellow fluorescent protein (YFP) in a subset of neurons in the brain. Using multiphoton microscopy, we are able to observe and monitor amyloid through cranial windows in PDAPP; Thy-1:YFP double transgenic mice using the <i>in vivo</i> amyloid-imaging fluorophore, methoxy-X04. Importantly, individual YFP-labeled dystrophic neurites adjacent to amyloid can be visualized over time due to their inherent fluorescence.</p> <p>Results: Preliminary studies using this system suggest that amyloid-associated dystrophic neurites are relatively stable structures in the absence of any treatments. However, an anti-Aβ antibody that induces clearance of Aβ deposits in the brains of PDAPP; Thy-1:YFP transgenic mice appears to acutely promote the morphological recovery of amyloid associated neuritic dystrophy. We are currently investigating the extent to which anti-Aβ antibodies can benefit this aspect of AD-like neuropathology.</p> <p>Conclusions: This analysis should yield new insight into the nature of amyloid toxicity and suggests that axonal and dendritic damage secondary to amyloid is at least in part, reversible.</p> <p>Supported by: NIH AG13956, AG05681, AG1135 (DMH), AG19547 (RPB), AG020570, EB00768 (BJB), AG08487, a Pioneer Award from the Alzheimer Association (BTH), and Eli Lilly and Co.</p> <p>Session Title: Poster Session Session Type: Poster Session Start: 7/19/2004 12:30:00 PM Session End: 7/19/2004 2:45:00 PM Location: Hall B</p>

Brunkan

P4-283

Endoproteolysis of Presenilin-1 Is an Intramolecular Autocatalytic Event

Topic: Molecular Mechanisms of Neurodegeneration - Presenilins

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Presentation Number: P4-283

Keyword: familial Alzheimer's disease, amyloid precursor protein (APP), beta-amyloid

BACKGROUND: Mutation in the presenilins (PS1, PS2) or the amyloid precursor protein (APP) account for all known causes of familial Alzheimer's disease (FAD). APP is cleaved in a presenilin-dependent manner to form the A β fragments that accumulate in plaques in AD brains. The signaling protein Notch is also cleaved in a presenilin-dependent fashion to form NICD. PS contain the active site for the γ -secretase complex that accomplishes this proteolysis in the presence of required co-factors Nicastrin, APh-1, and PEN-2. PS itself also undergoes proteolysis within its large hydrophilic loop to generate an N-terminal/C-terminal fragment complex (NTF/CTF). This highly regulated cleavage is hypothesized to be an autocatalytic event that may be important to convert the inactive full-length PS (FL-PS) into the active fragment complex. We have previously hypothesized that PS may exist as a homodimer in the active γ -secretase. **OBJECTIVE:** To further characterize the sequence specificity at the site of PS endoproteolysis and study the mechanism of PS1 autocatalysis. **METHOD:** PS1 molecules with subtle point mutations at the site of endoproteolysis were analyzed in PS1/2KO cells for PS1 endoproteolysis and γ -secretase activity. **RESULTS:** None of the point mutations completely abolished formation of PS1 fragments and the residual fragments produced from the mutants were sufficient to support γ -secretase activity in cleaving APP to form CTF γ and A β , and in cleaving Notch to form NICD. Furthermore, none of these mutant PS1 molecules had a dominant negative effect on endogenous PS. When the endoproteolysis-deficient active site mutant D257A was co-expressed with a wtPS1 molecule, the wtPS1 was unable to rescue endoproteolysis of the D257A mutant. When a PS1 endoproteolysis-site mutant was co-expressed with a wtPS1 molecule, the wtPS1 was also unable to rescue endoproteolysis of the deficient mutant as expected. **CONCLUSIONS:** FL-PS1 is likely to be a zymogen that is activated by intramolecular proteolytic cleavage that releases active site inhibition by the large loop. Removal of the loop produces the γ -secretase active site conformation that catalyzes APP and Notch cleavage. Further study of the regulation of endoproteolysis will clarify this mechanism of regulating γ -secretase activity.

Session Title: Poster Session
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Buckles

P2-315

Subjective Assessment of Understanding of Informed Consent

Topic: Social and Behavioral Research - Instrument Development

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Presentation Number: P2-315

Keyword: ethical issues, legal issues, cognition

Background: Assuring informed consent in cognitively impaired subjects is a challenge and at a minimum requires understanding of the elements of informed consent by the subject. Many variables can affect understanding of informed consent by demented individuals including the measurement tool and the range of dementia severity. We examine one method of measuring understanding. **Objective(s):** To evaluate a subjective/recall test to document understanding of informed consent by demented and non-demented research subjects. **Methods:** Elements of informed consent regarding participation in a longitudinal study of healthy aging and dementia were reviewed (read and discussed) with all new participants at their initial visit to the project. Following this one-hour informed consent process, subjects were interviewed with a 9-item open-ended questionnaire addressing the required elements of informed consent. Interviewers used standard prompts as needed and recorded answers verbatim. Participants then received a comprehensive dementia research assessment resulting in a diagnosis and rating of dementia severity (Clinical Dementia Rating, CDR). A total of 86 questionnaires was completed over one year. Participants were diagnosed as nondemented (CDR=0, n=31), very mildly demented (CDR=0.5, n=38) and mildly demented (CDR 1, n=17). After development of scoring rules, two trained raters scored the responses independently and resolved disagreements by consensus. In both the very mildly and mildly demented groups the most frequent diagnosis was DAT, although there was a small number of subjects with other non-DAT dementias. The predominant diagnosis (~70-80%) in both demented groups was dementia of the Alzheimer type (DAT) with remaining diagnoses of frontotemporal dementia, diffuse Lewy body disease, and uncertain dementia. **Results:** Groups did not differ on demographic variables of age, education, gender or race. We found, as expected, that performance decreased as dementia severity increased, with the greatest decrease seen in the mildly demented group (CDR 1). Median correct answers were 8 and 7 respectively for the nondemented and very mildly demented groups but dropped to 4 for the mildly demented group. **Conclusion:** In light of previous studies, we conclude that how understanding is evaluated or measured also influences the outcome. Dementia researchers should evaluate understanding and reach consensus on its measurement.

Commercial Relationship: V.D. Buckles, None.

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Buckner

IC-P-062 &

P3-081

Development of Automated Identification of Cortical Regions on MRI Scans in AD Patients

Topic: Monitoring the Progression Of Alzheimer's Disease

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Poster Board Number: IC-P-062

Background: MRI provides a useful tool for ante-mortem measurement of neuropathological changes. Most MRI studies related to AD have employed manual measures of the brain. There have been attempts to automatically identify the hippocampus on MRI scans, but cortical regions (such as the entorhinal cortex) have been challenging to evaluate. Recent improvements in computational and mathematical techniques, along with improved scanning procedures, now permit the development of anatomically accurate automated measurement of the cerebral cortex (Fischl et al., 1999, 2000, Dale et al., 1999).

Objectives: Our goal was to produce an anatomically accurate automated parcellation of the cortex. Of particular interest to the study of AD are measures of the entorhinal and perirhinal cortices.

Methods: Modified MP-RAGE MRI images were acquired on a Siemens 1.5 T MRI scanner. Scans were obtained from 4 controls and 4 patients with mild AD. The images were motion corrected and intensity normalized. Segmentation was performed to classify gray matter and white matter voxels. This information was used to generate surface representations of the gray matter (see Fischl et al. 1999, Dale et al., 1999). The entire cortical surface was manually subdivided into neuroanatomical ROIs on the basis of sulcal boundaries.

Results: The volume and thickness of the right and left entorhinal cortex was significantly reduced in the mild AD patients compared to the controls (p 's < 0.05). In addition, the volume of the perirhinal cortex showed a trend towards significance when the AD patients were compared to the controls. The next step is to apply the same manual boundaries to a set of 40 scans, and to use the 40 scans as a training set to automate the parcellation of the cerebral cortex into the same neuroanatomical ROIs we have labeled manually, using an algorithm for cortical parcellation (Fischl et al., 2004).

Conclusions: These results indicate that the manual parcellation scheme we have developed can detect changes in the medial temporal cortex in patients with mild AD. When automated, this method should provide a reliable means for identifying and following structural changes throughout the cerebral cortex in cases with both prodromal and established AD.

Commercial Relationship: R.J. Killiany, None.

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Burns
IC-P-024 &
P3-082

Subclinical Brain Lesions and Cognition in Nondemented Aging and Early-Stage Alzheimer Disease

Topic: Clinical Use of Imaging Differential Diagnosis

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Poster Board Number: IC-P-024

Background: Subclinical brain lesions including white matter changes and “silent” infarcts are prevalent in both nondemented aging and Alzheimer disease (AD). In nondemented aging, subclinical lesions are associated with reduced cognition and increased dementia risk. Their relationship with cognition in early-stage AD is unclear. **Objective(s):** Assess the relationship of subclinical lesions with cognition in early-stage AD and nondemented aging. **Methods:** Brain magnetic resonance imaging (MRI) and neuropsychological testing was performed in subjects enrolled in the Washington University Alzheimer Disease Research Center. All subjects were 60 years of age or older, without a clinical stroke history or transient ischemic attack, and were nondemented (Clinical Dementia Rating [CDR] 0, n = 86) or had very mild (CDR 0.5) or mild (CDR 1) dementia of the Alzheimer type (n = 62). At study entry, subjects were without medical, psychiatric, or neurological disease other than AD. MRI measures included deep (DWM) and periventricular (PVWM) white matter changes, infarcts, and normalized whole brain volume.

Standard measures of memory, language, reasoning, and visuospatial ability were used to generate a composite global measure of cognition based on prior factor analytic studies. Analyses include linear regression and analysis of covariance adjusting for age, gender, and education. **Results:** DWM and PVWM burdens were related to reduced global cognition (DWM $\Delta r^2 = 0.057$, $p < 0.01$ and PVWM $\Delta r^2 = 0.068$, $p = 0.001$). Silent infarcts were associated with reduced global cognition (mean difference, $z = -0.72$, $p < 0.05$). Separate group analyses indicated that DWM and PVWM were associated with reduced cognition in early-stage AD (DWM $\Delta r^2 = 0.076$, $p < 0.05$; PVWM $\Delta r^2 = 0.082$, $p < 0.05$) but not nondemented aging. Silent infarcts were associated with reduced cognition in both nondemented aging and early-stage AD (mean difference, $z = -0.938$, $p < 0.05$ and -0.438 , $p < 0.05$, respectively) and demonstrated a stronger relationship with cognition in early-stage AD than nondemented aging.

Conclusions: Subclinical brain lesions are associated with reduced cognition across a spectrum of early-stage AD and nondemented aging. The capacity of subclinical lesions to influence cognition is enhanced in early-stage AD vs. nondemented aging.

Commercial Relationship: J.M. Burns, None.

Session Title: Poster Session
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Burns

P3-173

Pathological Correlates of the Substantia Nigra in Alzheimer's Disease with Extrapyrarnidal Signs

Topic: Histopathology

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Presentation Number: P3-173

Keyword: parkinson's disease, alpha-synuclein, tau

Background: Extrapyrarnidal signs (EPS) are commonly encountered in Alzheimer's disease (AD) and increase in prevalence as AD advances. The precise neuropathological substrate responsible for EPS in AD remains to be fully characterized. **Objective:** To determine differences in substantia nigra pathology in AD-diagnosed individuals with and without EPS and examine their association with dementia severity when symptoms emerge. **Methods:** Subjects were participants in the Washington University Alzheimer Disease Research Center coming to autopsy from 1998 through 2002 and having a clinical diagnosis of AD that was confirmed by neuropathological examination. EPS presence during life was determined by clinical methods assessing bradykinesia, cogwheel rigidity, rest tremor, and parkinsonian gait. After excluding subjects with EPS and previous neuroleptic exposure, there were 28 subjects in the EPS group and 104 subjects without EPS. Neuron loss, synuclein labeled pathology, and tau labeled pathology in the substantia nigra were measured using semiquantitative techniques with higher scores representing increased pathological burden. **Results:** Presence of synuclein-labeled pathology was more common (50% vs. 28.9%, $p < 0.05$) and total burden higher (mean burden = 1.75 vs. 0.97, $p < 0.05$) in the EPS group vs. those without EPS. There was significantly more neuron loss in the EPS group (1.50 vs. 1.11, $p < 0.05$). Tau-labeled burden was not different by group comparisons although EPS onset in later stages of dementia severity was associated with increased tau-labeled pathology ($\tau_b=0.482$, $p < 0.01$) and remained after controlling for dementia severity at death. Additionally, moderate to severe tau burden was more common in pure AD (definite AD without a secondary diagnosis) with EPS (81.8% vs. 49.0% without EPS, $p < 0.05$). EPS was significantly associated with male sex and less commonly observed in those with the apolipoprotein E4 allele. Four subjects with EPS (14.3%) had little to no significant nigral pathological changes. **Conclusions:** Clinically detected EPS in Alzheimer's disease is associated with heterogeneous substantia nigra pathology including changes related to alpha-synuclein aggregation, hyperphosphorylated tau, and neuron loss. In some cases, limited nigral pathology suggests additional extranigral factors in the clinical symptoms of EPS.

Commercial Relationship: J.M. Burns, None.

Session Title: Poster Session

Session Type: Poster

Session Start: 7/20/2004 12:30:00 PM

Session End: 7/20/2004 2:45:00 PM

Location: Hall B

Carpenter

P2-410

Patient-Spouse Concordance in Health Care and Psychosocial Preferences

Topic: Social and Behavioral Research - Values, Beliefs and Help-seeking

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Presentation Number: P2-410

Keyword: family, ethical issues, caregiving issues

Background: Family members are often asked to provide information about patient preferences when individuals with dementia are no longer able to state their own desires. Yet little is known about the accuracy of those proxy reports.

Objective(s): This study examined the accuracy of spouses who were asked to predict their partner's health care preferences, psychosocial care preferences, and perceptions of their marital relationship.

Methods: Sixty-four participants provided self-reports regarding their health care and psychosocial care preferences. Spouses predicted their partner's responses. Both members of the couple provided an assessment of their current marital relationship. In half of the couples, one partner had mild dementia (CDR 0.5 - 1.0).

Results: Spouses demonstrated poor to fair accuracy when predicting patient health care preferences (ICCs = .24 - .42). Concordance regarding psychosocial care preferences was poor to moderate (ICCs = .25 - .54). Agreement regarding aspects of the marital relationship was higher (ICCs= .55 - .78). There were few differences in concordance between the dementia and no-dementia couples. In all domains there was wide variability in concordance, suggesting important intercouple differences. Flexibility and emotional closeness within couples were associated with better concordance.

Conclusions: Demographic factors were less predictive of spouse accuracy than contextual and relationship variables, suggesting that family education or counseling interventions may improve knowledge about the perspective of individuals with dementia.

Commercial Relationship: B. Carpenter, None.

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Carr
P2-307

Driving Retirement in Dementia of the Alzheimer's Type

Topic: Social and Behavioral Research - Human Factors and Environmental Design
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Presentation Number: P2-307

Keyword: driving, clinical care, caregiving issues

Driving Retirement in Dementia of the Alzheimer's Type.

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Background: As we experience an increasing number of older adults with dementia of the Alzheimer's Type (DAT), we can anticipate more demented drivers. There is a paucity of information on the characteristics of demented drivers at the time of driving cessation or knowledge regarding their reasons for driving retirement.

Objective: To describe the clinical and psychometric characteristics of DAT drivers that are at risk for driving cessation and to determine the reasons DAT drivers stop driving.

Method: This descriptive study examined DAT drivers who participated in a longitudinal study on memory and aging at our ADRC between 1981-2000. We identified drivers with DAT in our data base who stopped driving between two annual assessments. We examined the Clinical Dementia Ratings (CDR) the year prior to driving cessation to obtain a measure of dementia severity that might identify a driver at risk for cessation. We then abstracted information on the reasons for driving cessation that were provided by the collateral source in the research record.

Results: 112 DAT drivers (78.1 years, 40% male, 13.7 years of education) met criteria for our study. There were 11 CDR=0, 58 CDR=0.5, 42 CDR=1, and 1 CDR=2. We then compared our sample of 112 DAT drivers who had stopped driving with 65 active drivers with DAT (79.7 years, 51% male, 13.4 years of education) and found no difference between the two groups in their CDR at the last time of assessment, the number of times they had annual assessments, or any of the psychometric measures (all ps > .05). The reasons cited for driving cessation are listed as follows:

Reasons for Driving Cessation	% (Sum total great than 100 due to multiple reasons)
Dangerous Driver	22
Accident	20
Orientation	28
Visual, medical	12
Age	2
License	5
Other	13

Conclusions: The decision to stop driving in older adults in the mild stages of dementia is probably complex. In addition to the reasons cited in the table, there are likely other factors (financial and/or social) besides cognitive impairment that play a role.

Commercial Relationship: D.B. Carr, American Medical Association Development of educational materials with stipend; National Traffic Highway Safety Administration Specific research activities.

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Cirrito

P2-296

Apolipoprotein E delays A β elimination in the brain interstitial fluid of young PDAPP mice

Topic: Molecular Mechanisms of Neurodegeneration - APOE

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Presentation Number: P2-296

Keyword: APOE, beta-amyloid, metabolism

Background: Apolipoprotein E (apoE) is the only proven genetic risk factor for late-onset, sporadic Alzheimer's disease. Data from transgenic mouse models demonstrate that apoE can influence the structure and accumulation of amyloid- β within the brain. At older ages, PDAPP transgenic mice that lack mouse apoE (apoE^{-/-}) have significantly less fibrillar A β deposits (thioflavine S positive/Congo red positive) and a different anatomical pattern of A β deposition compared to mice that express apoE. It remains unclear however if apoE also has a direct *in vivo* effect on brain A β metabolism. **Objectives:** We performed a series of studies to dynamically assess A β elimination within the interstitial fluid in the presence and absence of apoE. **Methods:** We used an *in vivo* microdialysis technique to serially sample A β within the brain interstitial fluid of PDAPP mice. Combined with a potent γ -secretase inhibitor, we were able to determine the elimination half-life of A β in mice that contained or lacked apoE. **Results and Conclusions:** In addition to age-dependent, pathological changes, PDAPP apoE^{-/-} mice also have differences at younger ages that are independent of A β deposition. In three month old PDAPP animals, the absence of mouse apoE elevates the level of human A β in the extracellular compartments of the brain, namely the CSF and ISF. Altered A β levels prior to plaque accumulation suggests a possible role of apoE in brain A β metabolism/clearance. Using an *in vivo* microdialysis technique to determine the elimination half-life of A β within the brain interstitial fluid, we demonstrate that in the absence of mouse apoE, human A β is eliminated more rapidly from the ISF. This reveals that in addition to its effect on A β plaque structure and toxicity, apoE also has a direct role in A β metabolism/clearance *in vivo*. We are currently investigating if human apoE isoforms have differential effects on human A β metabolism/clearance in young PDAPP mice.

Funding: NIH AG13956, AG05681, AG11355, and Eli Lilly and Co.

Commercial Relationship: J.R. Cirrito, None.

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Location: Hall B

Crawley

P4-191

Differential Expression of Cholesterol 24-Hydroxylase and Cholesterol 27-hydroxylase in Alzheimer's disease.

Topic: Molecular Mechanisms of Neurodegeneration - Enzyme Activities

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Presentation Number: P4-191

Keyword: cholesterol, beta-amyloid, neuropathology

Background: Cholesterol is eliminated from neurons by oxidization, which generates oxysterols. Cholesterol oxidation is mediated by the enzymes cholesterol 24-hydroxylase (Cyp46) and cholesterol 27-hydroxylase (Cyp27). Recent studies show an association between the Cyp46 and Alzheimer's disease. The expression of Cyp46 and Cyp27 in Alzheimer's and control brain has not been investigated. In addition, although prior studies have examined the effects of synthetic oxysterols on the processing of amyloid precursor protein (APP), the actions of the naturally occurring oxysterols has yet to be examined.

Objective(s): To understand the role of cholesterol oxidation in AD, we compared the actions of 24 and 27 hydroxycholesterol on the processing of APP and analyzed the distribution of the two cholesterol hydroxylases.

Methods: The distribution of Cyp46 and Cyp27 was examined by immunohistochemistry and immunoblotting of Alzheimer and control brain. The regulation of APP processing was examined by immunoblot and ELISA in primary neurons, astrocytes and CHO cells.

Results: Immunocytochemical studies show that Cyp46 and Cyp27 are expressed in neurons and some astrocytes in the normal brain; in addition, Cyp27 is present in oligodendrocytes. In AD, Cyp46 shows prominent expression in astrocytes and around amyloid plaques. In contrast, in AD Cyp27 expression decreases in neurons, is not apparent around amyloid plaques but increases in oligodendrocytes. Both oxysterols inhibit production of A β in neurons to a much greater extent than in CHO cells or astrocytes, even after supplementation with retinoic acid. In addition, 24 hydroxycholesterol is approximately 1000-fold more potent than 27 hydroxycholesterol, with the IC₅₀ of 24 hydroxycholesterol for inhibiting A β secretion being about 1 nM. Oxysterols also selectively inhibited both protein kinase C activity and APP secretion following stimulation of protein kinase C in neurons but not in astrocytes or CHO cells.

Conclusions: The selective expression of Cyp46 around neuritic plaques and potent inhibition of APP processing in neurons by 24 hydroxycholesterol points to a potentially important role for this enzyme in the pathophysiology of AD.

Commercial Relationship: B. Wolozin, None.

Session Title: Poster Session
Session Type: Poster
Session Start: 7/21/2004 12:30:00 PM
Session End: 7/21/2004 2:45:00 PM
Location: Hall B

Csernansky

IC-P-091

Hippocampal Shape Predicts Response to Donepezil

Topic: Monitoring the Progression Of Alzheimer's Disease

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Poster Board Number: IC-P-091

Background:

There are few reliable predictors of the clinical outcome of donepezil treatment in patients with dementia of the Alzheimer type (DAT). Prior studies have suggested that neuroanatomical measures may predict such outcomes.

Objective(s):

We tested the hypothesis that an assessment of hippocampal structure could predict the outcome of donepezil treatment in DAT patients.

Methods:

The outcome of donepezil treatment was assessed using sum-of-boxes scores from the CDR, MMSE total scores and Neuropsychiatric Inventory (NPI) total scores in 31 DAT subjects treated with donepezil, 10 mg/day, for a minimum of 48 weeks. We used large-deformation, high dimensional brain mapping to quantify hippocampal volume and surface in each subject prior to treatment. Growth curve models were used to estimate the rate of change in the selected clinical measures.

Results:

The mean (SD) annual rate of change for the sum-of-boxes score was 0.069 (0.11) per year. The mean (SD) annual rate of change for the MMSE total score was -0.038 (0.1), and for the NPI total score was 0.027 (0.18). Among the first 10 eigenvectors, which explained greater than 80% of the variance in hippocampal shape, eigenvector 3 coefficients were correlated with cognitive decline. More negative eigenvector 3 coefficient values were correlated with increasing sum-of-boxes slope values ($r = -0.48$, $p = 0.0067$), decreasing MMSE slope values ($r = 0.36$, $p = 0.050$), but not with NPI scores. Hippocampal volume, total cerebral volume and total intracranial volume were not correlated with any measures of cognitive decline. The correlations persisted even after taking into account total cerebral brain volume, total intracranial volume, and age.

Conclusions:

The pattern of shape variation associated with the failure to respond to donepezil treatment resembled the pattern of shape deformation previously shown to discriminate DAT and nondemented subjects. In contrast, the absence of a specific deformation in hippocampal shape was associated with improvement during donepezil treatment. These results suggest that the hippocampal shape is a sensitive marker of the capacity of DAT patients to respond to cholinesterase inhibitor drug treatment.

Commercial Relationship: J.G. Csernansky, Pfizer Specific research activities.

Session Title: Poster Session
Session Type: Imaging Consortium
Session Start: 7/17/2004 12:00:00 PM
Session End: 7/17/2004 1:25:00 PM
Location: Convention Center

Csernansky

IC-P-111

Hippocampal Shape and Volume Predicts the Onset of Dementia in the Elderly

Topic: Early Detection

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Poster Board Number: IC-P-111

Background:

Structural deformity of the hippocampus has been found in individuals with very mild forms of dementia of the Alzheimer type (DAT). However, it is unknown whether such deformity is present in the nondemented elderly, and if so, whether its presence predicts dementia onset.

Objective(s):

The purpose of this study was to determine whether abnormalities of hippocampal shape, especially related to the CA1 hippocampal subregion, and volume predicts dementia onset in the nondemented elderly.

Methods:

Using high dimensional diffeomorphic transformations of a neuroanatomical template, hippocampal volumes and surfaces were defined in 49 nondemented elders. Within the neuroanatomical template, the hippocampal surface was partitioned into three zones corresponding to the CA1 subfield, the CA2-4 subfields and the subiculum, so that shape deformities specific to these anatomical subregions could be measured. Clinical assessments using the Clinical Dementia Rating scale (CDR) were then performed for a mean of 3.2 years (range 1.5-5.2 years), and conversion of a subject's CDR score from 0 to 0.5 was defined as the onset of dementia. Survival analysis (i.e., Cox proportional hazards models) was used to test our hypothesis.

Results:

Inward variation of the CA1 zone of the left hippocampal surface ($p=.019$) and left hippocampal volume ($p=.026$) predicted the onset of dementia in separate survival analyses. When hippocampal surface variation and volume were included in a single survival analysis, inward variation of the CA1 zone of the left hippocampal surface was selected as the only significant predictor of dementia onset ($p=.019$). Also, the pattern of hippocampal surface variation observed in the nondemented subjects who later developed dementia was similar to the pattern of hippocampal surface deformation previously observed to discriminate subjects with very mild DAT and nondemented subjects.

Conclusions:

These results suggest that inward deformation of the left hippocampal surface in an area corresponding to the CA1 subfield is an predictor of the onset of dementia in nondemented elderly subjects.

Commercial Relationship: J.G. Csernansky, None.

Session Title: Poster Session
Session Type: Imaging Consortium
Session Start: 7/17/2004 12:00:00 PM
Session End: 7/17/2004 1:25:00 PM
Location: Convention Center

Dong
P2-067

The Efficacy of Acetylcholinesterase Inhibitors in a Animal Model of Alzheimer Disease

Topic: Animal and Cellular Models - Animal Models, Transgenic

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Presentation Number: P2-067

Keyword: animal model, amyloid precursor protein (APP), cholinesterase inhibitor

Background:Cholinesterase inhibitors are the mainstay of treatment for dementia of the Alzheimer type (DAT). However, the capacity of such drugs to ameliorate behavioral deficits in an animal model of Alzheimer disease has not been well investigated.

Objective(s): The present study was performed to compare the effects of three acetylcholinesterase inhibitors – physostigmine, donepezil and galantamine – on memory-related behaviors in Tg2576 mice.

Methods: Multiple doses of the three cholinesterase (physostigmine 0.03, 0.1, 0.3mg/kg ; donepezil 0.1, 0.3 1.0mg/kg and galantamine 0.25, 0.5 1.0mg/kg) as well as vehicle were administered to Tg2576 mice and wild-type littermates in conjunction with two tests of spatial memory - special revisal learning and contextual fear conditioning. The animals were 9 months old at the time of testing and all drugs were administrated subcutaneously 30 minutes before testing on all testing days.

Results: On the spatial reversal learning task, physostigmine demonstrated a dose-dependent capacity to improve performance in both Tg2576 and wild-type mice (treatment effect - $F=3.6$, $p=0.02$). Also, because Tg2576 mice showed impairments in performance on this task relative to wild-type controls, physostigmine tended to normalize their performance. Donepezil, but not galantamine, showed similar trends (treatment effect for donepezil - $F=1.6$, $p=0.2$ and for galantamine - $F=0.8$, $p=0.50$). On the fear conditioning task, physostigmine also showed a dose-dependent capacity to improve performance, but only in Tg2576 mice (treatment effect - $F= 4.5$, $p=0.009$); group x treatment interaction – $F=3.8$, $p=.02$). Again, similar trends were observed for donepezil and galantamine (treatment effect for donepezil - $F=2.0$, $p=0.13$ and for galantamine - $F=9.1$, $p=0.002$). However, at the highest dosage of galantamine (1.0mg/kg, s.c.), both Tg2576 mice and wild-type mice showed impaired contextual memory.

Conclusions: These studies suggest that cholinesterase inhibitors can acutely ameliorate behavioral deficits in a animal model of AD, as they do in patients with DAT.

Commercial Relationship: H. Dong, None.

Session Title: Poster Session
Session Type: Poster
Session Start: 7/19/2004 12:30:00 PM
Session End: 7/19/2004 2:45:00 PM
Location: Hall B

Dong
P2-096

Effects of Isolation Stress on Hippocampal Neurogenesis, Memory, and Amyloid Plaque Deposition in APP (Tg2576) Mutant Mice

Topic: Animal and Cellular Models - Animal Models, Transgenic

Hongxin Dong¹, Brian Goico¹, Maureen Martin¹, Cynthia A. Csernansky², Amy Bertchume¹, John G. Csernansky¹, ¹Washington University School of Medicine, St. Louis, MO, USA; ²University of Queensland School of Medicine, Brisbane, Australia. Contact e-mail: dongh@psychiatry.wustl.edu

Presentation Number: P2-096

Keyword: animal model, amyloid, stress

Background: The Tg2576 transgenic mouse is a widely used animal model of Alzheimer Disease (AD). Tg2576 mice overexpress the human amyloid precursor protein, resulting in the deposition of β -amyloid plaques and deficits in performance on memory-related tasks beginning at 9 months of age. The effects of stress on plaque deposition, neurogenesis and behavioral deficits in Tg2576 mice have not been investigated

Objective(s): We investigated plaque deposition, the capacity for neurogenesis in the dentate gyrus of the hippocampus and performance on a task of contextual conditioning in Tg2576 mice that had been exposed to social isolation or control conditions.

Methods: Thirty-six Tg2576 mice and 36 wild type littermates were exposed to isolation stress from weaning until 6 months of age. A subset of these animals was administered fluoxetine administration (5mg/kg) at the end of their isolation period for 14 days. BrdU staining was used to assess the capacity for hippocampus neurogenesis. Contextual and cued fear conditioning task were used to assess memory-related behavior.

Results: Tg2576 mice had a decreased capacity for neurogenesis in the dentate gyrus of the hippocampus both before and after plaque deposition and the appearance of behavioral deficits. Social isolation for 6 months decreased hippocampal neurogenesis and impaired contextual, but not cued, memory in both Tg2576 and wild type mice, and the magnitude of this effect was larger in Tg2576 mice. Also, in Tg2576 mice, isolation was associated with increased deposition of β -amyloid plaques. Fluoxetine administration, increased neurogenesis in both isolated Tg2576 and wild type mice, and improved contextual memory in Tg2576 mice, but did not alter plaque formation.

Conclusions: These results suggest that the stress of isolation can accelerate β -amyloid plaque deposition and increase the functional consequences of β -amyloid plaque deposition in Tg2576 mice. Also, the effects of isolation stress on the functional consequences of β -amyloid plaque deposition can be mitigated by fluoxetine administration. These results provide evidence in an animal model of AD that stress may be an important factor in determining the onset of dementia in AD patients, and that treatment with fluoxetine might be useful in mitigating the effects of such stress.

Commercial Relationship: H. Dong, None.

Session Title: Poster Session
Session Type: Poster
Session Start: 7/19/2004 12:30:00 PM
Session End: 7/19/2004 2:45:00 PM
Location: Hall B

Fagan

P3-047

Probing for Antecedent Biomarkers of Alzheimer's Disease Through Analysis of Cerebrospinal Fluid From Middle-Aged Individuals

Topic: Diagnosis and Disease Progression - CSF Markers

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Presentation Number: P3-047

Keyword: biomarkers, beta-amyloid, APOE

Background: While there are presently no proven treatments that delay the onset or prevent the progression of Alzheimer's disease (AD), many promising candidates have this potential, some of which are currently being developed and slated for testing in humans. During therapy development and implementation, it will be critically important to have biomarkers that can identify individuals at particularly high risk for developing AD in order to target them for entry into therapeutic trials and to monitor therapy.

Objective(s): : Since AD neuropathology (preclinical AD) begins 10-20 years prior to the onset of clinical dementia, identification of antecedent biomarkers would allow us to identify individuals likely to be developing AD pathology but who are still cognitively normal (preclinical AD), a group in which targeted therapies would likely have the greatest clinical impact.

Methods: To this end, we are analyzing cerebrospinal fluid (CSF) samples from healthy, middle-aged individuals (age 45-64) and comparing them as a function of apoE genotype (the strongest genetic risk factor for AD) or family history of AD (having a parent with or without AD).

Results: Analysis of an initial subset of samples with a family history of AD demonstrates significantly lower levels of CSF A β ₄₂ in subjects who are apoE4+ (high risk, n=14) compared to apoE4- (lower risk, n=6). No significant differences were observed in levels of CSF A β ₄₀, tau, or p-tau₁₈₁ as a function of apoE genetic risk.

Conclusions: These preliminary data suggest that CSF A β ₄₂ may be useful as a potential biomarker for antecedent, preclinical AD. Analyses in progress include assessment of additional samples, quantification of A β species specifically associated with CSF lipoproteins, measurement of sulfatide and other possible candidate biomarkers, and stratification of data according to family history of AD.

Commercial Relationship: A.M. Fagan, None.

Session Title: Poster Session
Session Type: Poster
Session Start: 7/20/2004 12:30:00 PM
Session End: 7/20/2004 2:45:00 PM
Location: Hall B

Fryer

O1-03-04

THE LDL RECEPTOR REGULATES LEVELS OF CNS APOE AND ALZHEIMER'S DISEASE-LIKE PATHOLOGY

Topic: Molecular Mechanisms of Neurodegeneration - APOE

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Presentation Number: O1-03-04

Keyword: beta-amyloid, APOE, animal model

Background: Apolipoprotein E (apoE) has been shown to play a critical role in the deposition, structure, and clearance of the amyloid β (A β) peptide in Alzheimer's disease. Although apoE has several known receptors in the periphery, the primary apoE receptor regulating levels of brain apoE, or the role of specific apoE receptors in Alzheimer's disease, is unknown.

Objective(s): Our objectives were to determine the role of the LDL receptor in brain apoE metabolism and effects on Alzheimer's pathogenesis *in vivo*.

Methods: We utilize *in vitro* apoE uptake assays in cells deficient in low-density lipoprotein (LDL) receptor, LDL receptor-related protein (LRP), or double-null cells. We also utilize *in vitro* apoE uptake and degradation assays in CHO cells overexpressing other LDL receptor family members. Additionally, we examine apoE levels *in vivo* in the cerebrospinal fluid (CSF) of wild-type mice and mice deficient in the low-density lipoprotein receptor (LDLR^{-/-}). We also bred the PDAPP mouse model of Alzheimer's disease on an LDLR^{-/-} background to determine the role of this receptor in Alzheimer's pathogenesis.

Results: We show *in vitro* that the LDL receptor regulates cellular uptake and degradation of astrocyte-secreted apoE, whereas LRP, apoER2, and VLDLR showed no significant role in this process. Additionally, LDLR^{-/-} mice have significantly higher levels of apoE in CSF as well as significantly higher levels of the A β peptide. Furthermore, when the PDAPP mouse model of Alzheimer's disease was bred on an LDLR^{-/-} background, there were increased levels of A β measured by ELISA and significantly more plaques as measured by immunohistochemistry.

Conclusions: These results demonstrate that the level and function of the LDL receptor in the brain is likely to be critically involved in Alzheimer's disease pathogenesis and that strategies aimed at altering the level and function of the LDL receptor in the brain may be a good target for future therapeutic strategies.

Commercial Relationship: J.D. Fryer, None.

Session Title: Disease Mechanisms - APOE

Session Type: Oral

Session Start: 7/18/2004 4:00:00 PM

Session End: 7/18/2004 6:00:00 PM

Location: 201 ABC

Galvin
P1-118

Visuospatial and Psychomotor Deficits in Lewy Body Dementia: Improving Antemortem Diagnosis

Topic: Diagnosis and Disease Progression - Neuropsychological

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Presentation Number: P1-118

Keyword: dementia with Lewy bodies, neuropsychology

Background: Cognitive decline in Lewy Body Dementia (LBD) has been associated with deficits in attention, executive function, and visuospatial abilities, but because there are few prospective longitudinal studies of well-characterized individuals followed to autopsy, there is little information about the contribution Lewy bodies make to cognitive impairment when there is concurrent Alzheimer disease pathology.

Objective(s): We combined cognitive measures of memory, attention, visuospatial, and executive function to differentiate people with mixed Dementia of the Alzheimer type (DAT) and LBD from those with DAT alone and to refine the characterization of cognitive deficits attributable to LBD.

Methods: In a longitudinal study of memory and aging, 149 individuals followed to autopsy were administered annual psychometric tests tapping working, episodic, semantic, and nonverbal memory; attention; visuospatial, construction, and psychomotor function.

Results: In a postmortem sample of demented older adults (pure DAT = 77; mixed LBD and DAT = 72), we found a systematic pattern of group differences on psychometric test scores at entry into the study. The mixed DAT/LBD group performed significantly more poorly on Trailmaking A, and the DAT group perform significantly worse on WMS Logical Memory. A discriminant analysis was used to create a composite score that maximally discriminated people with DAT alone from people with mixed DAT and LBD. Based on this composite, we were able to predict the neuropathologic diagnosis with 77% accuracy (area under the ROC curve = .85). When the discriminant analysis was repeated using only these two tests, accuracy dropped only 6%, indicating that the bulk of the discrimination rests in these two tests.

Conclusions: Clinical criteria may accurately predict "pure" LBD cases but may not fully capture the more common occurrence of mixed Alzheimer and Lewy body pathologies, leading to an underestimation of the true prevalence of LBD. Using data from a well-characterized longitudinal sample, we were able to demonstrate meaningful differences between mixed DAT/LBD and DAT alone, which may improve the antemortem diagnosis of LBD.

Commercial Relationship: J.E. Galvin, None.

Session Title: Poster Session
Session Type: Poster
Session Start: 7/18/2004 8:00:00 AM
Session End: 7/18/2004 9:30:00 AM
Location: Hall A

Galvin

O1-01-01

Dysferlin In The Alzheimer's Disease Brain: Another Amyloid

Topic: Histopathology

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Presentation Number: O1-01-01

Keyword: amyloid, neuropathology

Background: Dysferlin is a cytoskeleton protein implicated in late onset limb-girdle muscular dystrophy and Miyoshi myopathy. It forms amyloid (Congo Red) deposits in affected muscle. Dysferlin is a member of the ferlin family, homologous to the fer1 protein in *Caenorhabditis elegans*. Dysferlin is expressed highly in muscle but has not been previously described in the brain.

Objective(s): We explored whether dysferlin also forms amyloid deposits in the brains of patients with Alzheimer disease (AD).

Methods: Twelve brains, staged using the Clinical Dementia Rating (CDR) were examined: 9 AD cases (CDR 1 = 3, CDR 2 = 3, CDR 3 = 3) with a mean age of 85.9y and mean disease duration of 8.9y, and 3 age-matched controls (CDR 0) with a mean age of 87.5y. Immunohistochemistry with antibodies to dysferlin, tau and amyloid β -protein (A β) was performed. We also performed Western blot analysis of total brain protein (RIPA) and serial extracted proteins from soluble (high salt and high salt/Triton X-100) and insoluble (SDS and formic acid) fractions.

Results: Dysferlin was found as a cytoplasmic protein in the pyramidal neurons of normal and AD brains. In addition, there were dysferlin-positive fibrillar structures resembling dystrophic neurites coursing through A β plaques in the neocortex and hippocampus in the AD brain, distinct from tau-positive dystrophic neurites. In RIPA extracted fractions, dysferlin was seen as 230-272 kD bands in normal and AD brains. In serial extractions, there was a shift of dysferlin from soluble fractions in high salt and high salt/Triton X-100 to the SDS fraction in CDR 1, 2 and 3 AD brains. No dysferlin was detected in formic acid extractions.

Conclusions: Dysferlin is a new amyloid described in the AD brain associated with neuritic plaques and A β at all stages of the disease. Dysferlin also forms amyloid deposits in muscle in adult onset muscular dystrophy. Dysferlin is thought to play a role in muscle maintenance and repair of plasma membrane damage. The deposition of dysferlin in the AD brain may be related to the inability of neurons to repair damage due to A β deposits accumulating throughout the progression of AD.

Commercial Relationship: J.E. Galvin, None.

Session Title:	Molecular Pathology/Histopathological
Session Type:	Oral
Session Start:	7/18/2004 4:00:00 PM
Session End:	7/18/2004 6:00:00 PM
Location:	113 ABC

Goate

S2-01-04

Progress Toward the Identification of Novel Genetic Risk Factors for Late Onset Alzheimer's disease

Topic: Epidemiology & Risk Factors: Genetic Factors

Alison M. Goate¹, Petra Nowotny¹, Tony Hinrichs¹, Scott Smemo², Keoni Kawe², Julie Williams³, Mike Owen³, Peter Holmans³, Lesley Jones³, Amanda Myers⁴, Fabienne Wavrant De Vrieze⁴, John Hardy⁴, Simon Lovestone⁵, Kit Lau⁶, Yonghong Li⁶, Andrew Grupe⁶, ¹Washington University, St. Louis, MO, USA; ²Washington University School of Medicine, St. Louis, MO, USA; ³University of Wales College of Medicine, Cardiff, United Kingdom; ⁴National Institute of Aging, Washington, DC, USA; ⁵Institute of Psychiatry, London, United Kingdom; ⁶Celera Diagnostics, Alameda, CA, USA. Contact e-mail: goate@icarus.wustl.edu

Presentation Number: S2-01-04

Keyword: molecular genetics, gene mapping, genotyping

Background: We have previously performed a genome-wide scan in late onset Alzheimer's disease (LOAD) families, and demonstrated linkage on chromosomes 9, 10 and 12. To follow up these findings our collaborative group has used two strategies: detailed analysis of candidate genes and large-scale genotyping of single nucleotide polymorphisms (SNPs) across the region of linkage. **Objective:** To identify novel genetic risk factors for LOAD. **Methods:** As part of a systematic analysis of our linkage signals we have performed SNP genotyping across the peaks on chromosomes 10 and 12. An exploratory sample was used for this screen and SNPs giving a $p < 0.05$ were then genotyped in additional samples and followed up with additional SNPs. We also genotyped SNPs spanning two candidate genes on chromosome 10: IDE and VR22 in multiple case control series. We tested for allelic and genotypic association in these series, examined marker-marker linkage disequilibrium across the genes and tested for haplotypic association. **Results:** Our systematic genotyping on chromosome 12 identified a region exhibiting strong association with disease in our exploratory sample. Follow-up with additional SNPs in the same sample confirmed the observation. However, genotyping these SNPs in additional case control series provided only weak evidence for replication. In contrast, several SNPs on chromosome 10, which gave only modest evidence for association in the exploratory sample gave consistent evidence for association in multiple series and strong evidence for association in the combined sample. Our analysis of IDE and VR22 did not provide compelling evidence that these genes harbor major risk factors for LOAD. While individual SNPs or haplotypes in both genes exhibited association with disease in some datasets, no consistent evidence for association was observed for SNPs in either IDE or VR22 across multiple series. **Conclusion:** It is unclear whether our results on chromosome 12 represent type I error or locus heterogeneity of a complex disease. Despite our strong linkage signal on chromosome 10 we have not observed compelling association with SNPs in any single candidate gene, raising the possibility that the strong linkage signal results from two or more susceptibility genes in the region.

Commercial Relationship: A.M. Goate , None.

Session Title: Genetics
Session Type: Symposia
Session Start: 7/19/2004 10:30:00 AM
Session End: 7/19/2004 12:30:00 PM
Location: 103 ABC

Hartman

P2-068

Age-independent and age-dependent learning and memory abnormalities in PDAPP mice on a C57Bl/6 background.

Topic: Animal and Cellular Models - Animal Models, Transgenic

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Presentation Number: P2-068

Keyword: behavioral symptoms, animal behavior, cognition

Background: PDAPP transgenic mice express a mutant form of human amyloid precursor protein that is associated with early-onset Alzheimer's Disease, and they develop age-dependent A β deposition. However, these mice exhibit impairments in spatial learning and memory on the water maze navigation task long before plaque deposition begins.

Objective(s): Finding a learning test that demonstrates age and/or plaque-related impairments is important for the development of therapeutic treatments targeting A β .

Methods: Chen et al. (Nature, 2000; 408:975-9) found that old, but not young, PDAPP mice on a mixed genetic background demonstrated increasingly poor performance on a water maze task in which the location of the escape platform changed 5 times over the course of testing. The deficits in old mice were correlated with plaque load in the brain, suggesting that A β deposition played a role in the cognitive decline. Using a similar task, the spatial learning abilities of PDAPP \pm mice on a pure genetic background (C57Bl/6) were assessed in a longitudinal and cross-sectional study to determine the behavioral effects of A β accumulation with age. At 4 and 17 months of age, the PDAPP \pm and littermate WILDTYPE mice were subjected to a 5-week water maze protocol in which the escape platform's location changed weekly, requiring the mice to repeatedly learn new information.

Results: PDAPP mice were markedly impaired in water maze spatial navigation learning as early as 4 months of age (pre-plaque). This may be due to APP overexpression rather than A β deposition. The water maze performance of both wildtype and PDAPP mice declined with age.

Conclusions: The observation that PDAPP mice exhibit a greater deterioration with age suggests that A β deposition may play a role in the cognitive decline. Analysis of brain and plasma A β levels in these mice is currently underway.

Commercial Relationship: R.E. Hartman, None.

Session Title: Poster Session
Session Type: Poster
Session Start: 7/19/2004 12:30:00 PM
Session End: 7/19/2004 2:45:00 PM
Location: Hall B

Hecimovic

P4-271

Mutagenesis of APP Reveals No Correlation Between A β and AICD Production

Topic: Molecular Mechanisms of Neurodegeneration - Presenilins

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Presentation Number: P4-271

Keyword: gamma-secretase, amyloid precursor protein (APP), presenilin

Background: Understanding the molecular mechanism of β -amyloid (A β) generation is crucial for Alzheimer's disease pathogenesis as well as for normal APP function. The transmembrane domain (TM) of APP appears to undergo presenilin-dependent cleavage at two topologically distinct sites: a site in the middle of the TM domain that is crucial for generation of A β -peptides ($\gamma_{40/42}$ -site), and a site close to the cytoplasmic border (ϵ -site) that leads to production of APP intracellular domain (AICD). The PS-dependent dual intramembraneous cleavage has been described for several other γ -secretase substrates Notch1 and CD44. **Objective(s):** We aimed to determine whether the two topologically distinct PS-dependent cleavages of APP are mediated by a mechanism that requires sequential cleavage events or are independent of each other. **Methods:** In order to test this we introduced a series of mutations in APP and monitored A β and AICD generation both in the cell and in an *in vitro* γ -secretase assay. A β production was analyzed by ELISA assay and mass spectrometry, while AICD levels were monitored by western blotting. Subcellular localization of APPwt and its mutants was determined by confocal microscopy. **Results:** Since APP-FAD mutations that lie between the γ_{42} and ϵ -site exert a consistent effect on A β production (increasing A $\beta_{42/40}$ ratio), we tested whether such mutations would generate similar effects on AICD generation. T714I and L723P significantly decreased AICD levels, but other mutations had little or no effect. Furthermore, extensive mutagenesis between the γ_{42} and the ϵ -site did not reveal any correlation between total AICD levels and either an increase or a decrease of any A β species. Inhibition of cleavage at one site did not affect cleavage at the other site, indicating that APP processing events at $\gamma_{40/42}$ and ϵ -sites are not tightly linked. **Conclusions:** The observation that the PS-dependent generation of A β and AICD is not related suggests that $\gamma_{40/42}$ and ϵ -cleavages of APP may involve different enzyme activities/cofactors or may occur in mutually exclusive subcellular compartments. Thus, it may be possible to develop pharmacologically distinct γ -secretase inhibitors that would affect A β , but not AICD production.

Commercial Relationship: S. Hecimovic, None.

Session Title: Poster Session

Session Type: Poster

Session Start: 7/21/2004 12:30:00 PM

Session End: 7/21/2004 2:45:00 PM

Location: Hall B

Holtzman

P1-240

Static and Dynamic AFM Studies of β -amyloid Aggregates and Their Interaction with Relevant Biological Macromolecules

Topic: Molecular Mechanisms of Neurodegeneration - Beta-amyloidosis

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Presentation Number: P1-240

Keyword: antibody, APOE, amyloid

Extensive data suggest that the conversion of the amyloid- β (A β) peptide from soluble to insoluble forms is a key factor in the pathogenesis of Alzheimer's disease (AD). In recent years, atomic force microscopy (AFM) has provided useful insights into the physicochemical processes involving A β , and it can now be used to investigate other factors that may either inhibit or promote fibrillogenesis. Two of these factors are anti-A β monoclonal antibodies and astrocyte secreted lipoproteins containing apoE3, apoE4 or apoJ. Recently, there has been considerable interest in the role of anti-A β monoclonal antibodies as potential inhibitors of A β fibrillogenesis, as disrupters of already formed aggregates, and as clinical diagnostic tools. ApoE and apoJ are the two most abundant apolipoproteins produced in the central nervous system (CNS). As with other lipoproteins, CNS lipoproteins play a central role in handling hydrophobic (by)products of metabolism.

The first part of this presentation will describe the results of AFM studies of the A β fibrillogenesis in the presence of anti-A β antibodies directed against different domains of A β (m3D6 directed against an N-terminal domain of A β and m266.2 directed against the central domain of A β). The effect of antibodies was quantified through custom quantitative analysis of AFM images, including the determination of the number of fibrils per μm^2 and aggregate size analysis. Fibril formation was considerably retarded in the presence of m3D6, and it was completely suppressed by m266.2.

The second part will describe the results of three-dimensional shape and size analysis of plasma lipoproteins and astrocyte secreted lipoproteins (ASLPs) performed with the aid of *in situ* AFM, which has the unique ability to study three-dimensional nanostructures under physiological conditions. Main emphasis will be placed on the comparison of ASLPs containing apoE3, apoE4, or apoJ with high density lipoprotein particles, thought to be their closest analogs in plasma. The interactions of (ASLPs containing) apoE3, apoE4, and apoJ with A β and their impact on fibrillogenesis will be also discussed.

Commercial Relationship: T. Kowalewski, None.

Session Title: Poster Session

Session Type: Poster

Session Start: 7/18/2004 8:00:00 AM

Session End: 7/18/2004 9:30:00 AM

Location: Hall A

Holtzman

P3-021

Plasma A β Levels Following A β Antibody Administration in Young Mice Predicts Brain Amyloid Burden at an Older Age.

Topic: Diagnosis and Disease Progression - Blood/Plasma/Serum Markers

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Presentation Number: P3-021

Keyword: plaques, antibody

Background: Previously we reported that administration of an anti-A β antibody; m266.2, resulted in a rapid and sustained increase in plasma A β levels which were significantly correlated to brain A β /amyloid burden.

Objectives: To investigate whether plasma A β 40 and/or A β 42 levels following a single administration of an anti-A β antibody; m266, at a young age (4 or 8 months of age) could predict the amount of brain amyloid burden which would occur at an older age (12 months of age).

Methods: Two cohorts of APP^{V717F} transgenic mice at 4 and 8 months of age were administered a single dose of m266 (500 μ g). Twenty four hours after antibody administration, plasma A β 40 and A β 42 levels were determined by ELISA. Mice were subsequently aged an additional 8 and 4 month, respectively. At 12 months of age, amyloid burden was determined using quantitative immunohistochemistry.

Conclusions: There was a highly significant correlation between plasma A β 42 and A β 40 at 8 months and brain A β burden quantified at 12 months. Plasma A β 42 levels measured in 4 month old mice following administration of m266 were significantly correlated to brain amyloid burden at 12 months of age. These data suggest that plasma A β levels following a single administration of m266 at a young can accurately predict the amount of brain amyloid burden that will develop as these tg mice age.

Commercial Relationship: S.M. Paul, None.

Session Title: Poster Session
Session Type: Poster
Session Start: 7/20/2004 12:30:00 PM
Session End: 7/20/2004 2:45:00 PM
Location: Hall B

Hosto
P1-020

The Influence of Depression in the Prediction of Cognitive Performance in Persons with DAT

Topic: Diagnosis and Disease Progression - Clinical

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Presentation Number: P1-020

Keyword: depression, clinical assessment

Background: While informants have been shown to provide generally accurate information regarding the presence and degree of cognitive dysfunction in subjects with DAT, such information may be confounded by the co-existence of depression. Questions remain about the accuracy of informants in predicting cognitive performance in depressed DAT subjects. **Objective(s):** To examine whether depression influences the accuracy of informants in predicting performance on particular cognitive tasks in subjects with very mild or mild DAT, compared with subjects with no dementia. **Methods:** Data were collected from 406 entry assessments of subjects and informants interviewed in a dementia research clinic over a four-year period. The mean age of the subjects was 77.23 years, with 65% being female and a mean of 13.27 years of education. Only subjects with a diagnosis of no dementia (mean MMSE 28.6, n=136), very mild DAT (mean MMSE 24.9, n=145), or mild DAT (mean MMSE 21.1, n=125) were included. An accuracy score was derived from the informants' predictions of cognitive abilities and subjects' actual cognitive performance. Other measures included physicians' clinical dementia rating (CDR), physicians' clinical judgment of the presence of depression, subjects' self-reports of depression, and informants' ratings of depression in subjects. **Results:** Informants reported more depressive features in DAT subjects than the subjects did themselves; the number of depressive features increased with dementia severity. Physicians diagnosed depression more frequently in subjects with mild DAT (29.6%) compared with very mild DAT (24.1%), with cognitively normal subjects having the lowest rate of depression (13.2%). On self-reports, all three subject groups reported few or no depressive features. Informants were most accurate in predicting performance on tasks of memory, orientation, judgment and problem-solving for cognitively normal subjects and least accurate for mildly demented subjects. For very mildly demented subjects only, informants were less accurate in predicting cognitive performance when there was co-existent depression. **Conclusions:** Informants accurately predict cognitive performance in the presence of depression for cognitively normal and mildly demented subjects, but co-existent depression in subjects with very mild DAT adversely influences the informants' ability to assess the subjects' cognitive performance.

Commercial Relationship: T.L. Hosto, None.

Session Title: Poster Session
Session Type: Poster
Session Start: 7/18/2004 8:00:00 AM
Session End: 7/18/2004 9:30:00 AM
Location: Hall A

Hu

P3-026

Identification of biomarkers for Alzheimer's disease using proteomics approaches

Topic: Diagnosis and Disease Progression - CSF Markers

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Presentation Number: P3-026

Keyword: biomarkers, cerebrospinal fluid, mass spectroscopy

Background: Despite the fact that Alzheimer's Disease (AD) is a distinctive disorder with identifiable neuropathological features, accurate clinical diagnosis of AD remains a challenge. The initial symptoms of AD occur on a continuum with normal age-related memory loss, making AD difficult to identify in its earliest stages. Moreover, a growing body of evidence suggests that the pathology of AD (particularly the build-up of A β in plaques) begins 10-20 years before even the earliest cognitive symptoms become evident. By the time individuals begin to display cognitive symptoms associated with AD, they not only have massive A β deposition in the brain, but also have already suffered significant loss of neurons in vulnerable brain regions. Therefore, identification of better biomarkers, especially antecedent markers will greatly aid us in AD prevention, diagnosis and treatment. **Objective:** Our overall goal is to identify biomarkers for AD. As part of our efforts, we applied a comparative proteomics approach to the PDAPP mouse model for AD to identify candidate proteins that can be further examined in human body fluid samples. **Methods:** To identify proteins that are present at different levels in PDAPP mouse hippocampus and cortex tissue as opposed to that of the control animals, we employed 2-D DIGE (2-dimensional difference gel electrophoresis) that allows different samples to be co-electrophoresed on the same gel and quantified. This technique allows identification of differences in protein levels with a high degree of confidence. Duplicate 2-D DIGE analyses (using the same protein lysate samples) were performed. Selected gel protein spots were excised, digested with trypsin *in situ* and the resulting peptides were analyzed using high mass accuracy (>100 ppm) tandem mass spectrometry. The resulting spectra were used to search the NCBI-NR protein database. All peptide sequences were qualified with manual interpretation of the peptide fragmentation spectra. **Results/Conclusions:** A number of proteins have been identified that demonstrate robust differences between PDAPP mice and controls. These candidate proteins are being validated using Western blotting and ELISA and their utility as biomarkers/antecedent biomarkers for AD will be assessed using clinically characterized human CSF samples. **Funding:** NIH AG05681, MetLife Foundation, and Eli Lilly and Co

Commercial Relationship: Y. Hu, None.

Session Title: Poster Session

Session Type: Poster

Session Start: 7/20/2004 12:30:00 PM

Session End: 7/20/2004 2:45:00 PM

Location: Hall B

Kopan
P4-263

Is Substrate Oligomerization Regulating Cleavage by gamma-Secretase?

Topic: Molecular Mechanisms of Neurodegeneration - Presenilins

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Presentation Number: P4-263

Keyword: cell cultures, gamma-secretase, oligomers

Background: Regulated Intramembrane proteolysis (RIP) is emerging as a widespread means of regulating the activity of membrane-bound signaling molecules such as the Notch 1 receptor in eukaryotic cells. Intramembrane Cleaving Proteases (I-CLiP's), such as gamma-secretase catalyze peptide bond hydrolysis within the transmembrane domains (TMD) of Notch, APP and ErB4, among others. RIP by most I-CLiP's requires a prior cleavage of the juxtamembrane region, a process regulated by ligand. It has been proposed that uncleaved juxtamembrane region suppresses I-CLiP activity through dimerization of the substrate.

Objective(s): Because these conclusions were reached with substrates containing heterologous TMD or extracellular domains, we have re-examined this mechanistic model.

Methods, Results: First, we examined the dimerization potential of TMD from several PS substrates using a bacterial assay for TM dimerization (TOX-CAT). Surprisingly, we find that the TMD of gamma-secretase substrates Notch 1 through 4 and APP exist as dimers. Furthermore, mutations that adversely affect gamma-secretase-dependent cleavage of Notch in mammalian cells, do not modulate TMD dimerization. When tested in mammalian cells, the Notch TMD alone is an effective gamma-secretase substrate. While the TMD appears to be a dimer by these criteria, a quantitative immunoprecipitation assay, designed to identify intermolecular interacting domains within Notch in mammalian cells, recognized only the extracellular EGF repeats of Notch 1 as an oligomerization domain.

Conclusions: Our results indicate active (gamma-secretase substrates) and inactive (not substrates for gamma-secretase) Notch molecules cannot be differentiated by their oligomerization state. Therefore, controlled transition of TMD from dimer to monomer is unlikely to underlie the regulatory mechanism of intramembraneous cleavage by gamma-secretase. Controlled oligomerization of the juxtamembrane region may underlie the regulation of alpha-secretase cleavage by Notch ligands.

Commercial Relationship: R. Kopan, Sigma Formal advisor activities (i.e., scientific boards); BD Biosciences PharmingenBD Biosciences PharmingenBD Biosciences Pharmingen Product royalty/licensing fees; Merck Formal advisor activities (i.e., scientific boards).

Session Title: Poster Session
Session Type: Poster
Session Start: 7/21/2004 12:30:00 PM
Session End: 7/21/2004 2:45:00 PM
Location: Hall B

Mach

P2-188

Labeling Amyloid Plaque-like Structures by Radioiodinated Ligands in Rhesus Monkey Brain

Topic: Diagnosis and Disease Progression - Neuroimaging

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Presentation Number: P2-188

Keyword: plaques, imaging

Background: The deposition of β -amyloid (A β) plaques is a hallmark of Alzheimer's disease (AD). We have previously developed and characterized several iodinated ligands, which specifically bind to A β plaques in AD and transgenic mouse (Tg) brains. Aged rhesus monkey brains have been shown through immuno- and thioflavin-staining to contain these plaques. While there are many similarities between monkey and human plaque characteristics, differences exist such as a higher proportion of A β 40 over 42 in monkey. **Objective(s):** Radioiodinated ligands were used to detect A β plaques in aged monkey brains and the results were compared with the detection of plaques in AD and transgenic mouse brain sections.

Methods: Various iodinated ligands were incubated with brain sections from rhesus monkey, transgenic mouse over-expressing A β , or human AD to visualize plaque staining. An emulsion autoradiogram was compared to thioflavin-stained and A β 40 and 42 antibody labeled sections to confirm binding specificity.

Results: Three radioiodinated ligands - K01-042, IMPY (label plaques containing A β 40 and 42) and IMSB (predominately labels plaques containing A β 40) - were used for the studies. All these ligands showed distinct labeling in monkey brain sections. Antibodies for A β 40 and 42 confirmed the specific labeling of A β plaques and noted a general co-localization pattern of A β 40 and 42 plaques. These ligands also labeled A β plaques in Tg mouse brains with a strong, uniform signal, while human plaque labeling sensitivity varied with the radioiodinated ligands from case to case. **Conclusions:** Aged rhesus monkey brains do contain A β plaque deposition, which is detectable by radioiodinated ligands. However, the nature of the plaques may be different from and do not have the heterogeneity of human AD cases. Therefore, aged monkeys may be a potential Alzheimer's animal model, but as with transgenic mice, the results may not be translated directly to AD in humans.

Commercial Relationship: C. Hou, None.

Session Title: Poster Session
Session Type: Poster
Session Start: 7/19/2004 12:30:00 PM
Session End: 7/19/2004 2:45:00 PM
Location: Hall B

Morris

P1-146

The Neuropathology of Nondemented Aging

Topic: Diagnosis and Disease Progression - Others

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Presentation Number: P1-146

Keyword: preclinical dementia, pathology, plaques

Background: Histopathologic lesions associated with Alzheimer's disease (AD) often are observed in brains of older adults who were not considered to be demented prior to death. These lesions have been variously interpreted to represent aging as well as AD, or a preclinical stage of AD in which brain pathologic burden as yet is insufficient to produce clinical deficits. Another possibility is that the decedents had unrecognized very mild dementia. **Objective:** Determine the frequency of AD neuropathology in older individuals whose cognitive function was carefully assessed before death.

Methods: Cerebral tissue from 97 clinically normal subjects (CDR 0) who were assessed pre-mortem in Alzheimer Disease Centers at Washington University, Duke University, University of Rochester, University of Kentucky, University of California San Diego, Oregon Health Sciences University, and Mayo Clinic was studied with standard neuropathological and immunohistochemical methods at a central laboratory. The presence and densities of diffuse and neuritic plaques, neurofibrillary tangles, neuropil threads, Lewy bodies, amyloid angiopathy, argyrophilic grains, and other lesions were determined. The neuropathologic diagnosis of AD was made in accordance with standard criteria, including Khachaturian (Washington University modification), CERAD, and NIA-Reagan. **Results:** Depending on the set of diagnostic criteria used, neuropathologic AD was present in 22 to 38 (23% to 40%) of the 97 cases. There were no significant differences in those with and without neuropathologic AD on demographic variables or MMSE scores prior to death (Table). For all sets of criteria, the diagnosis of AD correlated better with neocortical plaque densities than with limbic or neocortical tangle densities. **Conclusions:** AD frequently is present neuropathologically in nondemented older adults. Undetected very mild dementia prior to death is an unlikely explanation for this observation.

Variable	AD (n=38)		No AD (n=59)		P-value
	Mean	SD	Mean	SD	
Age at Death (y)	84.9	7.4	83.8	9.3	0.53
Interval (y) from last assessment	0.85	0.66	1.09	0.88	0.15
Education (y)	15.6	2.6	15.4	3.1	0.71
MMSE	28.0	2.4	27.5	4.3	0.60
Braak NF Stage (1-6)	2.68	1.23	1.58	0.79	<0.01

Commercial Relationship: J.C. Morris, Merck Specific research activities; Eli Lilly Specific research activities; Novartis Formal advisor activities (i.e., scientific boards); Janssen Formal advisor activities (i.e., scientific boards); Neurochem Formal advisor activities (i.e., scientific boards).

Session Title: Poster Session
Session Type: Poster
Session Start: 7/18/2004 8:00:00 AM
Session End: 7/18/2004 9:30:00 AM
Location: Hall A

Morris

S2-02-03

Is MCI Early Alzheimer's Disease?

Topic: Diagnosis & Clinical Course: Clinical/Neuropsychology

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Presentation Number: S2-02-03

Keyword: mild cognitive impairment (MCI), early detection, clinical assessment

Background: Mild cognitive impairment (MCI) has been proposed as a transitional state between normal cognitive aging and clinically recognized dementia. Many conditions, including reversible disorders, can cause MCI. The insidious onset of the dementia syndrome of Alzheimer's disease (AD) results in the disease course progressing through MCI to stages of greater dementia severity. The advent of rationale therapy for AD has placed a premium on identifying the illness in its earliest stages. A key question is whether clinical detection methods accurately identify those cases of MCI that represent the earliest symptomatic stage of AD as distinguished from non-AD causes of MCI. **Objectives:** To evaluate informant-based methods for the clinical detection of early-stage AD in individuals with MCI and to assess the validity of AD detection by monitoring dementia progression and by the presence of AD histopathology at autopsy. **Methods:** Clinical, cognitive, and (in some) neuropathological data were obtained in over 500 normal and very mildly cognitively impaired individuals enrolled in the longitudinal studies of an Alzheimer Disease Research Center. The presence or absence of cognitive impairment was determined with the Clinical Dementia Rating (CDR). Cognitive normality (CDR 0) or impairment (CDR > 0; CDR 0.5 characterizes MCI) was based on informant reports and subject examination.

Neuropsychological test performance was obtained independently of the CDR determination. **Results:** In this sample, CDR >0 individuals predictably progressed to greater stages of dementia severity (average length of follow-up was 4-6 years) and overwhelmingly demonstrated neuropathologic AD at autopsy. These outcomes were realized even in CDR 0.5 individuals too minimally impaired on psychometric performance to meet current criteria for MCI. **Conclusions:** In at least some individuals, MCI represents the earliest symptomatic stage of AD. Informant-based clinical methods allow for the accurate diagnosis of AD in MCI-equivalent individuals, including those who are less cognitively impaired than individuals currently labeled as MCI.

Commercial Relationship: J.C. Morris, Merck S; Eli Lilly S; Novartis F; Janssen F; Neurochem F.

Session Title: Preclinical AD and MCI

Session Type: Symposia

Session Start: 7/19/2004 10:30:00 AM

Session End: 7/19/2004 12:30:00 PM

Location: Ballroom A

Nowotny

O3-02-06

Linkage analysis of AD sib pairs indicates evidence of interaction between genes regulating beta-amyloid degradation

Topic: Genetic Factors of Alzheimer's Disease

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Presentation Number: O3-02-06

Keyword: beta-amyloid, late onset

Background: Late-onset Alzheimer's disease is characterised by neuronal loss and the build up of β -amyloid ($A\beta$) plaques and neurofibrillary tangles. Genes that influence the rate at which $A\beta$ is degraded are strong biological candidates to influence disease risk. Furthermore, since several proteases have been shown to degrade $A\beta$ in vivo, *interactions* between the genes encoding them may influence disease susceptibility more than individual gene effects. **Objective:** To test for interactions between genes encoding $A\beta$ degrading proteases in late-onset AD cases. **Methods:** Linkage analyses were carried out in approximately 450 affected sibling pairs of European origin at the locations of the following five genes encoding $A\beta$ degrading proteases: ECE1 (chromosome 1p), ECE2, NEP (both 3q), IDE (10q) and APP (21p). Likelihood-ratio affected sib pair analysis was performed with the identity-by-descent (IBD) probability at each locus modelled as a logistic regression. Interactions between the genes were modelled by including the estimated number of alleles shared IBD by the pair at another locus as a covariate. Dependence of these interactions on the presence of APOE4 in the genotypes of the sib pair was modelled by including an IBD*APOE term. All pairs of genes were tested, except for NEP and ECE2, making a total of 9 tests. **Results:** A significant interaction was found between ECE2 and APP, a lod of 0.66 at ECE2 allowing for APOE effects being increased to 3.92 by adding APP-IBD and APP-IBD*APOE terms ($p=0.004$). In APOE4 +/+ and +/- pairs, IBD was positively correlated between ECE2 and APP. In APOE4 -/- pairs, the correlation was negative. Analysing APP allowing for interaction with ECE2 gave the same effect, increasing the lod from 0.61 to 3.63 ($p=0.005$). A positive correlation in IBD between ECE2 and IDE was also observed, a lod of 1.05 at IDE being increased to 2.33 ($p=0.009$), and a lod of 0 at ECE2 being increased to 1.32 ($p=0.029$). This correlation was independent of APOE. **Conclusions:** Further studies are required of the association between late-onset AD and genes encoding the $A\beta$ degrading proteases, particularly ECE2, IDE and APP, taking account of gene-gene interactions. **Commercial Relationship:** P. Holmans, None.

Session Title: Genetic Factors 1

Session Type: Oral

Session Start: 7/20/2004 3:00:00 PM

Session End: 7/20/2004 5:00:00 PM

Location: Ballroom A

Nowotny

P4-046

Association of late onset Alzheimer's disease with genetic variation in multiple members of a gene family involved in neuronal apoptosis

Topic: Genetic Factors of Alzheimer's Disease

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Presentation Number: P4-046

Keyword: genotyping, late onset, diagnosis

Background: While several genes have been implicated in the development of the early-onset autosomal dominant form of Alzheimer's disease (AD), the genetics of the late onset form of the disease is complex. Multiple loci on various chromosomes are known to be associated with the disease, but so far only *APOE* has been consistently shown to be a risk factor accounting for <50% of the Alzheimer's cases.

Objective: To identify risk factors for late onset Alzheimer's disease (LOAD). **Methods:** We performed a comprehensive, large-scale single nucleotide polymorphism (SNP) based association study on chromosome 12p, a region that had shown linkage with LOAD. Three case-control sample collections totaling 1,089 LOAD patients and 1,196 control subjects were used. **Results:** The study led us to identify one SNP that was significantly associated with LOAD in one sample set ($p < 0.005$) and was replicated in another case-control sample set ($p < 0.06$). We next tested markers in 5 genes that are homologous to the significant one and are located within other reported linkage regions, leading to the identification of one other gene with significant association to LOAD ($p < 0.002$, Cochran-Mantel-Haenszel test of all 3 samples combined) on chromosome 19. This marker appeared to be strongly correlated with age of disease onset before 75 years ($p < 0.00005$, all 3 samples combined). When we determined if other markers showed strong association with age of disease onset we identified another marker on chromosome 12q that was correlated to later age of onset (≥ 75 years, $p < 0.05$, all 3 samples combined). A significant trend between risk of LOAD and a multi-locus genotype among the three genes was observed in all individual sample sets at $p < 0.05$ and the combined sample set at $p < 0.001$ (Cochran-Armitage test of trend). **Conclusions:** These observations appear to suggest that variants in functionally similar genes may account for a potential series-to-series heterogeneity of disease risk and raise the possibility that the genes in this family are AD risk factors, which is consistent with the known role for one of the genes in neuronal apoptosis and neurodegenerative diseases.

Commercial Relationship: **A. Grupe**, Celera Diagnostics Full-time/part-time employment.

Session Title: Poster Session

Session Type: Poster

Session Start: 7/21/2004 12:30:00 PM

Session End: 7/21/2004 2:45:00 PM

Location: Hall B

Nowotny

P4-067

Genetic association studies of insulin-degrading enzyme (IDE) with late onset Alzheimer's disease (LOAD) - equivocal results from two large case-control studies

Topic: Genetic Factors of Alzheimer's Disease

Kit Fun Lau¹, Charlie Rowland¹, Kristina Tacey¹, Lisa Doil¹, Yonghong Li¹, Ryan van Luchene¹, Petra Nowotny², Scott Smemo², Veronica Garcia¹, Simon Lovestone³, Michael Owen⁴, Julie Williams⁴, Andrew Grupe¹, Alison Goate², ¹Celera Diagnostics, Alameda, CA, USA; ²Dept. of Psychiatry, Washington University, St. Louis, MO, USA; ³Dept. of Neuroscience, London, United Kingdom; ⁴Dept. of Psychological Medicine, University of Wales, Cardiff, United Kingdom. Contact e-mail: kit.lau@celeradiagnostics.com

Presentation Number: P4-067

Keyword: gene mapping, late onset, biostatistics

Background: Several linkage studies have shown that susceptibility to LOAD is linked to gene/genes on chr. 10. The gene encoding insulin-degrading enzyme (IDE) is a strong biological candidate in that linkage region as it is involved in the catabolism of A β . **Objective:** Evaluate whether genetic polymorphisms in IDE are associated with susceptibility to LOAD. **Methods:** Case-control studies have been carried out using two independent samples - 419 LOAD cases/ 375 controls collected in Washington University and 392 cases/ 407 controls collected in United Kingdom. Three SNPs within IDE gene and 1 SNP within the nearby KNSL1 gene were genotyped. **Results:** In the Wash. U. samples, genotypic association tests reveal that two SNPs in IDE and one SNP in KNSL1 are associated with LOAD only in the APOE4+ stratum with recessive mode (p-values 0.05, 0.06, 0.02 with odds ratios 0.3, 0.37 and 0.5). Diplotype analyses with logistic regression show that two diplotypes, D1 and D2, are associated with reduced risk of LOAD (D1 with OR 0.23 and p-value 0.01 while D2 has OR 0.3 and p-value 0.009). Global p-value is 0.001. In UK samples, however, the same analyses do not show significant results at 0.05 level. P-values for D1 and D2 are 0.37 and 0.07 respectively. Global p-value is 0.26. **Conclusion:** Results in the Wash. U. sample are not replicated in the UK sample. As in literature, and in another poster of ours using different markers and samples, results are equivocal on whether genetic polymorphism in IDE is associated with susceptibility to LOAD. Further studies, including meta-analyses of various datasets, may shed more light on this.

Commercial Relationship: K. Lau, None.

Session Title: Poster Session
Session Type: Poster
Session Start: 7/21/2004 12:30:00 PM
Session End: 7/21/2004 2:45:00 PM
Location: Hall B

Nowotny

P4-079

Is Variation in the Gene Encoding Insulin-Degrading Enzyme (IDE) a Risk Factor in Late-Onset Alzheimer's Disease?

Topic: Genetic Factors of Alzheimer's Disease

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Presentation Number: P4-079

Keyword: late onset, genotyping, risk factor

Background: LOAD is a complex disorder, caused by both genetic and environmental risk factors. We, and others have shown risk for LOAD and a QTL for plasma A β 42 levels is linked to a region on chromosome 10. A strong positional and biological candidate on chromosome 10 is the gene encoding the insulin-degrading enzyme (IDE), which is involved in the catabolism of A β . Polymorphisms in this gene have been examined by several groups for their association with AD and with several traits correlated with AD. While some groups have found no evidence for association with LOAD, others have shown association between several LOAD phenotypes and haplotypes spanning IDE. **Objective:** To systematically evaluate the role of variation in IDE in risk for AD in two independent LOAD samples.

Methods: We genotyped nineteen SNPs spanning 309kb in and around the IDE gene, including 3 "tagging" SNPs identified by Prince *et al.*, in two independent case control series. The first series contains approximately 500 cases derived from our linkage sample and 150 unrelated controls while the second is a case control series collected at Washington University. The tagging SNPs were genotyped by Pyrosequencing and the other SNPs were genotyped with allele specific PCR. **Results:** Analysis of the tagging SNPs demonstrated association with a single marker (allelic p-value 0.0012, stratified for APOE4-0.000225) and with a haplotype derived from all three SNPs (p-value 0.0003, APOE4- 0.0001) in the case/control set from the linkage sample. However we were not able to replicate the single marker (p-value 0.858, APOE4- 0.700) or the haplotype (p-value 0.3195, APOE4- 0.3195) results in the second case/control sample. Furthermore, the significant haplotype in our sample was different from that in the original study. None of the sixteen additional SNPs showed association with LOAD. **Conclusion:** We found a significant association with one IDE marker in one sample but we were not able to replicate our result or previously published observations. Further studies, including meta-analysis, will be helpful to decide if variants in IDE are risk factors in LOAD.

Commercial Relationship: P. Nowotny, None.

Session Title: Poster Session

Session Type: Poster

Session Start: 7/21/2004 12:30:00 PM

Session End: 7/21/2004 2:45:00 PM

Location: Hall B

Nowotny

P4-122

Genetic Association of an APP Binding Protein Gene with Late Onset Alzheimer's Disease

Topic: Genetic Factors of Alzheimer's Disease

Yonghong Li¹, Paul Hollingworth², Pamela Moore², Catherine Foy³, Nicola Archer³, Petra Nowotny⁴, Peter Holmans², Scott Smemo⁴, Kristina Tacey¹, Lisa Doil¹, Ryan van Luchene¹, Kit Lau¹, Joe Catanese¹, Veronica Garcia¹, Charlie Rowland¹, John Sninsky¹, Tom White¹, John Hardy⁵, Leon Thal⁶, Alison Goate⁴, John Powell³, Simon Lovestone³, Michael Owen², Julie Williams², Andrew Grupe¹, ¹Celera Diagnostics, Alameda, CA, USA; ²Dept of Psychological Medicine, University of Wales, Cardiff, United Kingdom; ³Dept of Neuroscience, Institute of Psychiatry, London, United Kingdom; ⁴Dept of Psychiatry, Washington University, St. Louis, MO, USA; ⁵NIA, Bethesda, MD, USA; ⁶Dept of Neuroscience, UCSD, San Diego, CA, USA. Contact e-mail: yonghong.li@celeradiagnostics.com

Presentation Number: P4-122

Keyword: amyloid precursor protein (APP), genotyping

Background: Alzheimer's disease (AD), the most common form of dementia among the elderly, is a complex neurodegenerative disorder affected by multiple genetic and non-genetic factors. Mutations in amyloid β precursor protein (APP) are known to be associated with familial AD and possibly also the late onset form of the disease (LOAD). A number of genes encoding proteins capable of binding to APP have been identified, but their relevance with AD remains largely unclear. Conceivably, mutations in these genes may play a role in affecting AD susceptibility, which appears to be substantiated by some genetic studies. **Objective(s):** To examine possible genetic association of an APP binding protein gene with LOAD. **Methods:** Genotyping single nucleotide polymorphisms (SNPs) using 3 LOAD case control sample sets. **Results:** We first genotyped 2 SNPs within the gene and identified one significant marker using a case-control sample collected in the UK. When we tested the marker in two other case-control sets, collected in different areas of the US, one sample set replicated the initial finding ($p < 0.1$), while the other did not. The SNP is located in an intronic segment that is homologous between the human and mouse genome. The association remained significant in a meta-analysis of all 3 sample sets ($p < 0.05$). However, association of the SNP with LOAD is most pronounced in subjects with disease onset before 75 years of age ($p < 0.0001$, Cochran-Mantel-Haenszel test with all 3 sample sets). We are currently studying other SNPs to determine the extent of linkage disequilibrium in this region. **Conclusions:** Our data raise the possibility that genetic variations in the APP binding protein may affect LOAD susceptibility.

Commercial Relationship: Y. Li, Celera Diagnostics Full-time/part-time employment.

Session Title: Poster Session
Session Type: Poster
Session Start: 7/21/2004 12:30:00 PM
Session End: 7/21/2004 2:45:00 PM
Location: Hall B

Nowotny

P4-110

A Systematic Scan of Chromosome 10 Single Nucleotide Polymorphisms Identifies Novel Candidate Genes Showing Strong Association to Alzheimer's Disease

Topic: Genetic Factors of Alzheimer's Disease

Lisa Doil¹, Kristina Tacey¹, Petra Nowotny², Ryan van Luchene¹, Yonghong Li¹, Peter Holmans³, Scott Smemo², Veronica Garcia¹, Charlie Rowland¹, Diane Leong¹, Goran Gogic¹, Anibal Cravchik¹, David Ross¹, Kit Lau¹, Joe Catanese¹, John Sninsky¹, Tom White¹, John Hardy⁴, John Powell⁵, Simon Lovestone⁵, Leon Thal⁶, Michael Owen³, Julie Williams³, Alison Goate², **Andrew Grupe**¹, ¹Celera Diagnostics, Alameda, CA, USA; ²Dept of Psychiatry, Washington University, St. Louis, MO, USA; ³Dept of Psychological Medicine, University of Wales, Cardiff, United Kingdom; ⁴NIA, Bethesda, MD, USA; ⁵Dept of Neuroscience, Institute of Psychiatry, London, United Kingdom; ⁶Dept of Neuroscience, UCSD, San Diego, CA, USA. Contact e-mail: andrew.grupe@celeradiagnostics.com

Presentation Number: P4-110

Keyword: late onset, genotyping, diagnosis

Background: Whole genome linkage studies for late onset Alzheimer's disease (LOAD) have repeatedly indicated a wide region of chromosome 10 to contain at least one disease risk locus. Although significant associations with several biological candidate genes on chromosome 10 have been reported in case control sample sets, these findings remain controversial, as they have not been consistently replicated in other sample sets. **Objective(s):** To identify chromosome 10 genes that are associated with risk of developing LOAD. **Methods:** We performed a chromosome 10 wide association study with 1345 gene based single nucleotide polymorphisms (SNPs, allele frequency $\geq 2\%$) to identify genes that are associated with risk of developing LOAD and therefore may contribute to the reported linkage signals. The scan covered 680 genes out of 1300 known or predicted genes; each gene was tagged with one or more markers, with the majority of markers resembling putative functional mutations. The initial testing was done in a Caucasian case-control sample from the St. Louis area with 419 cases and 377 controls. Markers that showed significant association in the exploratory analysis were followed up in two other Caucasian case-control sample sets to confirm the initial association. The replication case-control sets included a total of 670 cases and 819 controls, collected in the San Diego area and the UK. **Results:** Two markers, representing two genes, confirmed the initial association in the combined replication sets. A meta-analysis of all sample sets combined yielded allelic $p=0.00013$ and $p=0.00012$ for the two markers. After adjusting for other risk factors, i.e. ApoE4 status, gender, and age of disease onset, the p -values were $p=0.00002$ and $p=0.003$, respectively. **Conclusions:** These results indicate that variants in these two genes are risk factors for LOAD and implicate these genes in the pathogenesis of this disorder. We plan to evaluate the contribution of these variants to the chromosome 10-linkage signal and confirm their highly significant evidence for disease involvement in other sample sets.

Commercial Relationship: **A. Grupe**, Celera Diagnostics Full-time/part-time employment.

Session Title: Poster Session

Session Type: Poster

Session Start: 7/21/2004 12:30:00 PM

Session End: 7/21/2004 2:45:00 PM

Location: Hall B

Parasadianian

P4-358

In Vitro and In Vivo Characterization of Beta-Amyloid Antibodies Binding to Cerebral Amyloid Angiopathy (CAA) and the Selective Exacerbation of CAA-Associated Microhemorrhage

Topic: Therapeutics and Therapeutic Strategies - Therapeutic Strategies, Amyloid-based

Ronald B. DeMattos¹, Laura I. Boone¹, Deena L. Hepburn¹, Maia Parsadianian², Matthew T. Bryan¹, Daniel K. Ness¹, Kathy S. Piroozii¹, David M. Holtzman², Kelly R. Bales¹, Bruce D. Gitter¹, Steven M. Paul¹, Margaret Racke¹, ¹Eli Lilly and Co., Indianapolis, IN, USA; ²Washington University, St. Louis, MO, USA. Contact e-mail: demattos_ronald_bradley@lilly.com

Presentation Number: P4-358

Keyword: amyloid, cerebrovascular disease, immunotherapy

Background: Chronic administration of a monoclonal antibody directed against the amino-terminus of Beta-Amyloid (A β) to aged APP23 transgenic mice resulted in approximately two-fold increased frequency of microhemorrhage (Pfeifer et al., 2002). These findings suggest that the anti-A β antibody is exacerbating a pathological interaction between CAA and the arterial vessels. The mechanism likely involves a direct physical interaction between the antibody and CAA.

Objective: We performed a thorough biochemical evaluation of the binding properties of the monoclonal anti-A β antibodies 266 (13-28), 3D6 (1-5), 10D5 (3-6), and control IgG to both brain parenchymal and cerebral vessel amyloid. Additionally, we conducted a six-week study to determine whether 266 or 3D6 would facilitate CAA-associated microhemorrhage in aged PDAPP mice.

Methods: Immunohistochemistry was performed to test whether the monoclonal antibodies would bind to deposited A β in fixed and non-fixed Alzheimer's disease brain tissue. Procedures were developed to purify large quantities of "clean" cerebral vessels from aged PDAPP transgenic mice. ELISA based assays and biochemical extractions were developed in order to quantify the amount of specific IgG's that can associate with amyloid bearing cerebral vessels. *In vitro* and *in vivo* experiments were performed to determine to what degree the monoclonal antibodies would bind to cerebral amyloid in blood vessels. Antibody mediated effects on the incidence and severity of cerebral microhemorrhage were evaluated, relative to IgG control, following administration of 266 or 3D6 to 23 month-old PDAPP transgenic mice.

Conclusions: The immunohistochemical analyses demonstrated that the amino-terminally directed antibodies (3D6 and 10D5) bound with high affinity to deposited forms of the A β peptide; whereas the central domain antibody (266) completely lacked affinity for the deposited material. In concert with these findings, the binding preferences of the antibodies to CAA bearing microvessels were very similar (3D6 and 10D5 bound, whereas 266 binding was undetectable). Importantly, however, there was an increase in the incidence and magnitude and approximately two-fold increase in the severity of CAA-associated microhemorrhage when PDAPP transgenic mice were treated with 3D6, whereas mice treated with 266 had no effect. These results may have important implications for future immune based therapeutic strategies targeting the A β peptide.

Commercial Relationship: R.B. DeMattos, Eli Lilly Full-time/part-time employment.

Session Title: Poster Session

Session Type: Poster

Session Start: 7/21/2004 12:30:00 PM

Session End: 7/21/2004 2:45:00 PM

Location: Hall B

Pastor
P4-160

Haplotype structure of the 17q21 region in progressive supranuclear palsy and corticobasal degeneration

Topic: Genetic Factors of Non-Alzheimer Tauopathies

Pau Pastor¹, Mario Ezquerra², J. Christian Perez³, Sumi Chakraverty¹, Joanne Norton¹, Eduardo Tolosa², Brad A. Racette⁴, Alison M. Goate¹, ¹Department of Psychiatry, Washington University, Saint Louis, MO, USA; ²Department of Neurology, Hospital Clinic, Barcelona, Spain; ³Department of Genetics, Washington University, Saint Louis, MO, USA; ⁴Department of Neurology, Washington University, Saint Louis, MO, USA. Contact e-mail: pastorp@icarus.wustl.edu

Presentation Number: P4-160

Keyword: tau, molecular genetics, gene mapping

Background: Progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) are sporadic tauopathies that have been associated with polymorphisms in the tau gene.

Objective(s): To delimit the size of the haplotype associated with PSP and CBD in different populations and identify specific sub-haplotypes in the H1E haplotype background which show increased association with these diseases.

Methods: We genotyped 21 single nucleotide polymorphisms (SNPs) in the 17q21 region in two case-control samples from different populations (Spanish and North American). We used Pyrosequencing methods and RFLP analysis to genotype the samples.

Results: The SNPs that are associated with higher risk in homozygosis for the disease delimit a region of more than 1 Mb (1,139,950 bp in the Spanish sample, and of 1,032,439 bp in the American Sample). We found within this region 3 haplotype blocks that were strongly associated with PSP/CBD and showed high linkage disequilibrium (LD) among them. Pooling the patients with both, PSP and CBD, in the Spanish sample did not substantially change the extent and distribution of LD. The same LD measurements and haplotype block distribution was observed when pooling all the patients with tauopathies from different populations (Spanish and American population). However, in the control group the D' and r² measurements along the 17q21 region were not as strong as in the disease group. Haplotype analyses in the Spanish sample showed a specific sub-haplotype (H1E'1A) in PSP patients (16%) that was not present in the controls. The H1E'1A sub-haplotype originated on the most common haplotype (H1E'1) seen in the patient group. The other closest sub-haplotype (H1E'1B) was not associated with disease. In the American Sample, the H1E'1A sub-haplotype was present in 9% of the patients (PSP+CBD) and in 3% of the controls. Furthermore, the H2E'1 haplotype is rarely present in the disease group suggesting that it plays a protective role.

Conclusions: In the Spanish population, specific risk and protective haplotypes are associated with PSP. The identification of these specific sub-haplotypes that modify the risk for the disease in PSP/CBD supports the presence of a pathogenic allele in this region of chromosome 17 in a subgroup of PSP patients.

Commercial Relationship: P. Pastor, None.

Session Title: Poster Session
Session Type: Poster
Session Start: 7/21/2004 12:30:00 PM
Session End: 7/21/2004 2:45:00 PM
Location: Hall B

Roe

P2-276

Co-occurrence of Dementia of the Alzheimer's Type and Cancer

Topic: Epidemiology and Risk Factors of Alzheimer's Disease

Catherine M. Roe¹, María I. Behrens^{1,2}, Chengjie Xiong¹, J. Phillip Miller¹, John C. Morris¹, ¹Washington University School of Medicine, St. Louis, MO, USA; ²Universidad de Chile and Clínica Alemana de Santiago, Santiago, Chile. Contact e-mail: cathyr@wubios.wustl.edu

Presentation Number: P2-276

Keyword: epidemiology, longitudinal study

Background: Dementia of the Alzheimer's type (DAT) and cancer occur frequently in older adults. Review of the literature reveals little information regarding the co-occurrence of these conditions.

Objectives: To examine the relationship of the two disorders by assessing the development of cancer in individuals with DAT in comparison with nondemented older adults, and assessing the development of DAT in individuals with and without cancer.

Methods: Archival data from a longitudinal study conducted by the Washington University Alzheimer's Disease Research Center were used. Two sets of analyses were conducted. Participants with and without DAT were followed to compare the development of cancer within each group, and participants with and without cancer were tracked to assess group differences in the development of DAT. At each study session, clinicians conducted dementia assessments, and the collateral source of each subject reported whether the subject had a history of cancer.

Results: Two-hundred and ten nondemented participants, and 423 participants with DAT, had no history of cancer at study entry. Compared to nondemented participants, those in the DAT group had a slower rate of developing cancer according to the Kaplan-Meier Product-Limit log-rank test ($p = .0001$) and a Cox proportional hazard model controlling for sex, age, and education (HR = 0.36, 95% CI = 0.19 – 0.67, $p = .0015$) during a follow-up period ranging up to 12 years (mean = 3.8 y). Cancers commonly reported over the follow-up period included those of the skin (57.8%), prostate (11.1%), breast (8.9%), and colon (6.7%). Fifty-four subjects with cancer and 210 without cancer were nondemented at study entry. The log-rank test ($p = .1736$) and a Cox proportional hazard model controlling for sex, age, race, and education (HR = 0.61, 95% CI = 0.24 – 1.58, $p = .3104$) indicated that the rate of developing DAT across the follow-up period (range = 0.7 to 11.5 y, mean = 4.4 y) was lower for the cancer group, but this difference did not reach significance.

Conclusions: Individuals with DAT may be less likely to develop cancer than nondemented individuals.

Commercial Relationship: C.M. Roe, None.

Session Title: Poster Session
Session Type: Poster
Session Start: 7/19/2004 12:30:00 PM
Session End: 7/19/2004 2:45:00 PM
Location: Hall B

Snider
P4-063

Very Early Onset Familial Alzheimer's Disease With Lewy Bodies Without Known Causative Mutations

Topic: Genetic Factors of Alzheimer's Disease

B. J. Snider¹, Craig E. Hou¹, Ramiro Jervis², Corinne L. Lendon³, JoAnne Norton¹, Alison M. Goate¹, Dan W. McKeel, Jr.¹, John C. Morris¹, ¹Washington University School of Medicine, St Louis, MO, USA; ²Mount Sinai School of Medicine, New York, NY, USA; ³The University of Birmingham, Birmingham, United Kingdom. Contact e-mail: sniderj@neuro.wustl.edu

Presentation Number: P4-063

Keyword: early onset, neuropathology, dementia with Lewy bodies

Background: Early onset Alzheimer's disease (EOAD) accounts for less than 10% of all cases of Alzheimer's disease (AD). However, EOAD is important for understanding AD mechanisms. A subset of EOAD cases occurs with an autosomal dominant inheritance pattern (familial Alzheimer's disease, FAD). Mutations, most commonly in the presenilin 1 (PSEN1) gene, are found in slightly more than half of early onset FAD families.

Objective: We analyzed the clinical, neuropathological and genetic features of a family with very early onset FAD.

Methods: The proband underwent full clinical assessment. Family history was obtained through interviews with collateral sources and review of medical records. Post-mortem examination was obtained in the proband; limited pathological samples and autopsy records of 2 affected family members were available. The proband was screened for genetic mutations in the coding regions of the PSEN1 and PSEN2 genes, and in exons 16 and 17 of the amyloid precursor protein gene.

Results: A father and his son and daughter (the proband) developed dementia at a mean age of 27 years (range 26-27 years). All three had similar clinical features including myoclonus, seizures, and rigidity. All three were confirmed to have AD by neuropathology. Alpha-synuclein staining of the proband demonstrated sufficient Lewy body (LB) pathology in brainstem, limbic areas and neocortex to fulfill McKeith 1996 consensus criteria for Dementia with Lewy Bodies. No mutations were found in the coding regions of the PSEN1 and PSEN2 genes or in exons 16 and 17 of the APP gene. The proband was homozygous for the E3 allele of apolipoprotein E.

Conclusions: Mutations in PSEN1 result in a clinical phenotype characterized by early onset dementia, myoclonus, seizures, and rigidity. This very early onset FAD kindred demonstrated a very similar clinical phenotype. No mutations were present in any of the genes known to be associated with FAD, suggesting that additional unknown causative genes can present with a phenotype resembling that of PSEN1 mutations. To our knowledge, this kindred has the earliest reported onset of pathologically confirmed AD with widespread Lewy Body pathology; the relative contribution of the two pathologies to the clinical phenotype is unknown.

Commercial Relationship: B.J. Snider, None.

Keyword: early onset, neuropathology, dementia with Lewy bodies

Background: Early onset Alzheimer's disease (EOAD) accounts for less than 10% of all cases of Alzheimer's disease (AD). However, EOAD is important for understanding AD mechanisms. A subset of EOAD cases occurs with an autosomal dominant inheritance pattern (familial Alzheimer's disease, FAD). Mutations, most commonly in the presenilin 1 (PSEN1) gene, are found in slightly more than half of early onset FAD families.

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Commercial Relationship: B.J. Snider, None.

Session Title: Poster Session
Session Type: Poster
Session Start: 7/21/2004 12:30:00 PM
Session End: 7/21/2004 2:45:00 PM

Storandt

P1-073

Depression in the Early Stages of Alzheimer's Disease Does Not Affect Psychometric Test Performance

Topic: Diagnosis and Disease Progression - Neuropsychological

Martha Storandt¹, Kimberly K. Powlishta¹, Tammy A. Mandenach², Ellen Hogan¹, Elizabeth A. Grant¹, John C. Morris¹, ¹Washington University in St. Louis, St. Louis, MO, USA; ²St. Louis University, St. Louis, MO, USA. Contact e-mail: mstorand@artsci.wustl.edu

Presentation Number: P1-073

Keyword: depression, neuropsychology

Background: Depression can interfere with the normal expression of cognitive abilities in adults of all ages, but it is unclear if depression in demented people, which is relatively common, is associated with reduced cognitive performance beyond the effect of the dementia itself. **Objective:** To determine if depression adds to the cognitive deficit in dementia. **Methods:** This question was examined in 167 people with very mild (CDR = 0.5) and 155 people with mild (CDR = 1) dementia of the Alzheimer type using a battery of psychometric tests that measured memory and other cognitive functions. **Results:** Research clinicians judged depression to be present in 15% of the very mild and 24% of the mild group. There was no relation between the clinicians' diagnoses of depression and psychometric scores. Further, little relation was found between performance on the cognitive tests and the number of depressive features (out of a maximum of 9) reported by either the individual or a collateral source. The few statistically significant ($p < .05$) correlations were quite modest in size ($\leq .21$). **Conclusions:** Depression does not appear to worsen cognitive performance beyond the effect of dementia.

Commercial Relationship: M. Storandt, None.

Session Title: Poster Session

Session Type: Poster

Session Start: 7/18/2004 8:00:00 AM

Session End: 7/18/2004 9:30:00 AM

Location: Hall A

Wahrle

P2-291

ABCA1 Knock-out Mice Have Decreased ApoE in Brain and CSF and Secrete Cholesterol Poor ApoE-Containing HDL from Astrocytes *in vitro*

Topic: Molecular Mechanisms of Neurodegeneration - APOE

Suzanne E. Wahrle¹, Hong Jiang¹, Maia Parsadanian¹, Justin Legleiter², Tomasz Kowaleski², David M. Holtzman¹, ¹Washington University, St. Louis, MO, USA; ²Carnegie Mellon University, Pittsburgh, PA, USA. Contact e-mail: wahrles@msnotes.wustl.edu

Presentation Number: P2-291

Keyword: APOE, cholesterol, lipids

Background: ABCA1 transports cellular cholesterol and phospholipids from cells onto high density lipoproteins (HDL) in plasma. Mutations in ABCA1 can cause Tangier's disease, which is characterized by extremely low plasma HDL and accumulation of cholesterol in several tissues. ABCA1 knock-out mice (ABCA1^{-/-}) have been produced and their abnormalities model those of Tangier's disease. However, neither Tangier's disease patients nor ABCA1^{-/-} mice have been examined to determine whether ABCA1 plays a role in influencing apolipoprotein, cholesterol or phospholipid metabolism in the central nervous system. **Methods/Results:** Using primary astrocyte cultures from ABCA1^{-/-}, ABCA1^{+/-}, and wild type mice, we have found that ABCA1 deletion results in a marked decrease in cholesterol and phospholipid secretion and reduces the size of astrocyte-secreted apoE-containing HDL-like lipoproteins. *In vivo*, both brain and CSF levels of apoE are decreased in ABCA1^{-/-} mice, but total cholesterol and phospholipids levels are the same. **Conclusions:** These results demonstrate that ABCA1 plays an important role in apoE metabolism in the central nervous system. Since apoE genotype is a major determinant of risk for Alzheimer's disease (AD), likely via apoE/A β interactions, ABCA1 may play a role in AD pathogenesis by modulating apoE/A β metabolism. We are currently determining how ABCA1 may influence A β metabolism via apoE versus other mechanisms. **Funding:** NIH AG13956, AG05681, AG11355, MetLife Foundation

Commercial Relationship: S.E. Wahrle, None.

Session Title: Poster Session
Session Type: Poster
Session Start: 7/19/2004 12:30:00 PM
Session End: 7/19/2004 2:45:00 PM
Location: Hall B

Walker

P4-309

Presenilin 2 Familial Alzheimer's Disease Mutations Decrease A β and CTF γ While Differentially Affecting NICD Production

Topic: Molecular Mechanisms of Neurodegeneration - Presenilins

Emily S. Walker, Maribel Martinez, Anne Brunkan, Silva Hecimovic, Jun Wang, Alison Goate, Washington University, St. Louis, MO, USA. Contact e-mail: eswalker@icarus.wustl.edu

Presentation Number: P4-309

Keyword: presenilin, amyloid precursor protein (APP), gamma-secretase

Background: Certain mutations in the presenilin genes (PS1 and PS2) are associated with early-onset familial Alzheimer's disease (FAD). To date 133 familial Alzheimer's disease (FAD) mutations have been identified in PS1 and 9 FAD mutations have been identified in PS2. The presenilins are necessary for the γ -secretase cleavage of Notch to N β and NICD, and amyloid precursor protein (APP) to A β and CTF γ .

The major A β isoform is 40 amino acids long, however, a small percentage is 42 amino acids long, and FAD mutations increase the ratio of A β ₄₂/A β ₄₀. **Objective:** Our objective was to determine if FAD mutations in PS2 affect production of A β , CTF γ and NICD in addition to affecting the ratio of A β ₄₂/A β ₄₀.

Methods: We tested 8 reported PS2 FAD mutations (R62H, T122P, S130L, N141I, V148I, M239V, M239I, D439A) and two PS1 FAD mutations at the residue homologous to PS2 M239 (PS1 M233T/V). All experiments were performed in PS1/2 knock-out mouse embryonic fibroblasts transiently transfected with PS2 and APP or Notch constructs. **Results:** PS2 R62H, S130L, V148I and D439A had no effect on the A β ₄₂/A β ₄₀ ratio while T122P, N141I, M239V and M239I increased the ratio greatly. These latter four PS2 FAD mutations caused decreases in both A β and CTF γ production. While most of the mutations tested had a similar effect on CTF γ and NICD production, the PS2 M239 and PS1 M233 mutants had a differential affect. **Conclusions:** It is unlikely that R62H, S130L, V148I or D439A are pathogenic FAD mutations since they do not affect the A β ₄₂/A β ₄₀ ratio significantly. Interestingly, most PS2 FAD mutations we examined severely affected A β , CTF γ and NICD production. This is in contrast to most PS1 FAD mutations we have studied which have little affect on CTF γ or NICD levels - this suggests that PS2 FAD mutations have a more severe effect on function. Finally, FAD mutations at PS2 M239 and PS1 M233 are the only PS mutations we have studied or seen published that decrease CTF γ production without affecting NICD production. This residue could be important for an interaction with APP.

Commercial Relationship: E.S. Walker, None.

Session Title: Poster Session
Session Type: Poster
Session Start: 7/21/2004 12:30:00 PM
Session End: 7/21/2004 2:45:00 PM
Location: Hall B

Wang

P4-286

The C-terminal PAL Motif in Presenilins Constitutes Part of the gamma-secretase Active Site Pocket

Topic: Molecular Mechanisms of Neurodegeneration - Presenilins

Jun Wang¹, Dirk Behr², Mark S. Shearman², Alison Goate¹, ¹Washington University, St. Louis, MO, USA; ²Merck Sharp & Dohme Research Laboratories, Harlow, United Kingdom. Contact e-mail: wangju@psychiatry.wustl.edu

Presentation Number: P4-286

Keyword: presenilin, gamma-secretase, beta-amyloid

Background: β -amyloid peptide ($A\beta$) is produced through sequential cleavage of the precursor protein APP by BACE and γ -secretase, an unusual aspartyl protease complex that contains at least four proteins: presenilin (PS), nicastrin, APH-1 and PEN-2. Considerable evidence now suggests that PS is the catalytic component of γ -secretase and the other three proteins are essential cofactors. Two aspartic acids in PS are believed to constitute the active site of γ -secretase. Signal peptide peptidase (SPP) is a PS type aspartyl protease that has a similar overall topology and overlapping inhibitor profiles with PS. PS and SPP have no primary sequence homology except at three highly conserved regions: the two aspartate-containing active site motifs and a PAL motif at the C-terminal, suggesting that the PAL motif is important for the proteolytic mechanism that SPP and PS may both share. Our previous data have documented that point mutations in this motif (P433L, A434D and L435R) render the enzyme completely inactive. The phenotypes of these mutations exactly mimic that of the active site aspartyl mutants.

Objectives: To understand the mechanisms by which the conserved PAL motif contributes to γ -secretase activity. **Methods:** We tested two hypotheses: 1. the PAL motif is important for PS1 association with essential cofactors; 2. the PAL motif constitutes part of the γ -secretase active site pocket. **Results:** Co-immunoprecipitation experiments showed that the P433L mutation did not affect PS1 or PS1 Δ E9 association with nicastrin, APH-1 or PEN-2. In addition, both mutants were functional in restoring nicastrin maturation which is dependent on PS. To test the hypothesis that the PAL motif constitutes part of the γ -secretase active site, we examined whether the P433L mutation affected PS1 or PS1 Δ E9 binding to γ -secretase transition state analog inhibitors, which presumably bind to γ -secretase active site. While both wtPS1 and PS1 Δ E9 were affinity precipitated by the inhibitor, introduction of the P433L mutation into either construct abolished binding to the inhibitor. **Conclusions:** Our data strongly support the hypothesis that PAL motif constitutes part of the γ -secretase active site pocket.

Commercial Relationship: J. Wang, None.

Session Title: Poster Session

Session Type: Poster

Session Start: 7/21/2004 12:30:00 PM

Session End: 7/21/2004 2:45:00 PM

Location: Hall B

Wilkins

P4-013

Vitamin D Status Predicts Impaired Physical Performance in Persons with AD

Topic: Epidemiology and Risk Factors - Others

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Presentation Number: P4-013

Keyword: risk factor

Background: In addition to the cognitive and behavioral symptoms associated with AD, there is a substantial decline in physical health as well. Though it is commonly assumed that the decline in physical health in persons with AD is related to immobility and/or nutritional factors as the disease progresses, recent findings note a decline in physical performance in those with very mild cognitive dysfunction.

Vitamin D deficiency is a possible contributor to this excess disability. **Objective:** The purpose of this study is to examine the relationship between vitamin D status and physical performance in persons with mild AD. **Methods:** A Physical Performance Test (PPT) and 25-hydroxyvitamin D level was performed on 80 subjects enrolled in the Alzheimer's Disease Research Center at Washington University. The sample included 40 individuals with no dementia (CDR = 0), 20 individuals with very mild dementia of the Alzheimer type (DAT) (CDR = 0.5), and 20 individuals with mild DAT (CDR = 1). **Results:** There were 50 females, 62 Caucasians and 18 African Americans in this population. The mean age was 74.8 and mean educational level was 14.4 years. There was a 10% decline in PPT score in the vitamin D deficient group. The PPT score decreased with decreasing vitamin D level and increasing dementia severity as measured by CDR score. The correlation for total PPT score with vitamin D level was $r_b = 0.259$ ($p = 0.02$). The correlation for total PPT score with CDR score was $r_b = 0.190$ ($p = 0.05$). Linear regression model of predictors of PPT found vitamin D to have a β coefficient of 0.267 ($p = 0.013$) when CDR and age are added to the model. **Conclusions:** This study confirms that physical performance worsened with decreasing vitamin D level despite presence or absence of mild dementia.

Commercial Relationship: C.H. Wilkins, None.

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Zerbinatti

P2-116

Neuronal overexpression of LRP selectively increases a detergent-soluble pool of hippocampal A β in PDAPP mice.

Topic: Animal and Cellular Models - Animal Models, Transgenic

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Presentation Number: P2-116

Keyword: animal model, beta-amyloid, immunohistochemistry

The low-density lipoprotein receptor-related protein (LRP) has been linked to Alzheimer's disease (AD) by both genetic and biochemical evidence. We generated a transgenic mouse that overexpress a functional minireceptor of LRP in the brain and bred it to PDAPP mice, an animal model that develops typical amyloid plaques. While A β plaque burden was not altered by the overexpression of LRP, PDAPP/LRP⁺ mice had increased carbonate-soluble brain A β levels at old age. More important, A β levels in carbonate-soluble extracts were highly correlated with deficits in spatial learning and memory in old mice. To further characterize the soluble pool of A β altered by LRP, we performed a 3-step tissue extraction, which included: 1) a Tris-buffered saline (TBS) fraction (extracellular soluble A β); 2) a TBS/Triton X-100 fraction (membrane- and/or cell-associated soluble A β); and 3) a guanidine fraction (insoluble, plaque-associated A β). Each extract was analyzed for A β 40 and A β 42 by ELISA. While TBS-soluble A β levels were similar between PDAPP/LRP⁺ (n = 14) and PDAPP/LRP⁻ (n = 15) (60.7 \pm 3.7 and 57.6 \pm 2.8 pg/mg hippocampus, respectively; p = 0.51 by Student's *t* test), A β levels in the TBS/Triton X-100-soluble fraction was significantly higher in PDAPP/LRP⁺ than PDAPP/LRP⁻ mice as young as 12 months of age (47.4 \pm 3.8 and 37.5 \pm 2.1 pg/mg hippocampus, respectively; p = 0.027 by Student's *t* test). Insoluble A β levels did not differ significantly between the two groups (PDAPP/LRP⁺ = 24,730 \pm 2940 pg/mg hippocampus; PDAPP/LRP⁻ = 21,170 \pm 2186 pg/mg hippocampus; p = 0.33 by Student's *t* test). There were no significant correlations between TBS-soluble A β levels or TBS/Triton X-100-soluble A β levels and insoluble A β (R² = 0.03; p = 0.38 and R² = 0.03; p = 0.33, respectively), indicating that these soluble pools are independent of A β plaques. By immunofluorescence of brain sections using A β -specific antibodies, we detected A β staining in neuronal cell bodies and processes, suggesting that this cell-associated A β is likely the pool represented in the detergent-soluble extract. In summary, LRP appears to preferentially increase a cell- or membrane-associated A β pool, consistent with the hypothesis that LRP facilitates APP beta-cleavage in the endocytic pathway.

Commercial Relationship: C.V. Zerbinatti, None.

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