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NEW STUDIES PROBE ROLE AND FORM OF BRAIN PROTEIN THAT MAY BE RESPONSIBLE FOR IMPAIRED MEMORY IN ALZHEIMER'S DISEASE

Findings advance debate about broad role of amyloid-beta in disease causation

Washington, DC — Evidence is growing that particular forms of a protein involved in Alzheimer's disease may be responsible for impaired memory and brain cell communications. The findings, presented at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health, support recently released discoveries showing that certain conformations and combinations of the amyloid-beta protein produce the damaging effects of Alzheimer's disease.

Scientists have long known that large, insoluble deposits of amyloid-beta protein form senile plaques in the brains of Alzheimer's disease patients. Until very recently, these plaques were believed to be responsible for disrupting brain cell communication, which leads to memory loss and other symptoms of the disease. However, recent research suggests that the cell destruction is more likely triggered by earlier and smaller aggregates of amyloid-beta, and scientists have been searching for configurations that may be the culprit. If confirmed, the findings may one day lead to new avenues of treatment that may be effective in preventing or treating this devastating brain disorder, which affects 4.5 million Americans and millions more around the globe.

The new findings report that:

- An early, soluble, clustered form of amyloid-beta, protofibrils, is found in high levels in the brains of people with Alzheimer's; in addition, a strong correlation exists between high levels of the protofibrils in the brains of transgenic mice models and the cognitive impairments associated with the disease (Frida Ekholm Pettersson, abstract 409.9, see attached summary).
- A therapy that targets amyloid-beta levels in the brain significantly reduces two neurological markers that represent early to mid-stage Alzheimer's disease — tau pathology and neuron loss. The research was conducted in two new mouse models that closely re-create aspects of the disease seen in humans (Donna Wilcock, abstract 510.7, see attached summary).

Other research findings being discussed at the meeting show:

- A two-molecule cluster, or "dimer," of amyloid-beta may play an important role in memory loss in Alzheimer's disease. Amyloid-beta dimers extracted from people with Alzheimer's disease were injected into research animals, which promptly experienced impaired memory and brain cell communication (see attached speaker's summary).
- Amyloid-beta is important in the healthy brain. Brain levels of amyloid-beta in people recovering from serious brain injuries increase as the patients progress toward recovery — and decrease if the patients experience a setback (see attached speaker's summary).

"One of the mysteries of the amyloid hypothesis — that the progressive accumulation of amyloid-beta initiates the cascade of events leading to Alzheimer's disease — has been that healthy elderly individuals and those with Alzheimer's both develop amyloid plaques," said press conference moderator Edward Koo,

– more –

PhD, professor in the department of neurosciences at the University of California, San Diego, School of Medicine. “These studies reflect and advance the hunt for what forms of amyloid-beta protein are actually toxic to brain cells and important for the disease’s progression, and highlight the crucial role of research in creating significant progress on a host of neurological diseases and disorders.”

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Abstract 409.9 Summary

Lead author: Frida Ekholm Pettersson, PhD **(46) 18-471-48-02**
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Soluble Protein Clusters of Amyloid-Beta Found Only in Brains of Alzheimer's Disease Patients

Amount of these protein clusters correlates with cognitive impairment in animal studies

A new study has found that the brains of people with Alzheimer's disease have high levels of amyloid-beta protofibrils, a soluble, clustered form of the amyloid-beta protein, which plays such a crucial role in the development of the disease. Researchers also found a strong correlation between the presence of high levels of protofibrils in the brains of transgenic mice models of Alzheimer's disease and the cognitive impairments associated with the disease. The study was released at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

These findings suggest new possibilities for treating Alzheimer's disease: drugs that could counteract either the formation or the toxicity of the protofibrils.

Scientists have long known that large, insoluble plaques of amyloid-beta form in the brains of people with Alzheimer's disease. Until very recently, these plaques were believed responsible for the brain cell destruction that leads to memory loss and other symptoms of the disease. Today, however, scientists believe that the cell destruction is more likely triggered by earlier and smaller aggregates of the amyloid-beta, such as the protofibrils.

In this new study, Frida Ekholm Pettersson, PhD, of Uppsala University in Sweden and her colleagues compared amyloid-beta protofibril levels of patients who had died with Alzheimer's disease to those of patients who had been diagnosed with another neurodegenerative disorder known as frontotemporal dementia. Also measured were the protofibril levels of people never diagnosed with dementia. "The amyloid-beta protofibrils could only be detected in the Alzheimer brains," said Pettersson, "which suggests that this particular form of amyloid-beta may be a highly sensitive and specific marker for the disease."

For one component of the study, the researchers developed a mouse model with high brain levels of amyloid-beta protofibrils and then tested the animals' cognitive skills. The mice exhibited cognitive impairments before plaques could be detected in their brains. Furthermore, when comparing the animals' protofibril levels and test results, the researchers found an apparent correlation between high levels of protofibrils and impaired test performance.

"This suggests that amyloid-beta protofibrils impair the ability to store new information, a symptom typically seen in Alzheimer's disease patients early on in the disease," said Pettersson.

The research was supported by the Swedish Research Council, Swedish Brain Foundation, Swedish Alzheimer Foundation, Bertil Hållsten Foundation, Uppsala University Hospital, and the Uppsala Berzelii Technology Center for Neurodiagnostics.

Scientific Presentation: Monday, November 17, 3–3:15 p.m., Washington Convention Center, Room 150B

409.9, Amyloid-beta protofibrils are present in Alzheimer disease brain and linked to early cognitive impairment in APP transgenic mice
F. EKHOLM PETTERSSON, A. LORD, H. ENGLUND, D. SEHLIN, R. BRUNDIN, F. CLAUSEN, L. HILLERED, M. N. GORDON, D. MORGAN, L. N. G. NILSSON, *L. LANNFELT; Publ. Health/Geriatrics, Neurosci., Uppsala Univ., Uppsala, Sweden; Mol. Pharmacol. and Physiol., Univ. of South Florida, Tampa, FL
TECHNICAL ABSTRACT: Senile plaque burden does not correlate well with cognitive functions in Alzheimer's disease (AD). However, soluble forms of amyloid-beta (Abeta) are likely to be toxic for neurons. The Arctic mutation (Abeta E22G) provides clinical support for Abeta protofibrils, one among several oligomeric Abeta species described as a being pathogenic. The aim of this study was to establish the presence of Aβ protofibrils in human brain and to assess the role of Abeta protofibrils with respect to cognitive functions, by quantifying and relating Abeta protofibril levels to measures of spatial learning in tg-mice. We also investigated the association of Abeta protofibrils with other biochemical and histological measures. We recently developed an Abeta protofibril-specific sandwich ELISA (Englund et al. 2007) which allows measurement of protofibrils in biological tissues. Moreover, we have created an animal model (tg-ArcSwe) displaying high levels of Abeta protofibrils in the brain. In this study, levels of Abeta protofibrils in tg-ArcSwe, tg2576 and in APP+PS1 transgenic mouse models were compared to biochemical and histological measures of Abeta, such as Congo and Aβ burden. Tg-ArcSwe, tg2576 and APP+PS1 transgenic mouse brains displayed elevated levels of soluble Abeta protofibrils before amyloid plaque deposition and the Abeta protofibril levels modestly increased with age in all of these models, although much less than total Abeta. In young tg-ArcSwe mice there was an inverse correlation between protofibril levels - but not total Abeta - and performance in the Morris water maze. The cognitive deficits in the tg-ArcSwe model correlated better with Abeta protofibril levels than with total Abeta levels. Furthermore, we have recently measured Abeta protofibrils in TBS extracts from human brains. In AD cortex, levels of protofibrils were approximately at the same level as in transgenic mice, whereas in healthy control brains protofibrils were below level of detection (<1 pM). We conclude that early forms of soluble Aβ, in the absence of senile plaques, result in cognitive impairments in the tg-ArcSwe model. Our findings suggest that Aβ protofibrils are important bioactive Aβ species early in the pathogenesis and a promising drug target in AD.

Abstract 510.7 Summary

Lead author: Donna Wilcock, PhD **(919) 668-3398**
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Amyloid-Beta Therapy for Alzheimer's Disease Reverses Memory Deficits and Protects Brain Cells

Research in more accurate model of disease supports effectiveness of therapeutic approach

A therapy that targets amyloid-beta protein levels in the brain can reverse memory deficits and prevent neuron loss in animals that accurately model Alzheimer's disease, according to new research. The study was released at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"These findings suggest that amyloid-beta is a viable therapeutic target for Alzheimer's disease," said Donna Wilcock, PhD, of Duke University.

The two mice models used in this study are the first to display all three diagnostic characteristics of Alzheimer's disease: amyloid plaques composed of the amyloid-beta protein, neurofibrillary tangles composed of tau proteins, and the loss of neurons. Past studies have shown that anti-amyloid-beta immunotherapy, which uses bloodborne antibodies against amyloid-beta, is effective in lowering levels of the protein fragment in the brains of mice. The effects of the immunotherapy on tau pathology and, most importantly, on neuron loss had not been tested, however.

For this study, Wilcock and her colleagues vaccinated both mouse strains with amyloid-beta antibodies when the animals were 12 months old. At that age, the mice exhibit a 30 percent loss of neurons and significant amounts of amyloid and tau pathology — neurological markers that represent early to mid-stage Alzheimer's disease.

As had been seen in other studies, the amyloid-beta levels in the vaccinated mice fell, and the animals' performance on a memory test significantly improved.

"Because our mice also display tau pathology and neuronal loss, we were able for the first time to show that the vaccination also significantly reduced tau pathology," said Wilcock. "Most important, the vaccination prevented further loss in neurons."

The vaccinated mice also developed significant numbers of microhemorrhages (small-scale bleeding in the brain), which have been a concern reported in other studies. "Further studies are needed to determine if earlier vaccination — before the onset of any pathology — will prevent the development of the disease without incurring the microhemorrhages," said Wilcock. "Timing of the vaccination will establish whether microhemorrhages are a result of amyloid removal or, more worryingly, of the amyloid-beta vaccination itself."

The research was supported by the Alzheimer's Association and the U.S. National Institute on Aging.

Scientific Presentation: Tuesday, November 18, 9:30–9:45 a.m., Washington Convention Center, Room 151B

510.7, Aß vaccination in the APPSw/NOS2-/- model of Alzheimer's disease lowers amyloid deposition and endogenous mouse tau hyperphosphorylation while reversing cognitive impairment
*D. M. WILCOCK, M. P. VITEK, N. GHARKHOLONAREHE, C. A. COLTON; Div. of Neurol., Duke Univ., Durham, NC
TECHNICAL ABSTRACT: Immunotherapy targeting Aß is a promising therapeutic approach to the treatment of Alzheimer's disease. The preclinical experiments in mice have been limited to models of amyloid deposition only or models with APP and tau mutations which all only

partially recapitulate human Alzheimer's disease. In the current study we have examined active immunization in the APPSw/NOS2-/- mouse model. This mouse model more completely models human Alzheimer's disease. An essential component of Alzheimer's in addition to amyloid deposition is native tau hyperphosphorylation and aggregation as well as neuron loss. The APPSw/NOS2-/- mouse achieves native mouse tau hyperphosphorylation (AT8-positive) and aggregation (thioflavine-S positive) and significant neuron loss in addition to amyloid deposition. We performed two-day radial-arm water maze (RAWM) testing on mice aged 54-56 weeks and found significant impairment in the APPSw/NOS2-/- mice compared to NOS2-/- controls as well as APPSw mice alone. We then began Aß vaccination using fibrillar Aß1-42 in Freund's adjuvant. Mice received a total of 4 inoculations over a 3 month period. Behavioral testing including the two-day RAWM testing was performed again during the 2 weeks following the final innoculation. At sacrifice brain tissue was collected and processed for biochemical and histological analysis. Serum was also collected to allow measurement of the antibody response.
All mice produced significant antiibody titers with no differences observed between genotypes. Importantly, NOS2 deletion did not affect antibody titer. Histological analysis revealed a significant reduction in amyloid deposition by immunohistochemistry. In addition, tau pathology as measured by AT8 immunocytochemistry appeared to be reduced in both the number of positive cells and also the staining intensity of the neuronal cell body. AT8 western blot revealed a 45% reduction in the APPSw/NOS2-/- mice immunized with Aß compared to APPSw/NOS2-/- mice receiving a KLH control vaccination. No reductions in AT8-positive tau have previously been observed in mice carrying APP and tau mutations. APPSw/NOS2-/- mice also showed a reversal in cognitive deficits showing significant improvement in the two-day RAWM after 3 months of vaccination. We plan to examine vaccination effect on neuron loss. For the first time we are able to test a potential therapeutic for Alzheimer's disease in a mouse model that has amyloid driven tau pathology and neuron loss. Our findings suggest that amyloid lowering therapeutics such as immunotherapy will also reduce tau pathology.

Speaker's Summary

Speaker: Dennis Selkoe, MD **(617) 525-5200**
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Boston, Mass.

Amyloid β-Protein Dimers Isolated Directly From Alzheimer Brains Potently Impair Hippocampal Synaptic Plasticity and Memory (409.4)

Tuesday, November 17, 1:45–2 p.m., Washington Convention Center, Room 150B

Despite research over more than three decades on Alzheimer's disease (AD) using human brain tissue and cellular and animal models, identifying the pathogenic agent present within the human cortex that induces synapse dysfunction and impairs memory and then showing that it can confer these key features of AD in a healthy animal have not been achieved.

Evidence from numerous labs supports the concept that small, soluble assemblies ("oligomers") of amyloid ß-protein (Aß) are the form of Aß that correlates best with degree of dementia in AD subjects. Moreover, soluble Aß oligomers made synthetically or obtained from the fluid of cultured cells can impair the electrical function of synapses in the hippocampus of rodents and actually decrease synapse number. A few studies suggest that small Aß oligomers decrease memory function in vivo in animals. While these various studies support a pathogenic role for soluble Aß oligomers, the application of this approach to learn exactly what types of oligomers are present in AD patients and whether they confer AD-like effects on synapses — and thus memory — has not been reported.

We extracted soluble Aß oligomers directly from the cerebral cortex of typical AD subjects. In these extracts, we detected small oligomers ("dimers" and "trimers", i.e., doublets and triplets) in addition to Aß "monomers" [the single unit of Aß that all cells (and thus people) normally make] in all of the 8-10 AD brains we've examined to date. We did not observe significant amounts of soluble Aß oligomers in the cortex of non-AD demented patients (e.g., Lewy body dementia and frontotemporal dementia) who did not have AD-type neuropathology, nor did we see them in aged normals. Some of the aged non-AD brains did contain insoluble Aß requiring a harsh solvent to extract. In one case, the brain of someone without clinical AD nonetheless had insoluble amyloid plaques, a well-known situation that gives rise to concerns that amyloid may not cause AD since normal persons can have quite a lot of it. This particular case had insoluble Aß species in brain extracts but few or no soluble Aß oligomers. Thus, our analyses in a relatively small number of human brains to date suggest that levels of soluble Aß oligomers correlate with a history of clinical AD symptoms, whereas insoluble Aß load correlates less well. The oligomer-lacking soluble extracts from our non-AD subjects served as controls throughout this study.

We found that extracts containing soluble oligomers from the cortex of AD brains acutely and potently inhibited an electrical correlate of learning and memory (long-term potentiation; LTP) in hippocampal slices from normal adult mice. We also found that soluble Aß oligomers enhanced long-term synaptic depression (LTD) in hippocampus. The soluble AD extracts actually decreased the number of dendritic spines (parts of synapses) in hippocampus. The latter finding is relevant to AD because loss of synapses is the strongest neuropathological correlate of the degree of dementia in AD subjects. Importantly, we also showed that the soluble AD cortical extracts containing Aß dimers and trimers disrupted the memory of a learned behavior in normal adult rats.

Using a biochemical separation method called size-exclusion chromatography, we found that the various effects described above were attributable to soluble Aβ oligomers, specifically to dimers. We confirmed the identity of the dimeric species we saw as true Aß dimers using mass spectrometry. Importantly, SEC-isolated soluble Aß monomers were without activity in the various assays mentioned above.
Of therapeutic relevance, co-administering (with the soluble dimers) antibodies specific to the Aβ N-

terminus prevented the LTP and LTD deficits, whereas antibodies to the mid-region or C-terminus of the peptide were less effective.
Intriguingly, the amyloid plaque cores isolated from the same AD brains did not impair LTP unless they were first dissolved by a harsh solvent to release Aβ dimers, suggesting that plaque cores are largely inactive as such but sequester Aβ dimers that are potentially synaptotoxic.

We conclude that soluble Aβ oligomers extracted directly from AD brains potently impair the structure and function of synapses in the hippocampus, and that dimers are the principal (and smallest) synaptotoxic Aß assemblies. Another outcome of this study is that Aß extracted from human brain can now serve as the most pathophysiologically relevant material for further analysis of the biochemical pathways in neurons that are altered by Aß, and also for preclinical validation of Aß antibodies or small molecules intended to neutralize Aß assemblies. Our findings fulfill one of the essential requirements for establishing disease causation in Alzheimer's disease.

Speaker's Summary

Speaker: David Brody, MD PhD **(314) 362-1381**
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St. Louis, Mo.

Amyloid-Beta Dynamics Correlate With Neurological Status in the Injured Human Brain (210.6)
Sunday, November 16, 2:15–2:30 p.m., Washington Convention Center, Room 151B

Hour-by-hour changes in the amount of amyloid beta, a protein that is believed to play a key role in Alzheimer's disease, have been described for the first time in the human brain. The main finding was that amyloid beta levels were strongly related to the patients' neurological status.

Aspects of this work have been presented at the Federation of European Neurological Societies Meeting in Geneva (July, 2008), and published in Science (August 29, 2008).

Amyloid beta levels were measured in 18 patients who were recovering from serious brain injuries in the intensive care units at Barnes Jewish Hospital in St Louis, and the Ospedale Maggiore Policlinico in Milan, Italy. Amyloid beta levels often started out low, and as the patients progressed along the road toward recovery, their brain amyloid beta levels increased. If patients worsened, amyloid beta levels fell. Amyloid beta levels were measured using a technique called microdialysis, which involves placing a small catheter into the brain tissue to sample the fluid in the spaces between cells. Patients' families in both St. Louis and Milan gave permission in advance, and the catheters were placed when the patients were having other monitoring procedures performed.

The results relate to an experiment Washington University researchers conducted three years earlier. In that study, scientists monitoring mice with microdialysis showed that brain cell communication was directly linked to the levels of amyloid beta. When there was increased communication between brain cells, amyloid beta increased. When there was reduced communication, amyloid beta decreased.

However, it was not known whether the same relationship between brain cell communication and amyloid beta levels would hold in humans. The data in humans appear to fit well with the previous results in mice, because improved neurological status is likely to go along with increased communication between brain cells.

The new results provide scientists important clues about the general origins of Alzheimer's. Further investigation is needed to answer the specialized question of why brain injury increases risk of Alzheimer's. One theory being tested is that brain injury accelerates harmful processes that cause Alzheimer's. A second explanation for the link between injury and Alzheimer's suggests that injury may reduce the brain's ability to compensate for Alzheimer's-related damage, making the symptoms of the disease evident much earlier than they would otherwise appear. Evidence exists for both models, and both could be valid in different settings.

The ultimate goal of this line of research is to develop interventions that can be applied after a traumatic brain injury to improve outcomes and reduce the long-term risk of Alzheimer's.