Chicago: 2nd Annual Symposium Explores ApoE

26 July 2011. Apolipoprotein E (ApoE) gene alleles are the strongest genetic modulators of late-onset Alzheimer's disease (AD) risk. Some 60 percent of AD individuals carry at least one copy of the ApoE4 variant. ApoE alleles have also been implicated in multiple processes in the periphery. "The 2cd Annual ApoE, ApoE Receptors and Neurodegeneration Symposium: ApoE in the CNS and Periphery" brought together researchers working on the central nervous system and the periphery to address emerging areas of ApoE research. The conference, held 6-7 June 2011 at the University of Illinois, Chicago, was organized by members of the NIA/NIH program project grant (PPG) on ApoE Receptor Biology and Neurodegeneration. The take-home message was twofold: Insights can be gained in the role of ApoE in the CNS by considering the actions of ApoE in the periphery, and whether ApoE4 represents a loss or a gain of function is a critical question that may be addressed using compounds that upregulate expression of the protein in vivo.

The meeting was also a great opportunity for cross-disciplinary fertilization. Perhaps one of the best examples was the Monday morning keynote lecture by **Ted Mazzone** from the University of Illinois, Chicago. Mazzone discussed the role of ApoE alleles in adipocytes and macrophages in the periphery. Within adipose tissue, ApoE is expressed by both these cell types. Adipocyte ApoE expression is regulated by multiple factors, including PPARγ agonists, hyperglycemia, oxidative stress, and obesity, said Mazzone. In turn, adipocyte ApoE is a key regulator of inflammatory cytokine release from adipose tissue, helping control peripheral inflammation. Indeed, Mazzone noted that ApoE-regulated inflammation was a critical aspect of obesity's role in cardiovascular disease. Hence, multiple factors are capable of regulating ApoE expression, which in turn is a strong modulator of inflammation.

Ling Li, University of Minnesota, Minneapolis, followed with her studies involving apolipoprotein A-I (ApoA-I). Since it is a major component of high-density lipoproteins (HDL) in the periphery, ApoA-I is cardioprotective. By using an APP/PS1/ApoA-I triple-transgenic mouse model, Li found that peripheral ApoA-I was also protective for the brain; increased ApoA-I in the periphery reduced learning and memory deficits in the APP/PS1 mice, at least partly by decreasing neuroinflammation and congophilic amyloid angiopathy. Overall, Li's results suggested that agents which increase peripheral ApoA-I may reduce AD risk.

Danny Michaelson from the University of Tel Aviv, Israel, presented two lines of study. The first set focused upon ApoE involvement in age-related macular degeneration (AMD). Working with ApoE-targeted replacement (ApoE-TR) mice, he reported that the E4 variant was protective in a model of laser-induced AMD, similar to the actions of ApoE4 in human AMD. Moreover, he linked this protection to decreased vascular endothelial growth factor (VEGF) production in the injured retina; increases in VEGF and associated neovascularization are a hallmark of AMD. The second set

of studies also used ApoE-targeted replacement mice to test if deleterious actions of ApoE4 may be ameliorated by diet. By starting weaning mice on a diet rich in the polyunsaturated fatty acid, docosahexanoate (DHA), injurious actions of ApoE4 on hippocampal excitatory and inhibitory synapses, as well as behavioral deficits in an object recognition test, were reduced to levels seen in ApoE3 mice. Overall, Michaelson interpreted his data as consistent with epidemiologic evidence that populations with a higher proportion of dietary fish oil (DHA) have reduced AD incidence, and he suggested that at least a portion of this protection may reflect an inhibition of ApoE4's actions.

Mary Jo LaDu, University of Illinois, Chicago, presented data on a new transgenic mouse model: 5xFAD mice crossed with ApoE-TR mice. In the "EFAD" (not to be confused with "early-onset familial AD") mouse brains, both Aβ42 and ApoE increased in the soluble fraction in E4FAD mice compared with E2- and E3FAD mice, providing an opportunity to study the interactions between ApoE and Aβ in vivo. Intraneuronal accumulation of Aβ42 was greatest in E4FAD mice and occurred prior to plaque deposition, although this accumulation appeared to be dependent on specific regions of the hippocampus. However, total plaque burden in six-month-old E2FAD and E4FAD mice was the same. E2FAD and E3FAD animals had diffuse plaques, while E4FAD mice had more dense-core plaques, consistent with data that Aβ extracted by formic acid was greatest in E4FAD mice The ApoE2-induced plaque deposition is novel in a Tg-mouse model and supports human data that plaque load does not quantitatively correlate with dementia. Although data are limited, this difference in plaque morphology also mirrors human patients in the "oldest of the old" studies where, with comparable total amyloid deposition, plaques in non-demented ApoE2 humans were mainly diffuse, while plaques in demented ApoE4 patients were dense core. In summary, this is the first report of a Tg-mouse model in which the ApoE isoform directly influences Aβ solubility, intraneuronal Aβ levels, and extracellular plaque morphology.

The final primary speaker of the morning session was **Britto Nathan** from Eastern Illinois University, Charleston. His research focuses upon ApoE as a mediator of estrogen's actions in neuronal regeneration. Nathan presented data that 17β-estradiol upregulates ApoE expression in vitro and in vivo. Using primary neuronal cultures from the brains of adult ApoE targeted replacement mice, Nathan showed that the neurite-promoting actions of estrogen were strongest in ApoE2 cells, less robust in ApoE3, and had non-existent in ApoE4 cells. Hence, ApoE is induced by, and may mediate at least a portion of the neuroprotective actions of estrogen.

The afternoon session focused upon AD genetics and began with a presentation by **Steve Estus**, University of Kentucky, Lexington. His research seeks to elucidate the molecular phenotypes (gene expression or RNA splicing) driven by AD-associated single nucleotide polymorphisms. He showed through three vignettes that, since brain is a heterogeneous tissue in terms of cell type, normalizing for variation in the proportion of

cell types between tissue samples can facilitate the detection of gene expression changes that are associated with AD SNPs. The three vignettes involved the CLU, VLDLR, and HMGCR AD risk genes. In the last, a SNP was associated with splicing of a critical HMGCR exon; since this SNP is also associated with statin responsiveness, it may confound recent statin trials in AD.

Steven Younkin, Mayo Clinic, Jacksonville, Florida, then reviewed the current state of AD genomewide association studies. A major issue he drove home was that prevention trials can be made feasible by using a high-risk group of individuals, and that genetics, particularly ApoE genetics, can be used to define this high-risk group. Addressing the frequently asked question, "How many people want to be treated for AD after they have the disease?", he emphasized that most scientists feel that once an individual is symptomatic for AD, a complete recovery is highly unlikely. Hence, AD prevention needs to be a focus of research efforts.

David Bennett, concluded the AD genetics session. Bennett, from Rush University Medical Center, Chicago, Illinois, addressed the role of pathology in ApoE-associated AD risk. By using a statistical approach wherein ApoE alleles and AD-associated neuropathology competed to assess which was associated with cognitive decline, Bennett presented a cogent argument that the actions of ApoE alleles on amyloid deposits, and, in turn, tau pathology, are sufficient to account for most of the association of ApoE with the declining cognition.

This symposium also included a series of "Hot Topic" platform presentations by young investigators, as well as an active and well-attended poster session. The Hot Topic presenters included **Chia-Chin Liu**, Mayo Clinic, Jacksonville, Florida who showed that Wnt signaling through LRP6 modulates APP processing, and Henry King from the University of Leeds, U.K., presenting data that ApoER2 splice variants differentially affect APP cleavage in transfected cells. In her Hot Topic talk, **Laura Comley** from the University of Edinburgh, Scotland, presented data suggesting ApoE isoform-specific effects on neurite regeneration, with proteomic analyses of healthy and regenerating nerves, suggesting several proteins that may be differentially expressed in ApoE3 versus ApoE4 backgrounds. Finally, **Kanchan Garai** Washington University, St. Louis, Missouri, characterized ApoE protein behavior under the acidic conditions of the lysosome.

The second day began with a Keynote Lecture by **Karl Weisgraber** from the Gladstone Institute and the University of California, San Francisco. Weisgraber showed that ApoE4 may have a toxic gain of function that is independent of the Aβ peptide. He presented several lines of evidence to support this hypothesis. An endogenous ApoE mouse model that mimics ApoE4 (Arg-61 ApoE) had synaptic, functional, and cognitive deficits in the absence of Aβ pathology. Additionally, several indices of neuronal synaptic function were reduced in glia/neuron co-cultures from ApoE4 but not ApoE3 knock-in mice. Wiesgraber also demonstrated that ApoE4

induces the unfolded protein response in astrocytes, inducing autophagy and leading to astrocyte dysfunction. Overall, his presentation suggested that astrocytic ApoE4 induces dysfunction within the astrocytes themselves, which then leads to neuronal synaptic dysfunction. Hence, ApoE4 may act independently of amyloid in a toxic, gain-of-function fashion.

This hypothesis was developed further by **Yadong Huang**, who noted that ApoE4 is associated with a greater AD risk in women than men. Huang, from the Gladstone Institute and the University of California, San Francisco, used this observation as a foundation to evaluate ApoE4-targeted replacement mice. He found that female, but not male, E4-targeted replacement mice have a loss of hilar GABAergic neurons. This was paralleled by deficits in learning and memory. Pentobarbital or picrotoxin (both GABAA receptor antagonists),or genetic deletion of tau blocked these ApoE4 effects. Overall, Huang's data again support a model wherein ApoE4 has a toxic, gain of function, this time in a sex- and tau-dependent fashion.

The morning concluded with a talk by **Gary Landreth** from Case Western Reserve University, Cleveland, Ohio. Landreth has worked for several years to identify ApoE-directed therapeutics. As one of the genes involved in cholesterol homeostasis, ApoE is regulated transcriptionally by PPARγ and liver X receptors, which both form heterodimers with the retinoid X receptor (RXR). Working with APP transgenic mice, Landreth found that an RXR agonist, bexarotene, induces brain ApoE quickly and robustly. This induction precedes a rapid decline in brain amyloid, an increase in plaque-clearing microglia, and improvements in cognitive performance. Landreth noted that bexarotene is an FDA-approved chemotherapeutic that readily crosses the blood-brain barrier and has minimal side effects, most of which are associated with its action on lipid homeostasis. Hence, this drug minimally represents a test of the role of Aβ in AD and, maximally, may represent an AD therapeutic, he suggested. The presentation elicited a discussion of the utility of the drug in ApoE4-positive individuals, i.e., if ApoE4 represents a toxic gain of function, then bexarotene-induced increases in ApoE4 may exacerbate AD. Landreth noted that the drug has been in use for roughly a decade without reports of cognitive deficits as a side effect.

This second annual meeting on ApoE biology and pathophysiology concluded with great excitement about potential therapeutic approaches to AD that take advantage of ApoE transcriptional regulation. The research highlighted important effects of ApoE on Aβ and important effects of ApoE independent of Aβ, and clearly profited from understanding of ApoE in multiple systems. Next year's meeting at the Mayo Clinic in Jacksonville, Florida, (4-5 June 2012) will continue to focus on new aspects of ApoE.

Submitted by Steve Estus, on behalf of the members of the ApoE Receptor Biology and Neurodegeneration program project grant, including Mary Jo LaDu, Bill Rebeck, Guojun Bu, and Ed Weeber.