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APOE in the Normal Brain

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Abstract

The APOE4 protein affects the primary neuropathological markers of Alzheimer's disease (AD): amyloid plaques, neurofibrillary tangles, and gliosis. These interactions have been investigated to understand the strong effect of *APOE* genotype on risk of AD. However, *APOE* genotype has strong effects on processes in normal brains, in the absence of the hallmarks of AD. We propose that CNS APOE is involved in processes in the normal brains that in later years apply specifically to processes of AD pathogenesis. We review the differences of the APOE protein found in the CNS compared to the plasma, including post-translational modifications (glycosylation, lipidation, multimer formation), focusing on ways that the common APOE isoforms differ from each other. We also review structural and functional studies of young human brains and control *APOE* knock-in mouse brains. These approaches demonstrate the effects of *APOE* genotype on microscopic neuron structure, gross brain structure, and behavior, primarily related to the hippocampal areas. By focusing on the effects of *APOE* genotype on normal brain function, approaches can be pursued to identify biomarkers of APOE dysfunction, to promote normal functions of the APOE4 isoform, and to prevent the accumulation of the pathologic hallmarks of AD with aging.

Keywords

APOE; apolipoprotein; lipoprotein; inflammation; glycosylation; mouse model; functional MRI

Introduction

For the past 25 years, a great deal of research has examined *APOE* genotype in the context of its profound effect on the risk of Alzheimer's Disease (AD) (Strittmatter et al., 1993). In this time, a literature has also developed on *APOE* genotype in the context of normal brain function (Di Battista et al., 2016; Iacono and Feltis, 2019; Wisdom et al., 2011). Knowledge of the effects of *APOE* genotype prior to AD could provide insight into normal cognitive strengths and weaknesses of individuals based on their *APOE* genotypes as well as their later risks of cognitive dysfunctions. As more people make use of commercial DNA

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sequencing tools (Campion et al., 2019) and discover their *APOE* genotypes at young ages, public interest in the effects of *APOE* genotype throughout life will increase.

Knowledge of the effects of *APOE* genotype in the brain is based in part on its effects on neural dysfunction. In addition to the long history with late onset AD (Raber et al., 2004), *APOE* associations been observed in other conditions, such as risk of Diffuse Lewy Body Disease (Hansen et al., 2019), recovery from traumatic brain injuries (TBI) (Kassam et al., 2016; Merritt et al., 2018), recovery from stroke (Cramer et al., 2012; Wagle et al., 2009), and risk of cognitive impairment after chemotherapy (Buskbjerg et al., 2019; Mandelblatt et al., 2018) or HIV infection (Chang et al., 2014). These various findings are supported in preclinical studies, including mouse models of TBI (Main et al., 2018), stroke recovery (Lei et al., 2012), and chemotherapy-induced cognitive impairment (Speidell et al., 2019). These conditions support a model in which *APOE* genotype effects in normal brain create conditions that make adverse responses to injury more likely (Mahley and Huang, 2012). In this context, aging can be considered a condition of accumulating brain damages that are affected by *APOE* genotype: populations of the oldest old show increased prevalence of the *APOE2* allele and decreased prevalence of the *APOE4* allele (Garatachea et al., 2015; Rebeck et al., 1994; Revelas et al., 2018; Schachter et al., 1994; Sebastiani et al., 2019).

Our overall hypothesis is that CNS APOE is involved in processes in the normal brain that in later years apply to processes of AD pathogenesis. In normal brain, these processes are related to clearance of debris for homeostasis, inhibition of inflammation, and promotion of neuronal network resilience (Figure 1A). In AD brain, these processes are related to clearance of Aβ oligomers, glial activation in response to protein aggregates, and neuronal dysfunction and death (Figure 1B).

In this review, we will first consider the APOE protein that is present in the central nervous system (CNS); this form differs in important ways from APOE found in the periphery. We will then synthesize data on the effects of *APOE* genotype on brain structure and function in the absence of signs of AD pathogenesis. Finally, we will speculate on ways that the structure of CNS APOE could be related to some of the observed effects of *APOE* genotype on CNS structure and function. These observations of how *APOE* genotype predisposes brains to damage are particularly important because they will direct the development of prevention methods for conditions such as AD. Furthermore, they will help in the targeted identification of biomarkers that can be used to test prevention approaches that do not rely on the phenotypes observed in the later stages of AD, such as cognitive impairment and the accumulations of the pathogenic proteins Aβ and phospho-tau.

CNS APOE protein structure

APOE is present both in the CNS and in the periphery, although the structure of the protein is different in these two systems. The mature APOE protein is 299 amino acids with a single amino acid substitution defining each of the three common isoforms: APOE2 (Cys112, Cys158), APOE3 (Cys112, Arg158) and APOE4 (Arg112, Arg158) (Rall et al., 1982) (Figure 2). The rare Christchurch variant consists of a Ser136 variant (Wardell et al., 1987). APOE has three main domains: an N-terminal, four helix, receptor binding domain; a C-

terminal, triple helix, lipid binding domain; and an intervening flexible hinge region (Chen et al., 2011; Lalazar et al., 1988; Nguyen et al., 2010; Sakamoto et al., 2008). In the periphery, APOE is synthesized and secreted by hepatocytes (Mahley, 1988) and macrophages (Kockx et al., 2008), and is involved in the HDL, exogenous, and endogenous cholesterol metabolism pathways. It associates with a wide array of varied lipoproteins, ranging from small (7–14 nm) plasma HDL particles (Otvos, 2002) to the larger (30–100 nm) and polyhedral VLDL particles (Yu et al., 2016), to the very large (75–1200 nm) chylomicrons (Dawson and Rudel, 1999; Mahley and Ji, 1999; Patsch, 1998). It functions in the transport of lipoproteins and regulation of plasma lipid levels, with additional functions such as immune modulation (Bennet et al., 2007; Mahley, 1988; Sing and Davignon, 1985; Tenger and Zhou, 2003; Vitek et al., 2009).

APOE CNS lipoproteins

APOE is the most abundant apolipoprotein in the brain, although other apolipoproteins are also present, including abundant APOA-I and less abundant apolipoproteins including APOA-II, APOA-IV, APOJ, APOD and APOH (Roher et al., 2009; Wang and Eckel, 2014). In the CNS, APOE is primarily expressed by astrocytes and to a lesser extent by pericytes, oligodendrocytes, choroid plexus, and neurons under stressed physiological conditions (Achariyar et al., 2016; Bruinsma et al., 2010; Nelissen et al., 2012; Pitas et al., 1987a; Xu et al., 2006). APOE is secreted by glia associated with lipids forming small (8–15 nm) discoid particles, which increase in size, becoming spherical as they accumulate lipids and flow into the CSF (12–20 nm with a fraction up to 30 nm) (Koch et al., 2001; LaDu et al., 1998; Pitas et al., 1987b). APOE lipoproteins produced in the choroid plexus are secreted directly into the CSF (Achariyar et al., 2016).

CNS APOE secretion and lipidation occurs in conjunction with the ATP binding cassette (ABC) proteins, ABCA1 and ABCG1 (Courtney and Landreth, 2016). These proteins are embedded in the cell membrane and act to pump lipid molecules into the extracellular space, where they bind apolipoproteins such as APOE and APOA-I (Tall, 2018). Like APOE, the expression of ABCA1 and ABCG1 is increased by the transcription factor LXR (either directly (Xu et al., 2013) or indirectly (Fan et al., 2018)) to promote APOE and lipid efflux (Courtney and Landreth, 2016). ABCA1 activity can also be increased through binding of specific peptides based on the sequences of APOA-I (Sherman et al., 2010) or APOE (Bielicki, 2016), leading to increased lipidation of APOE (Boehm-Cagan et al., 2016a; Chernick et al., 2018).

The three APOE isoforms, APOE2, APOE3, and APOE4, have different levels of lipidation and related functions. In CSF from both middle aged and older cognitively normal individuals, those who carried an *APOE4* allele had significantly smaller APOE containing particle distributions compared to those without an *APOE4* allele, and *APOE2.3* individuals had significantly larger APOE particle distributions (Heinsinger et al., 2016). In a study of viral construct expression of APOE2, APOE3 or APOE4, the APOE4 protein promoted the development of less lipidated APOE particles while APOE2 was more highly lipidated (Hu et al., 2015). A consistent isoform effect on lipidation is apparent in the related function of lipid efflux: APOE2 promoted significantly more lipid efflux from both astrocytes and

neurons compared to APOE3, which promoted more lipid efflux than APOE4 (Michikawa et al., 2000; Minagawa et al., 2009). Complete APOE lipidation alters the hinge region movement for access to the N-terminal receptor binding domain, according to APOE-lipid binding models (Chen et al., 2011). Thus, the level of lipidation of each APOE isoform is essential, not only for efficiency of lipid transport, but also for downstream effects involving receptor-binding interactions.

The C-terminal of APOE, from amino acid 244 onwards (from middle of Helix C2 (Chen et al., 2011)), critically affects any lipoprotein binding, driving APOE isoform specificity (Minagawa et al., 2009; Nguyen et al., 2010; Sakamoto et al., 2008; Westerlund and Weisgraber, 1993). Lipid binding differences of APOE isoforms are observed in the periphery, with APOE3 binding preferentially with the more protein-rich HDL while APOE4 binds more effectively to the lipid-rich VLDL particles (Minagawa et al., 2009; Nguyen et al., 2010; Sakamoto et al., 2008; Weisgraber, 1990). Isoform-dependent lipoprotein binding preference is due to the APOE4 protein being more dependent on the C-terminal region (273–299) for binding than APOE3 (Nguyen et al., 2010; Sakamoto et al., 2008). Variations within the most C-terminal region of the APOE4 molecule are therefore more likely to have an impact on its lipid binding properties.

APOE self-association may be another important binding-related property for CSF lipoproteins. Unlipidated APOE monomers form multimers including dimers and tetramers, and APOE can further aggregate to form fibrils. Although there is no major difference in the overall quaternary structure or stability of APOE tetramers between isoforms using a range of techniques (Garai and Frieden, 2010; Raulin et al., 2019; Wang et al., 2019), APOE4 has slight differences in two helical regions (amino acids 12–20 and 204–210) which may result in the reduced formation of tetramers (Chetty et al., 2017). These data indicate that isoform differences in tetramers are more associated with number rather than structure. There are also differences in binding domains and larger structures. The APOE4 molecule has been shown to again rely on the C-terminal domain for self-association: when amino acids 273–299 are removed self-association is lost in APOE4 but not APOE3, and APOE4 creates more homo-isomers than APOE3 (Sakamoto et al., 2008). Purified recombinant unlipidated APOE4 forms large oligomers that create fibril-like structures over time; APOE2 and APOE3 make these structures to a lesser degree over the same timeframe (Hatters et al., 2006; Raulin et al., 2019). The reduced HDL binding affinity of APOE4 may result in a larger proportion of unlipidated APOE that is more likely to aggregate (Hatters et al., 2006). The APOE4 large aggregates are more toxic to neurons than APOE2 and APOE3 aggregates (Hatters et al., 2006). Normal self-association up to tetramers, however, may be important for the construction of large complexes with lipoprotein particles able to hold at least two APOE proteins (Chen et al., 2011; Minagawa et al., 2009; Raussens et al., 2005). Consequently, changes in the C-terminal region of APOE4 may not only impact lipid-binding but also healthy oligomer formation.

APOE dimers

The APOE isoform differences at positions 112 and 158 in the N-terminal domain. They are cysteine-arginine substitutions, altering both the charge of the protein and its ability to form

cysteine-cysteine dimers (Mahley, 1988). Indeed, APOE4 contains no cysteine residues throughout the protein. Through the cysteine 112 residue, APOE can form disulfide bonds with other APOE proteins and with APOA-II proteins (Weisgraber and Shinto, 1991). As expected, CNS APOE3 isoforms in brain and CSF form APOE-APOE and APOE-APOA-II dimers, while APOE4 isoforms do not (Elliott et al., 2010; Rebeck et al., 1998), although the levels in CSF are much lower than in plasma (Weisgraber and Shinto, 1991). Dimerization at the cysteine 112 site in APOE3 negatively affects its interaction with HDL (Weisgraber, 1990), consistent with the existence of only HDL-like particles in the CSF. Levels of plasma APOE3 homo- and heterodimers correlate with HDL levels (Yamauchi et al., 2017).

APOE protein levels in the CNS

Individuals that express APOE4 have lower levels of APOE in the CNS than those that express APOE3. Some of these data derive from the study of *APOE* targeted replacement (*APOE* TR) mice (Riddell et al., 2008; Sullivan et al., 2011; Vitek et al., 2009). These mice express *APOE* alleles from the endogenous mouse *APOE* promoter (Sullivan et al., 1997), with the expected glial expression of APOE isoforms (Sullivan et al., 2004). This glial expression pattern is consistent with the observations from a mouse model of GFP expression under the mouse *APOE* promoter (Xu et al., 2006). *APOE4* TR mice have the lowest levels of APOE and *APOE2* TR mice the highest APOE levels in: frontal cortex brain extracts (Riddell et al., 2008); hippocampus brain extracts (which had overall more APOE than the frontal cortex (Riddell et al., 2008)); CSF (Fryer et al., 2005; Riddell et al., 2008), and interstitial fluid (Ulrich et al., 2013). Primary astrocytes grown alone show these same trends with APOE4 astrocytes exhibiting reduced APOE secretion compared to APOE3 astrocytes (Riddell et al., 2008).

Findings in the *APOE* TR mouse model are supported by studies in humans, with *APOE2* alleles having a positive impact on APOE protein concentration in the CSF and *APOE4* alleles having a negative impact. The CSF from *APOE2.3* individuals had the highest levels of APOE, and *APOE3.4* and *APOE4.4* individuals had the lowest levels (Cruchaga et al., 2012). This same trend has been found in other analyses of CSF APOE concentration (Castellano et al., 2011). A genome wide association study has shown that of the *APOE* genotype has a strong ($p=6.9\times10^{-13}$) association with CSF protein level and no other SNP reached genome wide significance (Cruchaga et al., 2012). Finally, astrocytes derived from lines of inducible pluripotent stem cells also demonstrated higher levels of cellular and secreted APOE3 than APOE4 (Lin et al., 2018).

APOE glycosylation

APOE is an *O*-glycoprotein that was initially shown to hold glycosylation at a site in the hinge region (Thr194) (Wernette-Hammond et al., 1989), but has since been shown to also hold glycosylation at sites within the N-terminus (Thr8 and Thr18), the C-terminus (Thr289, Ser290 and Ser296), and at a second site (at low abundance) within the hinge region, Ser197 (Flowers et al., 2019; Halim et al., 2013; Lee et al., 2010; Nilsson et al., 2009; Steentoft et al., 2011). Although identification of the attached glycan is a more technical challenge, APOE holds predominately monosialylated (Neu5Acα2–3Galβ1–3GalNAcα1-) and disialylated (Neu5Acα2–3Galβ1–3(Neu5Acα2–6)GalNAcα1-) core 1 *O*-glycan structures

(Flowers et al., 2019). APOE in the cell is more heavily glycosylated than the secreted forms (Lee et al., 2010; Zannis et al., 1986) and APOE from the CSF is more highly glycosylated compared to APOE isolated from the plasma (Flowers et al., 2019; Pitas et al., 1987c; Rebeck et al., 1998). Normal human CSF holds ten times more abundant glycosylation within the C-terminal lipid-binding domain (CSF 37.8%, Plasma 3.7%), and also holds a higher proportion of larger disialylated core 1 glycans compared to plasma derived APOE (Flowers et al., 2019). Plasma APOE, on the other hand, holds greater glycosylation on the N-terminal domain sites (CSF 0.2%, Plasma 15.8%). Finally, while the hinge domain glycosylation was more similar for both plasma and CSF derived APOE, the CSF APOE held more abundant glycosylation (CSF 26.8%, 11.4% plasma) (Flowers et al., 2019). These analyses have important implications for the binding properties of the APOE from these two compartments, with plasma APOE holding little glycosylation in the C-terminal lipid domain and having a more diverse lipoprotein binding profile. The CSF APOE, on the other hand, binds only the small HDL particles and has higher abundance of C-terminal glycosylation. These observations suggest that C-terminal APOE glycosylation may tailor the disparate lipoprotein binding requirements in the two compartments. In support of this hypothesis, when sialylation was removed from APOE with a neuraminidase that removes α2–3 linked and α2–6 linked Neu5Ac (the linkages since confirmed to be common on APOE (Flowers et al., 2019)), the de-sialylated APOE binding to HDL was more detrimentally impacted than VLDL binding (Marmillot et al., 1999). This binding deficit was then rescued by the re-addition of sialic acid, confirming the importance of complete normal glycosylation including sialylation to effective HDL binding (Marmillot et al., 1999).

Glycosylation differences have been shown by two-dimensional electrophoresis under certain physiological conditions and with *APOE* genotype. Cells stably expressing *APOE* under a CMV promoter when loaded with cholesterol showed decreased APOE secretion, and decreased APOE sialylation (Kockx et al., 2012). It is unknown whether the APOE2, APOE3, or APOE4 variants differ in specific aspects of glycosylation in the brain. Brain samples solubilized sequentially in Tris-buffered saline (TBS) and then 1% Triton X-100, to separate soluble and membrane associated fractions, showed that *APOE4* brains, both mouse and human, held more soluble higher molecular weight APOE compared to the *APOE3* brain samples (DiBattista et al., 2016). Isoelectric focusing of the two fractions showed differences in a series of post-translation modifications, indicating that APOE *O*-glycosylation is associated with the more soluble forms of APOE in the brain (DiBattista et al., 2016). Interestingly, these modifications were also linked to neuron health: when *APOE4* TR mice were treated with an non-steroidal anti-inflammatory drug, soluble, glycosylated APOE decreased and neuronal dendritic spine density increased (DiBattista et al., 2016).

The structural differences in APOE isoforms are outlined in Figure 2, highlighting the regions that are affected by genetic variation, the lipid- and receptor-binding domains, and the glycosylation sites.

CNS APOE genotype effects

The functional consequences of the different APOE isoforms in the CNS can be inferred from the effects of *APOE* genotype on cognition and behavior before the onset of AD.

However, the *APOE4* allele is associated with an earlier appearance of amyloid as determined by amyloid PET scans (Jansen et al., 2015) and more amyloid as defined in post-mortem studies (Rebeck et al., 1993; Schmechel et al., 1993), consistent with its correlation with an earlier age of onset of AD (Corder et al., 1993). A meta-analysis of Alzheimer's Disease Neuroimaging studies showed that very many *APOE4*-positive control individuals have positive amyloid PET scans by age 60 (Jansen et al., 2015). Thus, it is likely that studies of control individuals middle-aged or older include the effects of both amyloid and *APOE4* genotype on brain structures and function. Thus, in this review, we will focus on studies of young human populations, and on mouse models with normal brain *APOE* regulation and without engineered AD pathological processes.

Human studies

Brain structure

There is a mixed literature on whether *APOE* genotype affects normal grey matter structure in younger individuals as evaluated by Magnetic Resonance Imaging (MRI) (Alexopoulos et al., 2011; Dennis et al., 2010; DiBattista et al., 2014; Filippini et al., 2009b; Matura et al., 2014; O'Dwyer et al., 2012b). Several recent studies show no *APOE* genotype-dependent effects in very young populations (Bussy et al., 2019; Lyall et al., 2019; Lyall et al., 2013; Wisdom et al., 2011; Zheng et al., 2017). Effects may be limited to specific hippocampal substructures, e.g., entorhinal cortex, or they may change substantially with normal development. Different effects associated with the *APOE4* allele have been reported in small medial temporal lobe structures in infants, children, and young adults (Chang et al., 2016; Dean et al., 2014; Knickmeyer et al., 2014; O'Dwyer et al., 2012a; Shaw et al., 2007). White matter microstructure, as measured by fractional anisotropy and white matter intensities, is impaired in *APOE4* carriers compared to non-carriers (Heise et al., 2011; Lyall et al., 2019; Westlye et al., 2012), consistent with potential *APOE4*-related problems with brain connectivity and activity.

Brain activity

Blood Oxygen Level Dependent (BOLD) contrast imaging in functional MRI is a measure of brain activity. Resting brain activity, analyzed through co-activation of the default mode networks (DMN), showed that young *APOE4* individuals have higher co-activations that include the medial temporal lobe than young *APOE3* individuals (Filippini et al., 2009a; Shen et al., 2017). *APOE* genotype effects on the DMN are not only related to *APOE4*, but include effects of *APOE2* as well (Trachtenberg et al., 2012). These effects on the DMN may be related to differences in spontaneous brain activity (Zheng et al., 2017) or lower functional connectivity (Su et al., 2017). During active encoding tasks, *APOE* genotype is also associated with altered medial temporal lobe activity with increased BOLD signal in young carriers of the *APOE4* allele compared to non-carriers (Dennis et al., 2010; Evans et al., 2017; Filippini et al., 2009a). An increased hippocampal activity in *APOE4* carriers compared to non-carriers occurred in the cognitive generation of grid-cell-like representations (Kunz et al., 2015), this signal correlates with cerebrovascular reactivity to CO_2 (Suri et al., 2015). In contrast to increased activity during encoding tasks, *APOE4*-positive individuals showed decreased medial temporal lobe activity during executive

attention compared to *APOE4*-negative individuals, which is a task dependent on frontal lobe activation (Green et al., 2014). Thus, in the unimpaired brain, *APOE4* is associated with higher levels of medial temporal lobe activity during resting state as well as during functions that depend on its efficient function.

Several studies have identified differences in measures of brain utilization of glucose and oxygen dependent on *APOE* genotype, supporting a model with *APOE4*-positive individuals are unable to efficiently regulate cerebral metabolism compared to *APOE4*-negative individuals (Brandon et al., 2018). The FDG PET measure of glucose uptake was lower in *APOE4* individuals in posterior cingulate, parietal, temporal and prefrontal cortex (Reiman et al., 2004). Post-mortem analysis of brains from young individuals show *APOE* genotype had several effects on levels of brain glucose and lactate transporters, and on mitochondrial electron transport proteins (Perkins et al., 2016). The lower glucose metabolism associated with *APOE4* may cause alterations in specific brain activities, or the lower energy metabolism may be caused by alterations in brain activities from other *APOE4*-related effects.

Behavior

Differences in brain activity and connectivity, particularly related to structures in the medial temporal lobe, may affect behaviors. However, there is a lack of consensus on behavioral effects of *APOE* genotype in young individuals. Compared to non-*APOE4* carriers, young *APOE4* carriers perform better in tasks of executive function, verbal fluency and memory (Jochemsen et al., 2012; Mondadori et al., 2007; Rusted et al., 2013). *APOE4* individuals have altered navigational behavior, consistent with differences in grid cell-like activity (Kunz et al., 2015) and decreased associative memory (Bussy et al., 2019), compared to individuals without *APOE4*. A meta-analysis from 2011 concluded that *APOE4* was associated with worse measures of episodic memory and global cognitive ability (Wisdom et al., 2011), although many of those studies included older individuals, when *APOE4*-related impairments increase (Rusted and Carare, 2015; Wisdom et al., 2011), thus potentially lessening the magnitude of direct cognitive effects of *APOE4* (Reinvang et al., 2013).

Mouse APOE knock-in model studies

Studies of mouse models of APOE complement human studies, allowing more genetically and environmentally controlled experiments (although in the absence of important factors relevant to human disease). Normal expression of specific human *APOE* isoforms in AD mouse models (e.g., *EFAD* mice (Youmans et al., 2012)), are useful in understanding how *APOE* genotype affects processes such as the deposition of Aβ (Youmans et al., 2012) or inflammatory responses to its accumulation (Rodriguez et al., 2014), and how *APOE*-related treatments alter AD pathologies (Safieh et al., 2019). However, here we will consider the effects of *APOE* genotype in mice lacking overt AD pathological changes, comparing mice expressing only *APOE4* with those expressing only *APOE3*.

Brain structure

The ease of collection of mouse brain tissue has allowed detailed studies of microscopic neuronal structures. These studies have consistently revealed that *APOE4* mice have simpler structures compared to *APOE3* mice. As demonstrated with Golgi stain analyses, *APOE4* mice had simpler neuronal dendritic arborization in the amygdala (Wang et al., 2005), cortex (Dumanis et al., 2009; Neustadtl et al., 2017), and hippocampus (Maezawa et al., 2006), including less branching or reduced spine densities. Decreased complexity of neurons in *APOE4* brains is also seen in the entorhinal cortex (DiBattista et al., 2016; Rodriguez et al., 2013), consistent with the altered function of that brain region in humans (Kunz et al., 2015). In older mice, *APOE4* is associated with fewer inhibitory neurons in the hippocampus (Andrews-Zwilling et al., 2010). *APOE4* brains have a lower vascular density, associated with white matter damage (Koizumi et al., 2018) and smaller hippocampal regions (Speidell et al., 2019). Importantly, some structural effects can be modified in ways that make *APOE4* mice more like *APOE3* mice in terms of neuronal complexity, for example with the anti-inflammatory agent ibuprofen (DiBattista et al., 2016).

Brain activity

The ease of collection of mouse brain tissue has also allowed cellular studies of neuronal activity and synaptic measures. The electrophysiology of amygdala neurons showed reduced excitatory transmission in *APOE4* mouse brain (Wang et al., 2005). There are lower levels of inhibitory tone of the *APOE4* entorhinal cortex (Nuriel et al., 2017) and hippocampal hilus (Andrews-Zwilling et al., 2010). Evoked release of acetylcholine of hippocampal neurons is lower in older *APOE4* mice (Dolejsi et al., 2016). Effects of *APOE* genotype on hippocampal neurotransmission could account for fewer short wave ripples and reduced slow gamma wave activity in aged *APOE4* mice (Gillespie et al., 2016). Thus, there are changes to neuronal activity concomitant with the changes to neuronal structures seen in *APOE4* mice.

The molecular processes behind these effects of *APOE* genotype on neuronal activities remain to be defined, but there are many effects of the endogenous APOE protein on intracellular signaling processes (Hoe et al., 2005; Huang et al., 2017; Lane-Donovan and Herz, 2017). Presynaptically, *APOE4* mice show lower glutaminase levels (Dumanis et al., 2013) and altered levels of the vesicular glutamate transporter 1 (Boehm-Cagan and Michaelson, 2014; Dumanis et al., 2013) compared to *APOE3* mice. Effects of APOE4 on neuronal activity could be mediated by its effects on the family of low density lipoprotein receptors, such as ApoER2 (Beffert et al., 2004; Weeber et al., 2002). *APOE4* mice have lower levels of ApoER2 in the CA1 and CA3 neurons of the hippocampus (Boehm-Cagan et al., 2016b; Gilat-Frenkel et al., 2014). These in vitro and in vivo studies combine to demonstrate that APOE isoforms differentially affect neuronal cell signaling.

Behavior

Effects of *APOE* genotype on mouse brain structure and activity are reflected in numerous behavioral assays. It is important to reiterate that the *APOE*-driven differences in behavior reviewed here occur in the absence of pathological changes introduced by transgenes or exogenous agents, and thus do not reflect the effects of gross AD pathological changes.

Compared to *APOE3* mice, *APOE4* mice are impaired in spatial learning as measured in the Barnes maze (Rodriguez et al., 2013; Speidell et al., 2019), the Morris Water Maze (Boehm-Cagan and Michaelson, 2014; Bour et al., 2008; Knoferle et al., 2014; Salomon-Zimri et al., 2014), and Novel Place Recognition (Grootendorst et al., 2005). They are impaired in other memory related pathways, as evidenced by Novel Object Recognition (Boehm-Cagan and Michaelson, 2014; Salomon-Zimri et al., 2014), Contextual Fear Conditioning (Boehm-Cagan and Michaelson, 2014; Salomon-Zimri et al., 2014; Segev et al., 2013) and Y-maze active avoidance (Bour et al., 2008). Several studies demonstrated that deficits were particularly observed in older *APOE4* mice (Andrews-Zwilling et al., 2010; Bour et al., 2008), which would be consistent with the increased risk of AD in older individuals. These behaviors present opportunities to alter *APOE4*-associated phenotypes in the absence of AD pathological changes, relevant for the generation of early prevention approaches. For example GABA potentiation alleviated *APOE4*-related behavioral deficits in the Morris Water Maze (Andrews-Zwilling et al., 2012) and deficits in Morris Water Maze and Novel Object Recognition were alleviated by bexarotene (Boehm-Cagan and Michaelson, 2014) and an ABCA1 agonist (Boehm-Cagan et al., 2016b).

Thus, human and mouse studies are consistent in their findings that the *APOE4* genotype affects the activity and function of the hippocampus, reflected in behavioral differences. These effects may lead to, or be exacerbated by, the presence of the various pathological changes later in life.

CNS APOE structure-function relationships

The effects of *APOE* genotype on APOE protein, APOE levels, brain structure, and brain function in normal brains are logical targets for studies on the prevention of brain dysfunction in AD (Yamazaki et al., 2016). However, linking measures in the normal brain to prevention of later AD-associated symptoms is a difficult task (Gomez-Isla and Frosch, 2019).

Increasing APOE levels could aid in the clearance of debris, inhibition of inflammation, and delivery of lipids to neurons for increased resilience (Figure 1). APOE levels are increased through activation of various transcription factors related to lipid homeostasis (Cao et al., 2007). APOE levels are further affected by recycling through neuronal endocytic pathways, with deficits in this recycling evidenced with APOE4 (Heeren et al., 2004; Xian et al., 2018). Through interactions with APOE receptors, APOE can promote neural complexity (Lane-Donovan and Herz, 2017), reduce inflammation (Pocivavsek et al., 2009), and promote debris clearance (Rasmussen et al., 2018). Importantly, APOE and APOE-derived peptides have anti-inflammatory effects (Laskowitz et al., 2017; Vitek et al., 2012) through interactions with the family of lipoprotein receptors; increasing APOE functionality could address the connections of pathological changes (Perez-Nievas et al., 2013) and genetics (Malik et al., 2015) with inflammation.

Increasing APOE lipidation in brain-specific HDL can be accomplished using ABCA1 agonists (Boehm-Cagan et al., 2016b) and perhaps through altering C-terminal glycosylation events specific to the CNS (Flowers et al., 2019). Chemical and thermal denaturation studies

demonstrate that the APOE4 monomer tertiary structure is less stable and less structured than APOE3 and APOE2, prone to a molten globule state (Morrow et al., 2002; Ray et al., 2017). The altered folding of APOE4 can be targeted with small molecules that stabilize APOE4 (Petros et al., 2019) or prevent APOE domain interactions (Wang et al., 2018). More stable and lipidated forms of APOE have conformations that also promote receptor interactions (Frieden et al., 2017).

Conclusions

The effects of *APOE* genotype on APOE modification, lipidation, or levels could influence neuronal resilience, the time course or intensity of neuroinflammation, and the homeostasis of extracellular hydrophobic molecules (Figure 1A). These functions are the same ones that are hypothesized to contribute to AD pathogenesis (Figure 1B). Approaches to address these properties of the APOE4 protein are being pursued to treat or prevent the symptoms of AD (Safieh et al., 2019). These potential treatments to address deficiencies in APOE4 positive AD patients could be developed using assays in preclinical studies of normal mice and humans. Some approaches may depend on beginning treatments in advance of marked amyloid accumulation, since there may be adverse effects of the form of APOE4, which is bound chronically to plaques (Wisniewski and Frangione, 1992). Thus, assays need to be developed to monitor characteristics of APOE and its effects in normal brain, such as state of lipidation, basal inflammation, or ability to transport hydrophobic molecules. Ideally, these measures could be based on APOE analyzed in the peripheral circulation, perhaps including studies of glycosylated APOE isoforms that may pass from the CNS to the periphery if the blood brain barrier is impaired. Overall, APOE-directed studies under non-pathological conditions are necessary for testing preventative approaches in this large population genetically at risk for AD.

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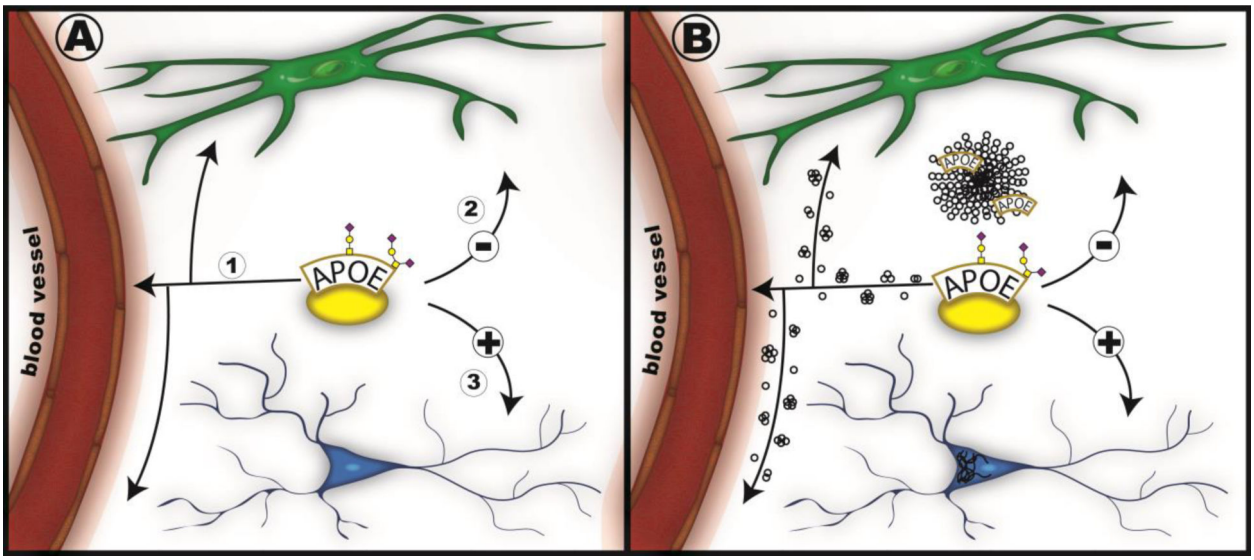

Figure 1. APOE functions in normal brain are reflected in functions in AD brain.

Secreted lipoproteins containing modified APOE are indicated as a yellow disk holding the APOE protein with two representative glycans. This CNS lipoprotein interacts with a variety of CNS cells to 1) clear debris through binding to molecules at the surface of the endothelial cells and basement membrane along CNS blood vessels (in red) and to CNS glia (in green); 2) inhibit activation of glia through signaling through cell surface receptors; and 3) promote neurite outgrowth and dendritic spine formation on neurons (in blue). In the AD brain, these functions act to promote clearance of Aβ monomers and oligomers (small collections of circles), to promote anti-inflammatory processes in response to Aβ plaques (round collection of circles containing APOE molecules), and to slow intracellular neurofibrillary tangle formation (black curved lines) and propagation.

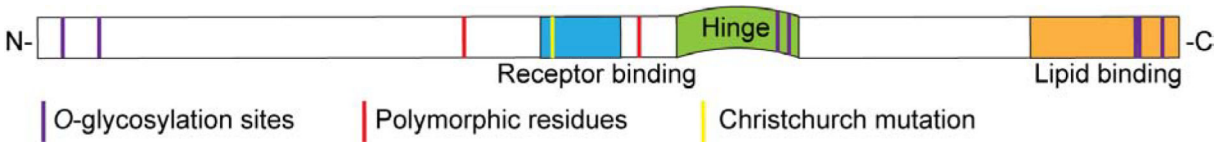

Figure 2. Structural components of APOE isoforms.

The 299 amino acid APOE protein consists of an N-terminal receptor-binding domain and a C-terminal lipid-binding domain (light orange) with an intervening flexible hinge region (green). The schematic also shows amino acids 112 and 158 (red) which determine APOE2, APOE3 and APOE4 status, and the rare Christchurch mutation at amino acid 136 (yellow). Glycosylation sites are in purple.