Keystone Symposia Meeting, Part 1—Alzheimer's Disease: Genes, Cellular Pathways and Therapies

By Minji Kim, Alice Lu, and Rudy Tanzi.

12 April 2006. This Keystone Symposia meeting on Alzheimer disease, organized by Rudy Tanzi, Massachusetts General Hospital, Boston, and Virginia Lee, University of Pennsylvania, Philadelphia, at Beaver Run Resort, Breckenridge, Colorado, focused on the molecular underpinnings of Alzheimer disease (AD) and frontal temporal dementia (FTD), covering genetic risk factors, cellular pathways, and emerging therapies. The program was enhanced by running parallel with another Keystone Symposia meeting organized by Jeff Kelly, The Scripps Research Institute, La Jolla, California, and Susan Lindquist, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, on the role of abnormal protein folding in disease.

Over the past two decades, studies of AD at the genetic, molecular, and cell biological level have revealed a host of genes, proteins, and biological pathways that impact the pathogenesis of this disease. Characterization of these pathogenic pathways has suggested several promising therapeutic strategies for treating and preventing AD, based on curbing the accumulation of the Aβ peptide in brain, regulating tau hyperphosphorylation, modulating inflammatory responses, and enhancing neurotransmitter function. This meeting covered the newest findings regarding the genetics of AD, Aβ generation and clearance pathways, regulation of tau, relevant animal models, novel therapeutic strategies, and advances in imaging and biomarkers for AD diagnosis. Overall, it was a very exciting meeting, especially since all of the speakers mainly presented new and unpublished findings. In many ways, this meeting granted a sneak peek into what will be presented at this year's international AD meetings in Geneva and Madrid, and from many of the top researchers in the field. We also had the "three S" formula that makes for a great Keystone meeting: Great Science, Bright Sun, and Incredible Snow. The meeting was very well attended with a capacity ~300 at the AD meeting alone. As the Keystone staff put it, there was clearly a great "buzz" at this meeting with a feeling of great movement and progress in the air. Many of the participants also commented that this was the best AD meeting they have attended in several years.

***Keynote Session*: Sangram S. Sisodia and Cynthia Kenyon**

Sangram Sisodia, University of Chicago, Illinois, focused on the molecular neurobiology of Alzheimer disease, including an overview of APP processing pathways and the roles of BACE (β-secretase) and of the presenilins (as part of the γ-secretase complex). He described the effects of PS1 mutations, including impaired transport of APP, kinesin, and Trk receptors in axons; inhibition of enrichment-mediated proliferation and survival of progenitor cells in the dentate gyrus; and promotion of vulnerability of cortical neurons to lesions. He also reviewed data from his recent paper in Cell showing in vivo evidence that exercise attenuates AD pathology in transgenic mice. Mice housed under conditions of environmental enrichment, including robust running wheel activity, exhibited decreased levels of brain Aβ—potentially via up-regulation of neprilysin (an Aβ degradation enzyme)—and also transthyretin (TTR) in hippocampus. Following up on these data, Sisodia showed that genetic reduction of TTR accelerated Aβ deposition in transgenic mice (TTR +/-). Based on his data, Sisodia argued that simple physical exercise can be an effective preventive measure against AD.

Cynthia Kenyon, University of California, San Francisco, illustrated the importance of controlling aging by altering genes involved in the aging process. She described the role of *C. elegans* DAF-2, which encodes a hormone receptor similar to the human insulin and IGF-1 receptors. Mutations that reduced the activity of DAF-2 doubled the lifespan of the roundworms. Longevity of DAF-2 mutants required DAF-16, the normal function of which is to extend lifespan. As DAF-2 is turned down and DAF-16 is turned up, FOXO transcriptional activity is increased. Together, DAF-2 and DAF-16 control a battery of downstream genes whose functions affect lifespan. In addition, Kenyon reported that heat shock factor-1 (HSF-1) has to be present in order for DAF-2 mutants to live long, suggesting a role for heat shock and stress-induced proteins. Many of the genes affected by DAF-2/DAF-16 are regulated by FOXO transcription factor, and included various antioxidant, chaperone, anti-microbial, and metabolic genes. Kenyon also demonstrated that the lifespan of *C. elegans* was influenced by its perception of soluble and volatile substances in the environment. Finally, a nematode that reached the very ripe age of 142 days was revealed; however, no details regarding its genetic makeup and growth conditions were presented.

Keystone Symposia Meeting, Part 2—Genetics and Epidemiology of AD

13 April 2006. **Steve Younkin**, Mayo Clinic at Jacksonville, Florida, presented the recent progress in identification of multi-locus genotypes in novel late-onset AD (LOAD) genes, particularly presenilin 1 (PSEN1), insulin-degrading enzyme (IDE) and ubiquilin 1 (UBQNL1). First, Younkin emphasized the need to identify the remaining AD genes, and stated that beyond the major LOAD risk factor, ApoE4, the majority of the remaining AD genes will likely exhibit relatively modest effects on risk and protection. Accordingly, he argued that for case-control studies, up to 10,000 matched cases and controls will be necessary to identify the remaining AD genes. He then described seven DNA variants in the conserved non-coding regions of the gene encoding the insulin-degrading enzyme on chromosome 10. These variants form five common haplotypes, and four risk-conferring and nine protective multi-locus genotypes. Younkin also provided RT-PCR data showing that the protective IDE genotypes were functionally associated with increased IDE mRNA expression in brain. Other genetic data were also presented to support the existence of risk-conferring variants for LOAD in the ubiquilin 1 and presenilin 1 genes.

Mario Bengtson, Genomics Institute of Novartis Foundation, San Diego, California, presented a tauopathy phenotype in the lister mutant mouse. The responsible gene, called "listerin," was found to encode a novel E3 ubiquitin ligase. The pathogenic mutation in this gene leads to a short, in-frame internal deletion. The mutant mouse shows progressive loss of hind limb reflex extension, motor neuron degeneration, and accumulation of hyperphosphorylated soluble forms of the tau protein. He emphasized that the lister mutant mouse is significant as a new mouse model for motor neuron/tauopathy disease and as the first mutation in a ubiquitin ligase identified in the disease. However, Bengston regretted that he could not reveal the identity of the gene or the human ortholog.

Lars Bertram, Massachusetts General Hospital, Boston, provided an overview of the recently developed "AlzGene" database. The database provides systematic and objective summaries of all peer-reviewed publications in the area of AD genetics and covers over 300 candidate genes and over 800 polymorphisms. Bertram emphasized the dire need for such a database, given the increasing number of AD gene papers and confusion created by mixed positive and negative results for the hundreds of AD candidate genes tested to date, especially since most exhibit modest to moderate effects on risk and protection. He also pointed out the usefulness and necessity of such a database for diseases that are characterized by complex genetics. The Alzgene.org site was described with regard to the data collection process and certification of the methods employed in the meta-analyses—ApoE4 results were used as an example of a positive control gene. Besides ApoE4, nine candidate gene polymorphisms were found to be positive so far by AlzGene meta-analyses: ApoE (promoter region), apolipoprotein C1 (ApoC1), angiotensin-converting enzyme (ACE), cystatin 3 (CST3), estrogen receptor 1 (ESR1), insulin-degrading enzyme (IDE), prion precursor protein (PRP), presenilin 1 (PSEN1), and transferrin (TF). He went on to describe results of family-based association analyses in NIMH and CAG samples, which revealed genetic association of AD with the ubiquilin 1 gene on chromosome 9 and the IDE gene on chromosome 10. Several negative candidate genes on chromosome 19 were also reviewed.

Ellen Wijsman, University of Washington, Seattle, outlined her strategy for identification of late-onset AD genes following up a genome scan in the Seattle LOFAD pedigrees using Markov Chain Monte Carlo oligogenic linkage analysis. Age-at-onset was used as an AD quantitative trait and ApoE as a major gene covariate for adjustment. She presented evidence of a new genetic association of LOAD with five single nucleotide polymorphisms in a locus on chromosome 19p at a position near 33 cM, but could not reveal the identity of the candidate gene.

Lisa Paige, Metabolon Inc., Research Triangle Park, North Carolina, presented a comparative metabolomic analysis of plasma from patients with AD or mild cognitive impairment (MCI) as a means to search for metabolic biomarkers for AD. After an overview of the metabolon process, she provided proof-of-concept data based on a strong correlation between metabolomic and clinical lab measures of creatinine, regarding both sensitivity and specificity. No metabolic markers were yet found in AD versus control plasma, emphasizing the challenges in developing biomarkers for the disease.

Keystone Symposia Meeting, Part 3—Amyloid Precursor Protein Function

14 April 2006. **Sam Gandy**, Thomas Jefferson University, Philadelphia, reviewed the trafficking pathways of APP from the endoplasmic reticulum (ER) to the trans-Golgi network (TGN) to the plasma membrane (PM), including regulated ectodomain shedding by α-secretase. He discussed the search for phospho-state-sensitive modulators of ectodomain shedding (PMES) candidates, including munc13, munc18, PKD2wt, and PKD2KD, all of which boosted basal and regulated shedding. However, none of these candidates were found to regulate shedding in a phospho-state-sensitive fashion. Gandy then addressed the evidence that PS1 plays a role in trafficking of APP or other membrane proteins. In experiments using APP:furin chimeric mice, he showed that brain Aβ42/40+42 ratios were similar to ratios associated with FAD-mutant PS1, suggesting that altered trafficking of APP out of the TGN could underlie PS1-linked FAD. Similar to PS1 FAD mutants, distribution of an APP:furin chimera in CNS neurons in vivo was more restricted to the TGN than was that of APP:APP. Moreover, APP:furin expressed in CNS neurons generated more Aβ42/40+42 than APP:APP. Based on these and other findings, Gandy suggested that impaired TGN egress of APP may enhance the generation of Aβ42.

Roberto Malinow, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, reviewed the effects of APP and Aβ on synaptic function. He presented data showing that when synaptic activity is increased, processing of APP to Aβ and secretion of the peptide is increased. Aβ was able to depress synaptic function, thus forming a potential negative feedback loop. Malinow also showed that in hippocampal slice cultures, both Aβ-induced depression and plasticity-induced synaptic depression reduce dendritic spine density, and reduce numbers of synaptic NMDA and AMPA receptors. These events, which required calcineurin and p38-MapK, partially occluded metabotropic glutamate receptor-mediated long-term depression. Overall, these studies provided further support for a possible role of APP and Aβ in modulating synaptic activity.

Thomas Südhof, University of Texas Southwestern Medical Center in Dallas, described CSPα (cysteine-string protein-α) as a synaptic vesicle protein that contains a DNA-J domain typical for Hsp40-type co-chaperones. CSPα activates the ATPase activity of Hsc70, and co-assembles with Hsc70 and the small glutamine-rich tetratricopeptide repeat (TPR)-containing protein (SGT) into an enzymatically active chaperone. In mice, CSPα prevented presynaptic neurodegeneration and acted at least in part on SNARE proteins. Overexpression of α-synuclein suppressed neurodegeneration induced by deletion of CSPα, whereas endogenous α-synuclein protected against neurodegeneration. In its capacity as a neuroprotective molecule, α-synuclein did not appear to act as a chaperone, but bound lipid coats of synaptic vesicles and influenced the activity of SNARE proteins. In contrast, overexpression of human α-synuclein caused neurodegeneration, but by an unrelated mechanism, most likely involving oligomerization and aggregation.

In the short talk slated for this session, **Constanze Reinhard**, Center for Human Genetics, KU Leuven, presented data from her search for novel receptors for secreted forms of APP (sAPP). By establishing a system to evaluate sAPP binding properties, she showed that sAPP binding occurs via two different mechanisms: a heparan sulfate-dependent interaction and a protein-protein interaction. In the neuroblastoma

cell lines, N2A and B10-3, sAPP preferentially binds through a protein-protein mediated interaction.

Keystone Symposia Meeting, Part 4—Presenilin and γ-secretase

17 April, 2006. The third day of the meeting started with a session on presenilin and γ-secretase. **Dennis Selkoe**, Harvard Medical School, addressed the three principal stages of the life of Aβ: production, degradation, and aggregation. With respect to Aβ production, Selkoe reported that he and his colleagues were able to purify a γ-secretase complex from mammalian cells that produced physiological ratios of Aβ40 and Aβ42. Using electron microscopy and single particle image analysis, they performed structural analysis of the presenilin/γ-secretase complex. The 3-D reconstruction revealed that γ-secretase is a 100-angstrom particle with a central chamber of irregular diameter and two openings, one up top and one on the side. The structure looked like an oblong bead resembling a mini-proteasome, possibly made up of two presenilin (PS) molecules.

Regarding Aβ degradation, Selkoe showed that amyloid-β precursor protein (APP) transgenic mice lacking neprilysin (NEP) had increased amyloid plaque burden and Aβ immunoreactivity. The NEP-negative mice also developed amyloid angiopathy and had increased levels of Aβ40 and Aβ42. Selkoe also presented evidence for Aβ oligomers in flotillin-rich lipid rafts. He demonstrated that soluble Aβ oligomers, but not monomers, abrogated hippocampal LTP, in vivo and in vitro, and that this could be prevented by both active and passive immunotherapy and by small, orally bioavailable compounds (see Townsend below). Soluble Aβ oligomers could also transiently interfere with the memory of learned tasks in animals, and they caused loss of dendritic spines in organotypic hippocampal slice cultures. The spines could be rescued after 10 days with an Aβ-specific antibody (6E10).

Finally, Selkoe presented results of in utero electroporation of APP RNAi into rat cortex at E13. Knockdown of APP at this stage of development blocked neuronal migration to the cortical plate and led to retention in the intermediate zone. Similar results were obtained for knockdown of APP-like proteins APLP1 and APLP2. Interestingly, migration could be restored with APP C-terminal constructs, suggesting a role for APP adapters, for example, Fe65 family proteins.

Christian Haass, University of Munich, Germany, employed a γ-secretase reporter assay to show that when APP was retained in the endoplasmic reticulum (ER) or trans-Golgi network (TGN)—using brefeldin A or monensin, respectively), there was no cleavage by γ-secretase. Similar results were obtained for tannic acid, which prevents fusion of synaptic vesicles with the plasma membrane. Inhibition of endocytosis with a dominant negative mutant of dynamin was used to show that γ-secretase cleavage mainly takes place at the plasma membrane and in endosomes.

Next, Haass presented the identification of a conserved protease motif, GxGD, in various aspartyl proteases including the nematode presenilin homologue Spe4. A PS1/Spe4 active site chimera cleaved APP, but not Notch. He showed that a single amino acid at position x of the GxGD active site motif of PS is implicated in APP/Notch substrate selection of γ-secretase, and that final substrate selection of γ-secretase may occur very close to the active site. If the position between the two glycines is filled by phenylalanine, Notch intracellular domain (NICD) generation is blocked while AICD production from APP is allowed. If leucine lies between the glycines, both Notch and APP are cleaved. He pointed out that all signal peptide peptidases have GxGD motifs. Mutagenesis of the aspartate residue within the GxGD

motif of signal peptide peptidases SPPL2 and SPPL3 in zebra fish resulted in a loss-of-function phenotype, indicating that SPPLs are aspartyl proteases of the GxGD family. Finally, he identified the type 2 transmembrane protein, TNFα, as a substrate for SPPL2. SPPL2 performs a γ-secretase-like dual intramembrane domain cleavage, which produces both an ICD-like fragment and a secreted C-terminal fragment.

Bart De Strooper, Center for Human Genetics, KU Leuven, Belgium, presented a nine transmembrane model for presenilin, and demonstrated that at least four different γ-secretase complexes exist. The complexes have different biological functions: Aph1A-/- mice had impaired embryological development, while Aph1BC-/- showed a normal phenotype—Aph1 is one of the subunits of γ-secretase. Interestingly, Aph1BC-/- mice had impaired γ-secretase activity in the adult brain, as well as impaired sensory gating (startle response), suggesting that the Aph1BC complex is a potentially interesting drug target for AD and that Aph1BC may have an important role in the development of dopaminergic pathways in the CNS.

The γ-secretase complexes also have different biochemical properties: Other γ-secretase subunits were affected in Aph1ABC-/- mice—there was no maturation of nicastrin (another γ-secretase component), no endoproteolysis of PS, and decreased levels of the fourth γ-secretase member, Pen-2. De Strooper also warned that a commercially available FRET-based assay for γ-secretase activity is not selective for γ-secretase. In studies of the role of the AICD, De Strooper stated that he could not confirm the recently reported effects of AICD on neprilysin expression whether using PS double knockouts, γ-secretase-inhibitors, or APP/APLP knockout mice.

Jie Shen, Brigham and Women's Hospital and Harvard Medical School, Boston, described the use of Cre/loxP technology to generate presenilin double conditional double knockout mice, FB-PS cDKO, in which inactivation of presenilins was restricted spatially and temporally to the adult cerebral cortex. These FB-PS cDKO mice had mild memory deficits at 2 months with normal performance in rotarod and open field tests. However, at 6 months, they had severely impaired spatial learning and memory and severely impaired hippocampus- and amygdala-dependent memory, indicating that with age, memory impairment worsens. At 2 months, FB-PS cDKO also had short-term plasticity deficits: impaired LTP, impaired paired LTP, and reduced NMDA responses. Shen also showed an age-dependent impairment of NMDA receptor (NMDAR) responses in FB-PS cDKO mice. She proposed that presynaptic PS1 is a trans-synaptic regulator of postsynaptic NMDAR. Finally, She stated her preference for the hypothesis that FAD mutations in PS cause AD by attenuating PS function at least partially in an Aβ-independent manner.

Homira Behbahani, Karolinska Institute, Stockholm, discussed the role of PS1 and PS2 on mitochondrial membrane potential and oxygen consumption in mice embryo. She showed that in mouse embryonic fibroblast (MEF), there was a higher fractional area of mitochondria in PS1-/- cells, reduced COX1 levels in PS2-/- and DKO, low mitochondrial membrane potential in PS2-/- and DKO cells, and a low basal respiratory rate in PS2-/- cells.

Taisuke Tomita, University of Tokyo, presented the identification of PS1 as a molecular target of potent dipeptidic inhibitors, DAPT, CE, and DBZ. He demonstrated that DAPT specifically bound PS1 C-terminal fragment (CTF), but not the N-terminal fragment (NTF), that CE and DBZ bound to PS1 NTF, but not CTF,

that CE and DBZ also bound to SPP and other polypeptides. He concluded that differences in the mode of binding might reflect enzyme specificity of the inhibitor.

Keystone Symposia Meeting, Part 5—β-secretase

19 April 2006. **Donald Price**, Johns Hopkins University, Baltimore, presented results of BACE knockout mouse studies. By filter trap and immunostaining methods, he showed that the BACE-/- mouse had no Aβ plaques in the brain. The knockout mouse also exhibited alterations in behavior, as monitored by the Morris water maze and the radial water maze. But the mice exhibited no developmental abnormalities or adult onset neuropathology. Interestingly, memory deficits, but not emotional and cognitive deficits, in APPswe/PS1ΔE9 mice could be rescued by BACE deletion. Finally, as a potential therapeutic intervention, he introduced a Tet-off conditional expression system coupled to lentiviral RNAi injection strategies for attenuating BACE and demonstrated successful clearance of plaques at the injected site of APPswe/PS1ΔE9 mouse brain.

Jordan Tang, Oklahoma Medical Research Foundation, Oklahoma City, elaborated on a mechanism of internalization and recycling of BACE, otherwise known as memapsin2 (M2) and APP, and the binding proteins responsible for their transport. By pull-down experiment and immunoprecipitation assay, he demonstrated that APP formed a complex with ApoER2 and the APP adapter molecules X11α and X11β. Tang also provided evidence that ApoER2 mediates Aβ production. If ApoER2 was overexpressed, alone, in N2a-APPswe cells, Aβ secretion was reduced. However, if ApoER2 was expressed in the presence of ApoE or very low-density lipoprotein (VLDL) in N2a-APPswe cells, Aβ secretion was enhanced. This led to the suggestion that a protein complex of ApoE, ApoER2, X11, and APP undergoes endocytosis to endosomes and enhances Aβ production.

Edward Lee, Hospital of the University of Pennsylvania, presented the paradoxical finding of inhibition of amyloid deposition following greater than a sevenfold overexpression of BACE in transgenic mice. Using biochemical markers for APP maturation, he demonstrated that BACE overexpression led to decreased axonal transport of APP and consequent depletion of synaptic Aβ in APP x BACE transgenic mouse, in vivo phospho-APP labeling, and sciatic nerve ligations. By use of a microdialysis system, he also showed that Aβ1-40 in the hippocampal interstitial fluid of the APP x BACE transgenic mouse was reduced as compared to the APP transgenic mouse. The axonal depletion of APP by BACE overexpression was not coupled with changes in other transport proteins, for example, kinesin. Finally, Lee raised the question of whether increased BACE activity might increase APP turnover in the cytosol, leading to less axonal transport and less secretion of Aβ at synapses. In the short talk slated for this session, **Michael Willem**, Adolf-Butenandt-Institute, Munich, Germany, presented evidence that BACE expression is high early in postnatal CNS. He then showed that sensory neurons of BACE1-deficient mice are hypomyelinated. Willem elucidated that the poorly ensheathed neurons resulted from changes in type 3 neuregulin-1 (NRG-1) cleavage. He pointed out that therapeutic strategies for AD using BACE inhibitors or γ-secretase inhibitors might interfere with NRG-1 function, since NRG-1 is a substrate for both secretases.

Keystone Symposia Meeting, Part 6—Tau and FTD

19 April 2006. **Lester Binder**, Northwestern University, Chicago, Illinois, described the changes in tau conformation that correlate with the stages of neurofibrillary tangle (NFT) formation. Two distinct conformations of tau, Alz50 and Alz66, can exist in the same neurons, but are separated temporally. Carboxy truncation of tau by caspase (tau-C3) facilitates tau polymer formation in vitro, but only occurs after tangles begin to coalesce in situ such that native tau→ tau Alz50 positive→ Alz50 and tau-C3 positive→ tau-C3 and Alz66 positive. Phosphorylation at S422 and tangle formation appears to predate tau truncation in situ. N-terminal truncations and modifications of tau showed that the amino terminus of tau potentiates aggregation. Thus, Binder argued that tau assembly into filamentous aggregates is governed by more than just the tau repeat regions.

Karen Duff, Nathan Kline Institute, Orangeburg, New York, described experiments employing mice overexpressing p25 crossed with mutant tau mice, as well as an inducible p25 cell line. P25/Cdk5 activity was found to influence APP processing in vitro and in vivo. Levels of BACE, C99, and Aβ were elevated in p25 mice. In p25-expressing cells, levels and activity of BACE were increased, and the synthesis rate of BACE was also altered. Several sites in the BACE promoter or 5'UTR are potentially affected by phosphorylation by p25/cdk5, including MEF2 and STAT sites. Effects of Cdk5 activity on the endosomal system may underlie altered substrate/secretase availability or activity. P25/Cdk5 can enhance both Aβ and tangle formation, but neither plaques nor tangles were seen in the p25 mice. Duff also showed that in mice, inhibition of GSK3 using lithium correlated well with reduced insoluble tau and functional decline, but not with pathological (immunohistochemical) markers. The effect of lithium was not mediated through AKT, or other Li-related pathways, since a second GSK3 inhibitor (AR) had a similar effect on insoluble tau in two different mouse models.

Eva-Maria Mandelkow, Max Planck Institute, Hamburg, Germany, described the role of tau in APP and BACE trafficking and presented data showing that tau inhibits anterograde transport of APP into axons and dendrites. However, inhibition of APP transport by tau overexpression could be rescued by MARK kinase. Tau-induced accumulation of APP in the cell body did not lead to increased levels of Aβ, and transfection with tau retarded the secretion of APP and of Aβ. Mandelkow also presented data indicating that vesicles carrying APP or BACE had different movement characteristics, and APP and BACE1 were likely carried by distinct vesicles (in disagreement with recent results reported by Larry Goldstein's laboratory at UCSD). APP was transported faster than BACE, and APP processing was not observed during the transport of APP down axons. There was no measurable cleavage of APP during vesicle transit: The ratio of N-terminal and C-terminal ends remained constant. Mandelkow also presented data in tau-inducible transgenic mice showing that tau aggregation starts in the entorhinal cortex at 3 months in transgenic mice with a proaggregation mutant. Tau proaggregation mutants aggregated early, while tau anti-aggregation mutants did not aggregate at all. Tau aggregation also progressed with age. Proaggregation mutant mice exhibited evidence for early phosphorylation of S262 tau. Aged transgenic mice revealed neuronal loss in the dentate gyrus. It was also pointed out that Mandelkow's inducible tau-δK280 mice express tau at only 1.7-fold endogenous levels, but still lead to early aggregates and disease. (In contrast, the

tau P301L mutant mice published by Karen Ashe, University of Minnesota, Minneapolis, overexpress tau at >sixfold endogenous levels.)

Michael Hutton, Mayo Clinic, Jacksonville, Florida, reviewed 34 mutations in the tau gene, MAPT, that cause of FTDP-17 in >90 families. FTDP-17 mutations can have a number of effects. Group 1 consists mainly of coding mutations that disrupt microtubule binding and enhance tau aggregation. Group 2 consists of splicing mutations that disrupt alternative splicing of exon 10 and increase four repeat (4R) tau. Mutations that increase 4R tau show clear linkage to disease; absolute increase in 4R tau (not 4R:3R ratio) appears to underlie the rate of aggregation. Hutton also demonstrated that risk for 4R tauopathies (progressive supranuclear palsey [PSP]/corticobasal degeneration [CBD]) and Parkinson disease is associated with the H1/H1 genotype of tau. The SNP rs242557 is also part of the H1 haplotype and associated with risk for PSP. This SNP sits in the LBP-1/LSF/CP2 transcription site in the tau promoter, and the risk allele (as well as the H1 allele) is associated with increased expression of tau. Using a genome-wide association study (Affymetrix 500k SNP chip) for PSP, MAPT was confirmed as a major risk factor genome-wide. In addition, association with PSP (O.R. = 2.1) was observed for SNPs on chromosome 11p11.2 contained within a single block of linkage disequilibrium spanning the genes for DNA damage binding protein (DDB2) and lysosomal acid phosphatase (ACP2).

Also from the Mayo Clinic in Florida, **Chad Dickey** introduced the hypothesis that depletion of CHIP, a tau-specific ubiquitin ligase, leads to increased abnormal tau accumulation. He showed that in CHIP-/- mice, a large subset demonstrated prenatal morbidity and marked accumulation of cerebral phospho-tau levels. Prenatal CHIP-/- mice developed marked accumulation of soluble, ubiquitin-negative, phospho-tau species. CHIP-/- mice had robust increases in phospho-tau, but no indications of aberrant folding. Caspase-3 activation was increased in CHIP-/- mice and was associated with increased apoptosis and elevated levels of caspase-3 cleavage of tau at Asp421. PAR-1/MARK2 overexpression was able to prevent CHIP from recognizing tau.

In this session's short talk, **Kun Ping Lu**, Beth Israel Deaconess Medical Center, Boston, demonstrated that knockout of the gene Pin1 on chromosome 19, which catalyzes the conversion of certain phosphorylated Ser/Thr-Pro motifs in polypeptides between cis and trans conformations, led to the accumulation of cis-pAPP, which increased amyloidogenic APP processing and increased Aβ plaque formation. Pin1 knockout also led to cis-p-tau accumulation, which resulted in hyperphosphorylated tau and tangles. Pin1 also changed the conformation of APP and tau from cis to trans. Pin1 knockout, alone or in combination with mutant APP, increased amyloidogenic APP processing in mice and elevated levels of Aβ42. These data suggest that Pin1-catalyzed prolyl isomerization may underlie a common pathogenic pathway leading to both Aβ and tau pathologies in AD.

Keystone Symposia Meeting, Part 7—Aβ Clearance

21 April 2006. **David Holtzman**, Washington University, St. Louis, Missouri, discussed the roles of ApoE and its three major isoforms—ε2, 3, and 4—on Aβ aggregation and clearance in FAD mutant APP mice expressing various ApoE isoforms. Hippocampal Aβ levels were suppressed in PDAPP transgenic mice expressing human ApoE isoforms, while the absence of ApoE resulted in altered half-life (50 percent increase) of interstitial fluid Aβ (normal half-life is ~2 hours). Holtzman suggested that ApoE plays a role in the transport and clearance of soluble Aβ. He also showed a role of LDLR (but not LRP) in regulating ApoE levels in the CNS of human ApoE knock-in mice. Finally, he presented data showing that ABCA1, which normally lipidates apoprotein with HDL, also lipidates ApoE in CNS. In ABCA1-/- mice, ApoE levels were decreased while ApoE mRNA levels were unchanged. Holtzman suggested that the lipidation state of ApoE by ABCA1 may influence amyloidogenesis.

Berislav Zlokovic, University of Rochester, New York, highlighted the importance of regulation of Aβ clearance across the BBB via efflux of soluble Aβ by LRP binding and its re-entry into brain via RAGE in AD and other neurodegenerative disorders. He showed that Dutch/Iowa mutant form Aβ possessed low affinity for LRP1 and was poorly cleared in the transgenic mouse. When APPswe+/- mice were treated from 6-9 months with secreted LRP (sLRP) daily (40 ug/kg, S.C.), performance on a visual memory task was improved. The mechanism of action is believed to involve sLRP binding Aβ in plasma, preventing re-entry into brain. In addition, Zlokovic made the point that RAGE-dependent Aβ transport induces neurovascular stress, while RAGE blockage improved cerebral blood flow in Tg2576 mouse. A screen of CHO cells showed that tertiary amides could be used to block RAGE-Aβ interactions with high affinity, thus suggesting prospects for therapeutic intervention.

Louis Hersh, University of Kentucky, Lexington, presented recent studies of NEP and IDE as therapeutic targets for AD based on enhancing Aβ degradation. Neprilysin and IDE activities are decreased in AD brain, and both enzymes are sensitive to oxidation. Neprilysin contains steroid hormone response elements, and ovariectomy decreased neprilysin activity in mice after 45 days. This could be rescued with estrogen. Lentiviral transduction of NEP into CHO cells lowered extracellular Aβ42, and its administration into APP transgenic mice led to a decrease in Aβ deposits and dissolution of preformed amyloid deposits. Hersh also showed evidence that dynorphin B9 could allosterically induce IDE catalytic activity specifically toward Aβ without changing its insulin-degrading activity, thus supporting IDE as an attractive therapeutic target. Additionally, he provided data that a newly identified small molecule, NGX96992, increased Aβ-degrading activity of IDE twofold.

Matthew Townsend, Brigham and Women's Hospital, Boston, presented data regarding the neutralizing effect of the inositol derivative, AZD-103, on inhibition of long-term potentiation (LTP) by cell-derived oligomeric Aβ. AZD-103 does not destabilize Aβ oligomers but abrogates their effects on LTP before they come into contact with cells (not afterward). One possibility is that AZD-103 prevents Aβ oligomers from binding synaptic membranes. The rescue of LTP by AZD-103 was specific to Aβ and AZD-103 showed no overt toxicity, suggesting its potential benefit for AD therapy.

Keystone Symposia Meeting, Part 8—Animal Models and Therapeutics; Therapeutics and Imaging

Animal Models and Therapeutics

Cynthia Lemere, Brigham and Women's Hospital, Boston, discussed the Aβ vaccine approach to AD and progress toward developing a potential vaccine for AD that is both safe and efficacious. She provided an overview of the results of intranasal, subcutaneous, and transcutaneous immunizations using two types of Aβ1-15 immunogens (including dendromeric forms) with various adjuvants, including LT(R192G). She highlighted the promising outcomes, such as high antibody titers, reduced cerebral insoluble Aβ42 level, and lower plaque burden, when using the new immunogens together with various adjuvants. She also warned of recent reports that passive immunization with Aβ could lead to cerebral hemorrhage, especially in cases with high levels of congophylic amyloid angiopathy.

Charlie Glabe, University of California, Irvine, presented data from immunization of triple transgenic mutant mice with oligomeric Aβ antigen covalently coupled to colloidal gold. He indicated several advantages of immunization with the Aβ oligomer over fibrillar Aβ antigen. These included much more restricted immune response specifically to oligomers, a lower inflammatory response in terms of microglial activation, and equally effective reduction of amyloid deposition and improvement of behavioral performance. Targeting amyloid oligomers was suggested to be an effective strategy for developing an AD vaccine. Glabe also presented data on his pan-anti-oligomeric antibody, A11, which is able to detect many different soluble amyloidogenic oligomers (but not monomers) and prevent their toxicity.

Dora Kovacs, Massachusetts General Hospital, Boston, focused on the link between cholesterol and AD pathogenesis. She presented a review of the therapeutic effects of statins and ACAT inhibitors (CP-113,818 and CI-1011) on Aβ generation and Aβ pathology in a transgenic mouse model and cell-based assays. By providing 2D gel data showing decreased APP-binding of ER proteins upon CP-113,818 treatment, she illustrated that lack of ACAT activity perturbed APP processing in the ER, perhaps degrading a fraction of immature APP. She also showed that ACAT inhibitors reduce amyloid load more effectively than do statins in the transgenic mice. In exploring the mechanism by which ACAT inhibition and the resulting decrease in cholesteryl esters lowers Aβ generation, Kovacs found that ACAT inhibition retained APP back at the ER, thus impairing maturation and trafficking through the secretory pathway. This results in diminished secretion of APPs and Aβ. Kovacs also described a novel cleavage of APP in the N-terminus in response to cholesteryl ester levels. While searching for proteases that might carry out this clip, Kovacs discovered that HtrA2 was implicated. It was originally found as an APP interactor that preferentially binds immature APP holoprotein. Levels of immature APP were also found to be increased in HtrA2 knockout cells.

Abraham Fisher, Israel Institute for Biological Research, Ness Ziona, Israel, described the effects of AF267B, an M1 muscarinic agonist, on AD pathology in triple transgenic AD mice. AF267B is CNS-penetrable and attenuated all three major pathological hallmarks of AD in the triple transgenic mice: interneuronal and extraneuronal accumulations of Aβ, tau hyperphosphorylation, and cognitive impairment. After pointing out the uncertainty of the AD etiology and therapeutic need to target all AD hallmarks, Dr. Fisher suggested that AF267B might be useful

for both prevention and treatment of AD. The drug would not only treat the symptoms of AD (by ameliorating the cholinergic pathway), but based on the new transgenic data, could potentially delay AD progression by reducing Aβ and tau pathologies. The M1 agonist is now being tested in phase 1 clinical trials for AD (under the name of NGX267) by TorreyPines Therapeutics, Inc, La Jolla, California.

Steven Jacobsen, Wyeth Research, provided an overview of the results of passive immunization against Aβ in PDAPP transgenic mouse. Clearance of amyloid plaques was detected by treatment with 12A11, an anti-Aβ monoclonal antibody (mAβ), but not by mAβ 266. To assess effects on cognition, contextual fear conditioning (CFC) was performed, and revealed that contextual memory (CM) deficits occurred in the pre-plaque and the plaque-bearing transgenic mice; mAβ 266 led to acute reversal of the CM deficits. Effects of the anti-Aβ mAβs on improving CM deficits were different depending on the epitopic region, with mAβs directed at the N-terminal portion of Aβ showing the most efficacy.

In the session's short talk, **Thomas Bayer**, University of Saarland, Germany, presented the relationships of intraneuronal Aβ levels, neuron loss, and axonopathy in an APP/PS1 transgenic mouse model. He indicated that plaques did not influence neuronal integrity, while Aβ deposits led to intracellular Aβx-42 aggregates, suggesting a revised amyloid hypothesis.

Therapeutics and Imaging

Eddie Koo, University of California at San Diego, provided an overview of the role of nonsteroidal anti-inflammatory drugs (NSAIDs) in treating AD, demonstrating that a subset of NSAIDs modulate γ-secretase activity by reducing Aβ42, while increasing Aβ38. These effects required relatively high concentrations of NSAIDs and were found to be independent of COX inhibition, the primary mode of action of this family of compounds. NSAIDs had no effect on AICD, NICD, or other γ-secretase substrates at modulatory concentrations. Using PS1 mutations, in vitro γ-secretase assays, and FRET/FLIM, Koo showed that the effect of NSAIDs on Aβ42 might be mediated at the γ-secretase/substrate level, with some caveats. Koo also presented data from recent Phase 1 and 2 trials of the R-enantiomer of flurbiprofen, flurizan (Myriad). This NSAID analog with marginal COX-inhibitory activity modulated Aβ42 levels in cells and animal studies. However, in Phase 1, there was no effect on the measurements of Aβ in CSF (pre- and post-drug), perhaps due to the time of lumbar puncture. In Phase 2, 207 subjects treated for one year showed about a 30 percent slowing in cognitive decline in the 1,600 mg dosing (highest dose) cohort; mild AD patients benefited, while moderate-stage AD patients did not. Mild patients also had statistically significant benefits in Activities of Daily Living and Global Function. A larger trial is currently underway.

Christoph Hock, University of Zurich, Switzerland, provided an update on biomarkers for AD. He summarized the use of measurements of CSF-Aβ, plasma-Aβ, β-amyloid in brain by PET, absolute rCBF, and tau proteins as current biomarkers for AD. CSF levels of Aβ42 were low in AD, while both total tau and phosphorylated tau were increased. However, sensitivity and specificity of these biomarkers, even when combined, did not surpass clinical diagnostic accuracy using psychological testing. Reliable biomarkers for sporadic AD in plasma are not yet available, but strategies to improve diagnostic accuracy include multiplexing arrays, proteomic patterns, and discovery, as well as combinations with imaging measures, are being utilized.

Potential proteomic biomarkers mentioned include ApoA1, truncated transthyretin, and glutathione-conjugated transthyretin. Hock also pointed out that auto-antibodies to cross-linked Aβ peptide species (CAPS), which have been reported to be decreased in AD plasma, are worthy of further study. He concluded that direct in vivo measurement of brain β-amyloid and rCBF by PET and brain volume by MRI will also be potentially useful in diagnostic and therapeutic evaluation of AD.

Chester Mathis, University of Pittsburgh Medical Center Presbyterian Hospital, presented on in vivo imaging of β amyloid in brain using 6-OH-BTA-1, or Pittsburgh Compound B (PIB). 6-OH-BTA-1 fulfilled the properties needed for an Aβ PET radioligand: It bound selectively to and had a high affinity for Aβ; it crossed the blood-brain barrier well; it had rapid nonspecific clearance; it had no radio-labeled metabolites in the brain; and it worked in vivo in animal models. In human AD brains, PIB appeared in expected gray matter areas and was absent where there was no amyloid. In contrast, in control brains very little PIB retention was observed with an absence of retention in gray matter. PIB retention controls had no overlap between control and AD groups, but MCI subjects spanned the control and AD groups. Unusual patterns of PIB retention were observed in FAD cases and in Down syndrome, perhaps due to preference of the compound for cerebral blood vessel amyloid.

Ward Pedersen, Creighton University Medical Center, described the occurrence of insulin resistance in aged Tg2576 APPswe mice as a rationale for treating them with rosaglitazone, a thiazolidinedione used to treat insulin resistance in diabetics. He demonstrated that rosaglitazone attenuated the learning and memory deficits of Tg2576 mice. Interestingly, metyrapone, an inhibitor of glucocorticoid production, mimicked the effects of rosaglitazone on the learning and memory performance of Tg2576 mice, in a reversible fashion. Rosaglitazone also normalized IDE mRNA levels in the hippocampi of Tg2576 mice, and reduced Aβ42, but not Aβ40, levels with no effect on amyloid plaque burden. Rosiglitazone is now being tested in clinical trials for AD.