● **PERSPECTIVE**

ApoE2 and Alzheimer's disease: time to take a closer look

Alzheimer's disease (AD) is the most common form of dementia among the elderly. It currently affects approximately 5.1 million Americans, a number predicted to triple by 2050. AD is clinically manifested as progressive loss of memory and cognitive function, and is characterized pathologically by the formation of amyloid-beta (Aβ) plaques and neurofibrillary tangles (NFT). Since its discovery in 1906, extensive research has been undertaken to define AD pathogenesis and to develop treatments; however, the cause of AD remains largely unknown and no therapeutic success has been achieved in over 200 AD drug trials conducted in the past decade (Cummings et al., 2014). These challenges underscore the need for increased research focus to better understand AD risk mechanisms that would allow for the development of strategies aimed at AD prevention and early intervention.

Human apolipoprotein E (ApoE) is a 299-amino-acid protein with a molecular mass of 36 kDa. In the periphery, ApoE is primarily present in the liver, kidney, and spleen, where it plays a critical role in cholesterol and lipid transport and metabolism. In the central nervous system, ApoE is synthesized and secreted by astrocytes, microglia, and, to a lesser extent, neurons. Brain ApoE is involved in injury repair *via* the redistribution of lipids among neurons and the modulation of neurite outgrowth and cerebrovascular integrity. Human ApoE exists as three major isoforms, ApoE2, ApoE3 and ApoE4, which are the products of three alleles at a single gene locus on the long arm of chromosome 19. These isoforms differ structurally by two amino acid substitutions at residues 112 and 158: ApoE2 (Cys112, Cys158), ApoE3 (Cys112, Arg158), and ApoE4 (Arg112, Arg158) (**Figure 1**). ApoE consists of two functional domains joined by a flexible hinge region: an amino-terminal domain that contains a highly positively charged receptor-binding region composed mainly of arginine and lysine residues; and a carboxyl-terminal domain which includes a lipid-binding region (**Figure 1**). Substitutions of two amino acid residues in the three ApoE isoforms significantly alter their receptor-binding and lipid-binding affinities (Mahley and Rall, 2000). ApoE2 has a much lower binding affinity for low-density lipoprotein (LDL) receptors compared to ApoE3 and ApoE4. Furthermore, ApoE2 and ApoE3 preferentially bind to small, phospholipid-enriched high-density lipoproteins (HDL) whereas ApoE4 preferentially binds to larger, triglyceride-enriched lipoproteins. It has been postulated that the unique domain interaction between the residue Arg61 and Glu255 might underlie the detrimental effects of ApoE4 in the brain (**Figure 1**).

Human ApoE isoforms have been shown to confer differential susceptibility to AD. As the most common isoform, ApoE3 is present in approximately 75% of the population and is believed to play a neutral role in AD. ApoE2 is relatively rare, with only 5% incidence, and is considered to be a protective variant against AD. By contrast, as the most potent genetic risk factor for AD, ApoE4 exists in only about 20% of the population; however, it is present in nearly 50% of AD patients. It is estimated that individuals who carry two ApoE2 alleles or one ApoE2 allele and one ApoE3 allele are 40% less likely to develop AD than those who carry two ApoE3 alleles; whereas, people who have one ApoE4 allele and one ApoE3 allele or two ApoE4 alleles are 3.2 or 14.9 times more likely to develop AD than those carrying two ApoE3 alleles. While an immense amount of work has been done to examine the role of ApoE in AD pathogenesis, most studies have focused on identifying AD risk mechanisms conferred by ApoE4 through comparisons between ApoE4 and ApoE3 and between ApoE4 carriers and noncarriers. Clinically, ApoE4 has been associated with the accelerated rate and severity of cognitive decline, with a lower age of onset, and with altered response to AD treatments. On the molecular level, ApoE4-expressing brains have been demonstrated to be less efficient in Aβ clearance; this might be a consequence of reduced ApoE protein quantity and reduced

affinity of ApoE for Aβ binding, resulting in impaired ApoE-mediated efflux and transport of Aβ across the blood-brain barrier (Liu et al., 2013). In addition, ApoE4 brains have been associated with greater brain atrophy, decreased cerebral glucose metabolism, impaired synaptic function, and defective hippocampal neurogenesis (Liu et al., 2013).

Comparatively few studies have explored the role of ApoE2 in relation to AD; yet, overall, the results of these studies suggest that ApoE2 is neuroprotective. AD patients that carry ApoE2 are found to exhibit significantly reduced Aβ deposition in the neocortex (Nagy et al., 1995). In addition, ApoE2-expressing AD brains appear to express less NFT formation (Morris et al., 1995), although other studies demonstrate the opposite outcome (Berlau et al., 2009). These conflicting observations of the influence of ApoE2 on pathological manifestation in AD could be explained, in part, by the age difference of the test subjects in the studies. In addition, the protective effects of ApoE2 might vary with the stage of AD; the protection exerted by ApoE2 might occur only in the early stages of the disease but is masked in late-stage AD due to severe neuronal loss. ApoE2 has also been positively associated with cognitive functions in aging. An 8-year-long follow-up study in a large cohort of elderly, dementia-free subjects demonstrated that individuals who possess at least one ApoE ε2 allele (ε2/2 and ε2/3) exhibit improved episodic memory performance. By contrast, a decline in performance was found in subjects with the ε3/3 genotype, and a sharper decrease was found in those with at least one ApoE ε4 allele (Wilson et al., 2002). Consistent with an earlier study that found that children and adolescents who possess ApoE ε2 have the thickest entorhinal and medial temporal cortex (Shaw et al., 2007), results from the Alzheimer's Disease Neuroimaging Initiative (ADNI) study revealed that ApoE2 carriers have larger hippocampal volume and reduced hippocampal atrophy rate compared to the noncarriers (Chiang et al., 2010). Collectively,
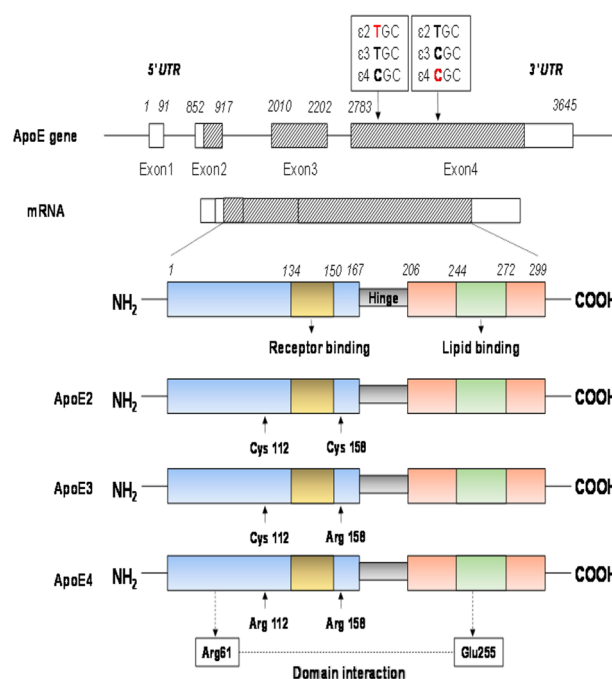

Figure 1 The schematic representation of the structural and functional domains of human apolipoprotein E (ApoE) isoforms.
Human *ApoE* gene is composed of four exons interrupted by three introns (pattern box: coding region; open box: untranslated region). The ApoE2, ApoE3 and ApoE4 isoforms are coded by ε2, ε3 and ε4 alleles of the *ApoE* gene, respectively. The isoforms differ from each other in two amino acid substitutions at residues 112 and 158 resulting from C→T or T→C point mutation in exon 4 as indicated. ApoE contains two functional domains: the N-terminal domain containing the receptor-binding region (residues 134–150) and the C-terminal domain containing the lipid-binding region (residues 244–272). The unique domain interaction between Arg61 and Glu255 in ApoE4 might underlie its detrimental effects in the brain.

these findings suggest that ApoE2 may play a positive role in preserving the structural integrity of the brain, which could account for its cognition-favoring properties in aging brains as well as for the increased resistance to pathological development in early-stage AD brains.

In addition to ApoE genotype, gender/sex is another variable risk factor for AD. Females have a greater lifetime risk of developing AD and constitute two-thirds of the current AD population; however, the mechanisms underlying the gender bias in AD remain poorly understood. Our research has recently demonstrated that female and male brains follow profoundly dissimilar trajectories as they age. Compared to male brains, female brains undergo a much earlier age-associated transition that could be associated with the onset of reproductive senescence. These early changes in female brains, including perturbed insulin-like growth factor 1 (Igf1) signaling and reduced mitochondrial bioenergetics, signal the onset of a hypometabolic phenotype, which, if not corrected, may predispose females to a weakened defense state against other age-related neurodegenerative stressors and thus put them at increased risk for the development of AD (Zhao et al., 2015). Additionally, increasing evidence indicates that sex interacts with ApoE genotypes to modify the risk for AD. A recent analysis of a multisite, longitudinal aging and dementia dataset, involving a total of 8,084 subjects (5,496 healthy controls and 2,588 mild cognitive impairment (MCI) patients), found that the risk of clinical conversion associated with ApoE4 was significantly greater for women than for men, and that ApoE4 and female sex interaction was present in both the conversion from healthy aging to MCI and in the conversion from MCI to AD. A significant interaction between ApoE2 and sex was also revealed in these analyses, in which a protective role of ApoE2 was detected in male but not female subjects (Altmann et al., 2014).

While there is an abundance of research demonstrating the neurodegenerative impact of the ApoE4 genotype, far less is known about the mechanisms by which ApoE2 exhibits neuroprotection. To address this research gap, our laboratory has recently initiated a series of novel studies designed to identify the differences at the molecular level that separate ApoE2 brains from ApoE3 and ApoE4 brains, which could contribute to the neuroprotective properties of ApoE2. Our recent analyses have demonstrated that human ApoE isoforms differentially modulate brain Igf1 signaling and downstream glucose uptake and metabolism. Compared to ApoE3 and ApoE4 brains, ApoE2 brains exhibited the most bioenergetically robust profiles, providing a possible mechanism whereby ApoE2 promotes neuroprotection (Keeney et al., 2015). Moreover, we have recently demonstrated for the first time that the three ApoE isoforms differentially modulate a key component of the catalytic domain of the V-type H^+-ATPase (Atp6v), a proton pump that mediates the concentration of neurotransmitters into synaptic vesicles and thus plays a crucial role in synaptic transmission. Specifically, our data demonstrate that ApoE2 brains express significantly higher levels of the beta subunit of Atp6v when compared to both ApoE3 and ApoE4 brains, providing a mechanistic rationale for the positive impact on cognitive function conferred by ApoE2 (manuscript under review). Taken together, our data indicate that the three ApoE brains are significantly different in two major areas—bioenergetically and synaptically—and that a more efficient and robust status in both areas may underlie the neuroprotective and cognition-favoring properties associated with ApoE2.

In summary, in the past 20 years, ApoE2 has been increasingly recognized as a neuroprotective variant; however, the underlying mechanisms have been largely unexplored. Our recent findings offer new perspectives for further in-depth studies that will increase our understanding of the roles of ApoE2 and of how ApoE genotypes interact with sex to modulate the adaptation and defense mechanisms in the aging brain. We propose a therapeutic approach that can possibly convert an ApoE4 brain into an ApoE2-like brain has the potential to reduce the risk of developing AD. Such an approach could be achieved *via* genetic modification, structural correction, or functional modulation. The rationale for currently attempted methods of genetic modification would be to introduce the ApoE2 gene using viral vectors, and as a result, offset some of the neurotoxic effects of ApoE4 in the brain. Similarly, the idea of structural correction would be to physically change the structure of the ApoE4 protein and make it behave more like the ApoE2 protein. However, the goal of functional modulation would be to potentiate the neuroprotective mechanisms conferred by ApoE2 thereby increasing the brain's natural ability to fight against AD, which, in our view, could represent a relatively safer and easier-to-accomplish strategy than the first two particularly in a chronic treatment regimen. Impaired glucose metabolism is associated with AD beginning in the earliest stages, perhaps even before synaptic dysfunction and long before onset of clinical symptoms. Based on our recent work, we are currently testing the hypotheses that bioenergetic robustness could serve as a major mechanism whereby ApoE2 delegates neuroprotection; and enhancing brain energy metabolism could hold promise for preventing or delaying the onset in an aging brain – in particular an ApoE4 brain – of AD.

Long Wu, Liqin Zhao[*]

Department of Pharmacology and Toxicology, School of Pharmacy (Wu L, Zhao L), Neuroscience Graduate Program, University of Kansas, Lawrence, KS, USA (Zhao L)

*Correspondence to: *Liqin Zhao, Ph.D., lzhao@ku.edu.*

References

Altmann A, Tian L, Henderson VW, Greicius MD (2014) Sex modifies the APOE-related risk of developing Alzheimer disease. Ann Neurol 75:563-573.

Berlau DJ, Corrada MM, Head E, Kawas CH (2009) APOE epsilon2 is associated with intact cognition but increased Alzheimer pathology in the oldest old. Neurology 72:829-834.

Chiang GC, Insel PS, Tosun D, Schuff N, Truran-Sacrey D, Raptentsetsang ST, Jack CR, Jr., Aisen PS, Petersen RC, Weiner MW, Alzheimer's Disease Neuroimaging I (2010) Hippocampal atrophy rates and CSF biomarkers in elderly APOE2 normal subjects. Neurology 75:1976-1981.

Cummings JL, Morstorf T, Zhong K (2014) Alzheimer's disease drug-development pipeline: few candidates, frequent failures. Alzheimer Res Ther 6:37.

Keeney JT, Ibrahimi S, Zhao L (2015) Human ApoE isoforms differentially modulate glucose and amyloid metabolic pathways in female brain: evidence of the mechanism of neuroprotection by ApoE2 and implications for Alzheimer's disease prevention and early intervention. J Alzheimers Dis 48:411-424.

Liu CC, Kanekiyo T, Xu H, Bu G (2013) Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. Nat Rev Neurol 9:106-118.

Mahley RW, Rall SC, Jr. (2000) Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet 1:507-537.

Morris CM, Benjamin R, Leake A, McArthur FK, Candy JM, Ince PG, Torvik A, Bjertness E, Edwardson JA (1995) Effect of apolipoprotein E genotype on Alzheimer's disease neuropathology in a cohort of elderly Norwegians. Neurosci Lett 201:45-47.

Nagy Z, Esiri MM, Jobst KA, Johnston C, Litchfield S, Sim E, Smith AD (1995) Influence of the apolipoprotein E genotype on amyloid deposition and neurofibrillary tangle formation in Alzheimer's disease. Neuroscience 69:757-761.

Shaw P, Lerch JP, Pruessner JC, Taylor KN, Rose AB, Greenstein D, Clasen L, Evans A, Rapoport JL, Giedd JN (2007) Cortical morphology in children and adolescents with different apolipoprotein E gene polymorphisms: an observational study. Lancet Neurol 6:494-500.

Wilson RS, Bienias JL, Berry-Kravis E, Evans DA, Bennett DA (2002) The apolipoprotein E epsilon 2 allele and decline in episodic memory. J Neurol Neurosurg Psychiatry 73:672-677.

Zhao L, Woody SK, Chhibber A (2015) Estrogen receptor β in Alzheimer's disease: From mechanisms to therapeutics. Ageing Res Rev 24:178-190.