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Review

A Quarter Century of APOE and Alzheimer's Disease: Progress to Date and the Path Forward

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Alzheimer's disease (AD) is considered a polygenic disorder. This view is clouded, however, by lingering uncertainty over how to treat the quasi "monogenic" role of apolipoprotein E (*APOE*). The *APOE*4 allele is not only the strongest genetic risk factor for AD, it also affects risk for cardiovascular disease, stroke, and other neurodegenerative disorders. This review, based mostly on data from human studies, ranges across a variety of *APOE*-related pathologies, touching on evolutionary genetics and risk mitigation by ethnicity and sex. The authors also address one of the most fundamental question pertaining to *APOE*4 and AD: does APOE4 increase AD risk via a loss or gain of function? The answer will be of the utmost importance in guiding future research in AD.

Introduction

Recent advances in gene-based therapeutics have led to startling responses in neurologic disorders that have previously proved resistant to treatment. Starting in the periphery, patients with neuropathy caused by transthyretin amyloidosis (OMIM#105210) have responded well to treatment with either RNAi or an antisense oligonucleotide (ASO) directed against the mutated *TTR* gene (Adams et al., 2018; Benson et al., 2018). ASO treatment has been FDA approved (though not without controversy; Aartsma-Rus and Krieg, 2017) for Duchenne muscular dystrophy (OMIM#310200), using an exon-skipping strategy (wherein the ASO prevents the mutated exon from being incorporated into the protein resulting in a shortened, but functional, form of dystrophin). Moving into the CNS, where treatment efforts are further complicated by the blood-brain barrier, neurology has entered a revolutionary new phase in our approach to fatal, neurodegenerative diseases. Spinal muscular atrophy (OMIM#253300), a previously, universally fatal motor neuron disorder of childhood, has recently proved amenable to intrathecal ASO treatment (Mercuri et al., 2017, 2018). Additional ASO studies are underway in Huntington's disease (OMIM#143100) and amyotrophic lateral sclerosis (ALS, OMIM#105400) due to superoxide dismutase mutations (Miller et al., 2013; Wild and Tabrizi, 2017), two diseases believed to result from a toxic gain of function in the mutated protein. Thus, ASOs have already proved highly versatile in their potential for treatment using disparate mechanisms including eliminating a toxic protein, excising a mutated, interior exon to spare the rest of the protein, and, critically, preventing transcription of the upstream open-reading frame that typically inhibits translation, thereby resulting in increased translation of a protein (Rinaldi and Wood, 2018). Across this remarkably broad spectrum of ASO-based strategies, the common theme in these initial gene-based treatment efforts is the targeting of a relatively rare, monogenic disorder.

Late-onset Alzheimer's disease (referred to in this review as AD, OMIM#104300) is generally considered a polygenic disorder (Escott-Price et al., 2015; Lambert et al., 2013). This view is clouded, however, by lingering uncertainty over how to treat the apolipoprotein E4 (*APOE*4) allele. What is a neurologist to make of a 56-year-old woman with AD and two copies of *APOE*4? Does she have a monogenic version of AD? If so, should the physician consider an ASO-based approach to treating her, knowing that this has the potential for serious side effects (Shugart, 2017)? Most critically, would the physician want to increase or decrease the availability of the APOE4 protein? We will use this thought experiment as a point of departure for considering the critical question of whether APOE4-related AD risk reflects a loss or a gain of APOE function. Several reviews, centered on rodent and cell work, have already critically advanced this dialog (Huang, 2010; Huynh et al., 2017a; Kim et al., 2009; Liu et al., 2013; Michaelson, 2014). The current review will focus mainly on human studies—ranging across a variety of topics from coronary artery disease to evolutionary genetics—in the hopes of settling on an evidence-supported answer to this fundamental question pertaining to APOE4 and AD.

A Brief History of APOE in Human Genetics

In 1973, Havel and Kane described a mysterious "arginine-rich" protein in the very low-density lipoprotein (VLDL) of patients with a rare disorder of lipid metabolism called familial hypercholesterolemia type III (OMIM#143890) (Havel and Kane, 1973). Utermann and colleagues further characterized this protein and dubbed it APOE (Utermann et al., 1975), with subsequent work characterizing the three main isoforms of the protein: APOE2, APOE3, and APOE4 (Zannis et al., 1982). These initial findings unveiled a role for APOE and its variants in lipid metabolism and cardiovascular disease (Mahley, 1998; Mahley and Rall, 2000). The three isoforms result from common polymorphisms at two nearby loci on the *APOE* gene. The genetic polymorphisms are labeled rs429358 (C > T) and rs7412 (C > T) and result in amino acid changes at position 112 and 158, respectively, of the APOE protein (Rall et al., 1982; Weisgraber et al., 1981). As shown in Figure 1A, the haplotype combination at the two SNPs defines the three APOE protein isoforms, where the most common isoform, APOE3, has a cysteine at position 112

A

	Variant allele frequency		Isoform amino acid (AA) differences	
	Control	*AD*	*AA 112*	*AA 158*
APOE2	8%	4%	Cys	Cys
APOE3	78%	59%	Cys	Arg
APOE4	14%	37%	Arg	Arg

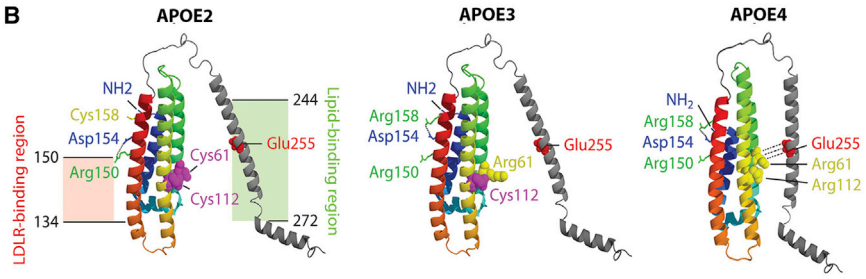

Figure 1. APOE Isoforms, Allele Frequencies, and Protein Structures

(A) The three main APOE isoforms APOE2, APOE3, and APOE4, respectively, encoded by the Apolipoprotein E2, E3, and E4 alleles, are the result of non-synonymous polymorphisms that cause amino acid changes at position 112 and 158 of the APOE protein (Rall et al., 1982; Weisgraber et al., 1981). *APOE*3 is the most common variant in the general population. The *APOE*4 variant is a major genetic risk factor for AD, while *APOE*2 is protective (Farrer et al., 1997).

(B) Structural models of lipid-free APOE are shown for each major isoform, based on X-ray crystallography, structure prediction, and circular dichroism spectroscopy (Zhong and Weisgraber, 2009). The N-terminal domain contains APOE's low-density lipoprotein receptor (LDLR) region at amino acid residues 134–150, while the C-terminal holds the lipid-binding region at residues 244–272. Amino acid substitutions in APOE4 promote a salt bridge between Arg61 and Glu255, which, compared to the APOE2 and APOE3 variants, drives increased domain interaction between the N- and C-terminal domains. In (A), APOE allele frequencies are obtained, with permission, from American Medical Association © Farrer, L. et al. JAMA. 278, 1349–1356 (1997). (B) is a reprint, with permission, from Annual Reviews © Yu, J. et al. Annu. Rev. Neurosci. 37, 79–100 (2014).

and an arginine at position 158. APOE2, the least common isoform, has a cysteine at both positions, and APOE4 has an arginine at both positions. These amino acid substitutions have been shown to drive a domain interaction in the protein such that the N-terminal and C-terminal domains, which are normally separated in APOE2 and APOE3, are joined by a salt bridge in APOE4 (Figure 1B).

The diligent reader will, in looking carefully at Figure 1A, wonder why there is not a genotype corresponding to an arginine at 112 and a cysteine at 158. It seems that the rs429358 and rs7412 SNPs are in almost perfect linkage equilibrium (The 1000 Genomes Project Consortium, 2015), so that the combination of an arginine at position 112 and a cysteine at position 158 is extremely rare and defines the APOE1 (also called APOE3r) isoform (Seripa et al., 2007). Only four subjects carrying the *APOE*1 variant have been reported in the literature: one healthy 70-year-old African Yoruba and three Italians (a 76-year-old with motor neuron disease and, from a separate family, a healthy mother and her autistic son) (Murrell et al., 2006; Persico et al., 2004; Seripa et al., 2007). The frequency (and potential clinical relevance) of this rare *APOE*1 variant on human diseases may have been underestimated, since it cannot be genotyped by standard sequencing or SNP-arrays but requires restriction-fragment length polymorphism (RFLP)-PCR.

These critical genetic and structural protein insights were already well established when the link between *APOE*4 and AD risk was made by Allen Roses and colleagues at Duke University in a pair of landmark studies published in 1993 (Corder et al., 1993; Strittmatter et al., 1993). The next year the same group demonstrated that the *APOE*2 allele was protective against AD (Corder et al., 1994). In the ensuing quarter century, roughly ten thousand papers have been published pertaining to some aspect of the relationship between AD and *APOE*. Several critical findings have been replicated and are now essentially indisputable. The risk of AD increases and the age at onset decreases with the number of *APOE*4 alleles (Corder et al., 1993; Farrer et al., 1997). The increase in risk varies substantially, as we will consider later, depending on ancestral background and sex, but as a rough estimate, having a single *APOE*4 allele increases risk 2- to 4-fold and having two *APOE*4 alleles increases risk about 8- to 12-fold (Farrer et al., 1997). The *APOE*4 allele also drives the age at onset down such that *APOE*4 carriers are, on average, about 12 years younger than non-carriers (Corder et al., 1993; Roses, 1996). The risk of AD decreases and the age at onset increases with the number of *APOE*2 alleles (Chartier-Harlin et al., 1994; Corder et al., 1994; Farrer et al., 1997). These associations of *APOE* alleles with age at onset have also been reported for early-onset AD due to mutations in the *amyloid precursor protein* (*APP*) and *presenilin* (*PSEN*) genes, although the size of the *APOE* effect tends to be diminished some on this autosomal dominant background (van Duijn et al., 1994; Sorbi et al., 1995; Vélez et al., 2016; Wijsman et al., 2005). Similar observations have been made in subjects with Down syndrome (OMIM#190685), who, due to trisomy of chromosome 21, carry three copies of *APP* and develop early-onset AD pathology (Coppus et al., 2008; Royston et al., 1996).

Before leaving the relative safety of solid findings about *APOE*4 and AD for shakier ground, it is worth making the point that the association between *APOE4* and AD is highly likely to be due to the *APOE*4 variant itself. This is never a foregone conclusion in genetic association studies. Many common SNPs that are associated with a given disease are merely markers that indicate that the actual, causal genetic change is nearby (and in linkage disequilibrium [LD] with the common SNP). This has been a particularly controversial issue in the *APOE* literature because the senior author from the landmark 1993 *APOE* studies made a case that *APOE*4's link to AD was actually mediated by a causal variation in a nearby gene called *TOMM40* (Roses et al., 2010). Roses and colleagues developed this hypothesis across a series of papers (Roses et al., 2016, 2014; Zeitlow et al., 2017), but two, much larger studies by independent groups failed to replicate the main finding (Cruchaga

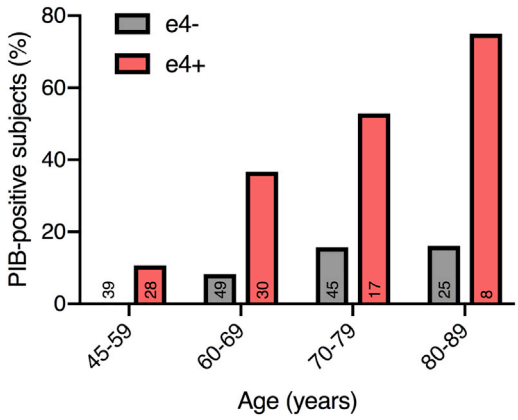

Figure 2. *APOE*4 Drives Amyloid Accumulation
Binding of Pittsburgh Compound-B (PIB) reflects cerebral amyloid accumulation. Graph displays, per age group and stratified by presence of *APOE*4 allele (number of subjects indicated at bottom of bars), the frequency of PIB-positive subjects in a cohort of cognitively normal individuals. *APOE*4 carriers more frequently display amyloid accumulation, an effect that becomes more pronounced with age. Figure is adapted, with permission, from John Wiley and Sons © Morris, J.C. et al. Ann. Neurol. 67, 122–131 (2010).

et al., 2011; Jun et al., 2012). While some groups continue to pursue this hypothesis, the general consensus in the field is that the association of *APOE*4 with AD is, in fact, mediated by the corresponding amino acid change in the APOE protein itself. The *TOMM40* saga serves, nonetheless, as an important reminder that a SNP identified in a genome-wide association study (GWAS) is rarely the causal variant itself but, instead, a nearby genetic co-traveler in LD with the causal variant. With *APOE* and AD, we have the rare good fortune of the association locus being a relatively common, non-synonymous exonic SNP, which decades of basic science in cell and animal models have all but proved to be the causal variant.

Role of APOE in Alzheimer's Disease Pathogenesis

Several outstanding reviews have covered the molecular and cell biology related to *APOE*'s role in cardiovascular disease and AD (Huang, 2010; Huang and Mahley, 2014; Kim et al., 2009). A few consistent findings are worth pointing out here to lay the groundwork for interpreting the human data in this review. In the periphery, APOE is produced mainly by the liver, but also by the adrenal gland and macrophages (Elshourbagy et al., 1985; Kockx et al., 2018). In the CNS, *APOE* is expressed mainly by astrocytes and microglia, but also, to a less clear extent, by neurons under stress conditions (Huang et al., 2004b; Kockx et al., 2018; Saura et al., 2003; Stone et al., 1997; Uchihara et al., 1995; Xu et al., 2006). APOE does not appear to cross the blood-brain barrier, and so the peripheral pool of APOE and the CNS pool of APOE are considered to be largely independent of one another (Linton et al., 1991; Liu et al., 2012). In the periphery and the CNS, APOE is a protein that shuttles cholesterol and other lipids between cells (Dietschy and Turley, 2001; Mahley, 1998). To do so, APOE interacts with other proteins (like ABCA1) that can add lipid moieties to APOE, transforming it into a lipoprotein (Koldamova et al., 2005; Wahrle et al., 2004). APOE then delivers its cholesterol cargo to cells via one of several receptors belonging to the low-density lipoprotein receptor (LDLR) family (Herz and Bock, 2002). Much research on APOE in the CNS has focused on its critical role in shuttling cholesterol to neurons for the maintenance of cell membranes and synapses, and for their repair after injury (Lane-Donovan et al., 2014; Pfrieger, 2003).

Quality?

With the ability to measure beta-amyloid peptides in the spinal fluid and plaques in the brain using positron emission tomography (PET), it is now well established that even among cognitively healthy, older controls, *APOE*4 carriers are more likely to have abnormally high amyloid levels (Figure 2) (Fleisher et al., 2013; Morris et al., 2010). Conversely, while there is a bit less evidence, given the relative rarity of *APOE*2 carriers, it appears that the *APOE*2 allele is associated with a reduced amyloid burden in healthy older controls (Fleisher et al., 2013; Morris et al., 2010). Given the primary role of beta-amyloid in AD, it is presumed that the effect of APOE4 on beta-amyloid accumulation is the chief, though perhaps not the sole (Conejero-Goldberg et al., 2011; Shi et al., 2017; Reiman et al., 2001; Yu et al., 2014), mechanism linking *APOE*4 to AD risk. While it is clear *that* APOE4 is associated with accumulation of beta-amyloid, it is not clear *how* APOE4 does this. A landmark study (now widely replicated; Dickson et al., 1997; Wisniewski and Frangione, 1992) established early on that APOE is co-deposited with Aβ in amyloid plaques, suggesting that a direct protein-protein interaction might link APOE to amyloid aggregation (Namba et al., 1991). This was later expanded by observations in mice that the APOE isoforms differentially affect Aβ clearance from the brain (Castellano et al., 2011; Verghese et al., 2013). For many years, the leading hypothesis, derived mainly from mouse models, was thus that APOE4 results in impaired processing and clearance of amyloid (Huynh et al., 2017a; Kim et al., 2009). Recent work in human stem-cell derived astrocytes and microglia supports this view (Lin et al., 2018). Conversely, studies of human stem-cell derived neurons also found a direct increase in beta-amyloid production and secretion by *APOE*4 neurons (Huang et al., 2017; Lin et al., 2018; Wang et al., 2018). Another recent discovery is that microglia appear to upregulate *APOE* expression as part of a unique phenotype that develops in the context of neurodegeneration and amyloid pathology (Keren-Shaul et al., 2017; Krasemann et al., 2017). This phenotype in turn is critically regulated by signaling of TREM2, a microglial surface receptor, that interacts with Aβ oligomers and APOE to enable amyloid clearance and APOE-mediated immune regulation (Zhao et al., 2018a; Yeh et al., 2016). These insights are particularly compelling, given that many microglia-associated genes, such as TREM2, are significantly associated with risk for AD (Pimenova et al., 2018).

Or Quantity?

While the basic mechanisms remain ill defined, one contributing factor of the APOE effect on amyloid and AD risk may relate to the availability of the protein. Measuring APOE, whether in plasma or CSF, can be challenging. Most approaches make use of ELISA-based strategies for APOE detection, but these are hobbled by intrinsic assay variability, bias from antibody isoform-specific binding preference, and a lack of isoform specificity (Baker-Nigh et al., 2016; Martínez-Morillo et al., 2014a). In response to these concerns, a few studies have used mass spectrometry approaches to decrease measurement

bias (Baker-Nigh et al., 2016; Martínez-Morillo et al., 2014a, 2014b). The mass spectrometry approaches tend to show similar general trends, but with smaller effect sizes. That is, *APOE* genotype-dependent differences in APOE protein levels appear less pronounced in mass spectrometry studies compared to ELISA-based methods, supporting concerns about isoform-specific binding preference in ELISA (Baker-Nigh et al., 2016; Rasmussen et al., 2015). Given the limited sample sizes, differing strategies in lipoprotein extraction, and varying analytical standards of these mass spectrometry studies, future large-scale studies are needed to determine the optimal measurement technique. Ultimately, the goal would be to have reliable, reproducible, and isoform-specific assays, so that, in a person with the *APOE* (3/4) genotype, one could measure the amount of plasma or CSF APOE3 and APOE4, rather than just the total amount of APOE.

Bearing these caveats in mind, several large studies have now shown that plasma APOE levels vary, consistently, as a function of *APOE* genotype (Corsetti et al., 2016; Cruchaga et al., 2012; Khan et al., 2013; Rasmussen et al., 2015; Schiele et al., 2000). From *APOE*2 to *APOE*3 to *APOE*4, there tends to be a linear decline in APOE levels. The Copenhagen study group in Denmark has also shown that plasma APOE levels tend to be lower in patients with AD compared to age-matched controls, even after controlling for *APOE* genotype (Rasmussen et al., 2015, 2018). That is, even among *APOE*4 carriers, lower plasma APOE levels are associated with greater risk of AD. As noted above, the plasma and CSF pools of APOE appear to be independent of one another, with plasma APOE derived mainly from hepatocytes, CSF and brain APOE derived mainly from astrocytes, and little to no capacity for APOE to move across the blood-brain barrier (Linton et al., 1991; Liu et al., 2012). As such, it may not be surprising that the consistent story of *APOE* genotype impacting APOE levels in the plasma is not replicated in human CSF studies. Some have shown a similar *APOE*2 > *APOE*3 > *APOE*4 effect on CSF APOE levels (Cruchaga et al., 2012; Toledo et al., 2014), but others have not (Bekris et al., 2008; Talwar et al., 2016). CSF APOE measurements also appear to vary with age, sex, and with the amount of beta-amyloid in the CSF (Baker-Nigh et al., 2016; Bekris et al., 2008; Cruchaga et al., 2012; Darreh-Shori et al., 2011; Toledo et al., 2014). A step closer to the pathological substrate, findings on brain APOE levels have also been marked by heterogeneity. As with CSF, some studies of APOE levels in brain have found the plasma-like pattern of *APOE*2 > *APOE*3 > *APOE*4 (Beffert et al., 1999; Bertrand et al., 1995), while others have not (Conejero-Goldberg et al., 2011; Glöckner et al., 2002). An inverse correlation between brain APOE levels and beta-amyloid has also repeatedly been observed (Beffert et al., 1999; Glöckner et al., 2002; Lambert et al., 2005; Shinohara et al., 2013). Across these studies, age, degree of pathology, and brain region studied vary considerably, which prohibits making a solid conclusion about the effect of *APOE* genotype on APOE levels in the brain. In summary, plasma APOE levels have consistently been reported to depend on *APOE* genotype, while levels in the CSF and brain tissue, likely in part due to small sample sizes, measurement issues, and other covariates, do not yet abide by a conclusive and replicable pattern across genotypes. Some factors that may contribute to *APOE* genotype affecting APOE levels (as in plasma) are the differential effects of APOE isoforms on receptor binding preferences, instability due to N- and C-terminal domain interaction, lipidation status, beta-amyloid sequestration, and differential clearance efficiency (for reviews, see Huang and Mahley, 2014; Huang et al., 2004b).

APOE Pleiotropy

As is clear from the brief history above, *APOE* is not just an AD-relevant gene but has been strongly implicated in cardiovascular disease as well. Its role in other disorders of the nervous system is less clear cut, but association studies have been done in nearly every neurologic disorder known to humankind. The various implications of APOE isoforms across disorders is relevant to shed light on its gain- or loss-of-function properties and to guide therapy development. In this section, we will review both the firm and the less firm associations between *APOE* and diseases other than AD. An overview of the main association between *APOE* genotypes and these different pathologies is summarized in Figure 3.

Cardiovascular Disease

The *APOE* genotypes show a pronounced, stepwise effect on several, interrelated cardiovascular phenotypes. Levels of plasma APOE and, generally also, high-density lipoprotein cholesterol (HDL-C) decrease in a perfect parametric fashion across the six *APOE* genotypes (2/2 > 2/3 > 2/4 > 3/3 > 3/4 > 4/4; Figure 4A), and the reverse is true for levels of low-density lipoprotein cholesterol (LDL-C; Figure 4B) (Bennet et al., 2007; Khan et al., 2013; Rasmussen, 2016). A similar stepwise pattern is seen in the risk for coronary artery disease (CAD) and myocardial infarction (MI), where *APOE*2 carriers have the least risk, *APOE*4 carriers have the greatest risk, and *APOE*3 carriers fall in the middle (Anand et al., 2009; Bennet et al., 2007; Wang et al., 2015; Xu et al., 2016; Figure 4E). For comparison, the risk for AD across genotypes is shown in Figure 4D. The impact of *APOE* genotype on CAD is undoubtedly linked, in large part, to the effect of *APOE* on LDL-C and HDL-C (Goldstein and Brown, 2015). Some studies have suggested that CAD risk may be above and beyond that attributable solely to the cholesterol level changes, while others have not (Corsetti et al., 2012; Rasmussen et al., 2016; Ward et al., 2009). These effects are relatively strong with a single *APOE*4 allele increasing LDL-C by roughly 10% and increasing the risk of CAD by 6% (Bennet et al., 2007; Khan et al., 2013; Rasmussen, 2016). Two copies of *APOE*4 increase LDL-C by 20% and the risk of CAD by 20%. In contrast to these linear trends in HDL-C, LDL-C, and CAD, the effect of *APOE* genotype in triglycerides (TGs) is quite distinct (Figure 4C). *APOE*2 carriers and *APOE*4 carriers are both at increased risk of hypertriglyceridemia (Bennet et al., 2007; Dallongeville et al., 1992; Huang, 2010; Marais et al., 2014). A small portion of *APOE*2 homozygotes, already the rarest of the six genotypes, are prone to a severe disorder of lipid metabolism called type III hyperlipoproteinemia (OMIM#617347), characterized by extreme elevations in TGs and total cholesterol. These patients, in addition to their risk for early CAD, can develop changes such as palmar xanthomata (orange creases in the palms) and tuberous xanthomas (nodular lipid deposits) typically appearing on skin over the knees and elbows (Marais et al., 2014).

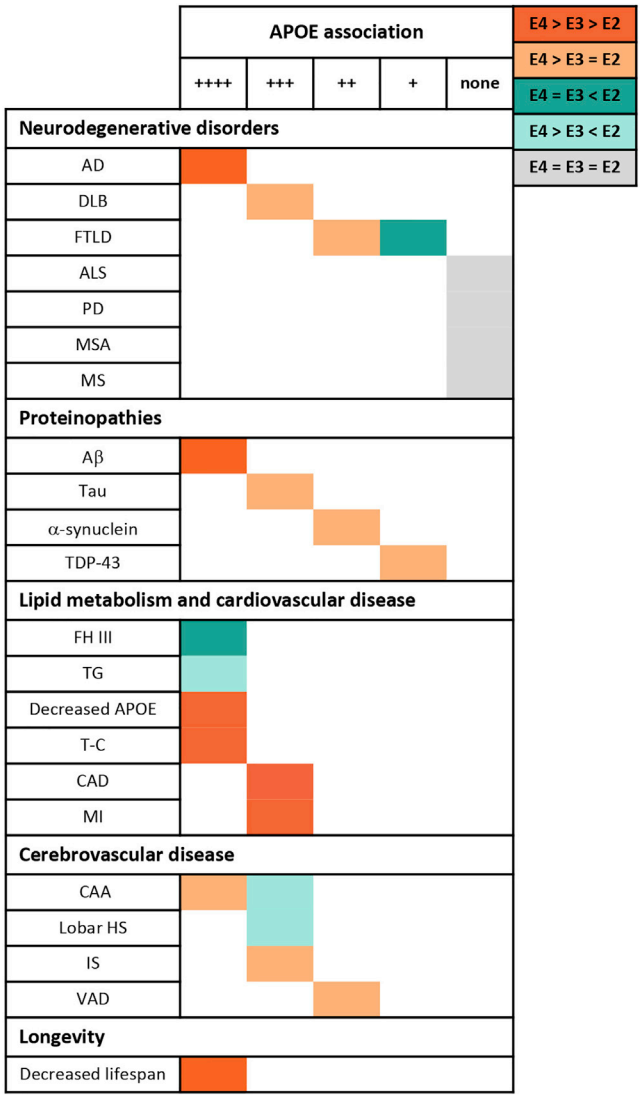

Figure 3. *APOE* Is a Pleiotropic Gene
The table illustrates the relationship between the three major *APOE* genotypes and their association with various diseases and pathologies. Position on the matrix, from left to right, indicates the strength of association, while color marks the *APOE* genotype relationship. AD, Alzheimer's disease; DLB, dementia with Lewy bodies; FTLD, frontotemporal lobar degeneration; ALS, amyotrophic lateral sclerosis; PD, Parkinson's disease; MSA, multiple systems atrophy; MS, multiple sclerosis; FH III, familial hypolipoproteinemia type III; TG, triglycerides; CAD, coronary artery disease; T-C; total cholesterol; MI, myocardial infarction; CAA, cerebral amyloid angiopathy; HS, hemorrhagic stroke; IS, ischemic stroke; VAD, vascular dementia.

Cerebrovascular Disease

Given the prominent effects of *APOE* genotypes on cholesterol and CAD, it is not surprising that there would be parallel effects in the CNS. Indeed, the risk of ischemic stroke is roughly 30% greater for *APOE*4 carriers (Wei et al., 2017). It is unclear whether this increased risk is solely attributable to the risks of hypercholesterolemia and CAD (both of which predispose to stroke), or whether there are additional brain-specific effects at play, for example, the role of APOE in astrocytes and pericytes at the blood-brain barrier (Bell et al., 2012; Halliday et al., 2016). The *APOE*2 allele does not appear to be protective against ischemic stroke. In terms of hemorrhagic stroke, the role of the *APOE* genotype is likely mediated in part by cerebral amyloid angiopathy (CAA, resulting from amyloid deposition in blood vessel walls). CAA predisposes to lobar hemorrhages, which are more common in *APOE*2 carriers and *APOE*4 carriers, resulting in a U-shaped curve when looking at hemorrhage risk across the six *APOE* genotypes (Viswanathan and Greenberg, 2011; Figure 4F). While *APOE*2 and *APOE*4 both increase risk of CAA and related hemorrhage compared to *APOE*3, the risks are qualitatively distinct. *APOE*4 carriers tend to have a greater burden of amyloid in vessels, leading to microbleeds, but *APOE*2 carriers appear to be more prone to such vessels rupturing and causing larger hemorrhages (Biffi et al., 2011; Charidimou et al., 2015; Yu et al., 2015). This increased propensity of *APOE*2 carriers to hemorrhage from CAA is also supported by a recent paper demonstrating that they are much more likely to show superficial siderosis on MRI scans (Pichler et al., 2017). The importance of beta-amyloid deposits in vessels has been highlighted recently in the replicated finding that APOE4-positive AD patients treated with anti-amyloid antibodies are at increased risk for amyloid-related imaging abnormalities (ARIAs). *APOE*4 carriers are more prone both to the edematous and hemorrhagic forms of ARIA, prompting some pharmaceutical companies to adjust the antibody dose for *APOE*4 carriers in these studies (Sevigny et al., 2016; Sperling et al., 2012).

Vascular Dementia

Vascular dementia (VAD) is, for lack of a better term, a squirrelly diagnosis that lacks standard pathological criteria and can be difficult to operationalize for research purposes (O'Brien and Thomas, 2015). It comes in several flavors, including post-stroke dementia, multi-infarct dementia, and small vessel ischemic disease (also called subcortical vascular dementia or, to use an older term, Binswanger's disease). To complicate matters further, cerebrovascular disease and AD often co-occur, particularly in older patients (Attems and Jellinger, 2014; Grinberg et al., 2010). Because *APOE* impacts the risk of both ischemic and hemorrhagic stroke, it is bound to have an impact on the risk for VAD. Indeed, earlier reviews and meta-analyses suggest an increased risk in *APOE*4 carriers (Dwyer et al., 2013; Rohn, 2014; Sun et al., 2015; Verghese et al., 2011). A more interesting question is whether, when controlling for stroke burden and other potential confounds, an *APOE*4 stroke patient is more likely to develop post-stroke dementia than an *APOE*3 stroke patient. These are challenging studies to undertake and there is a good deal of methodological variability across studies. For now, the jury is still out with several studies suggesting that *APOE*4 carriers are more prone to post-stroke cognitive decline and dementia (Ballard et al., 2004; Wagle et al., 2009, 2010), and several other studies suggesting no effect of *APOE*4 (Bour et al., 2010; Qian et al., 2012; Rowan et al., 2005).

Other Neurodegenerative Disorders

The reader will forgive us if we lump multiple sclerosis (MS) in with the neurodegenerative disorders, but this is an increasing defensible position (Calabrese et al., 2015; Eshaghi et al., 2018). Numerous studies have looked at whether the *APOE*4 allele increases risk for MS, and it appears, on balance, that it

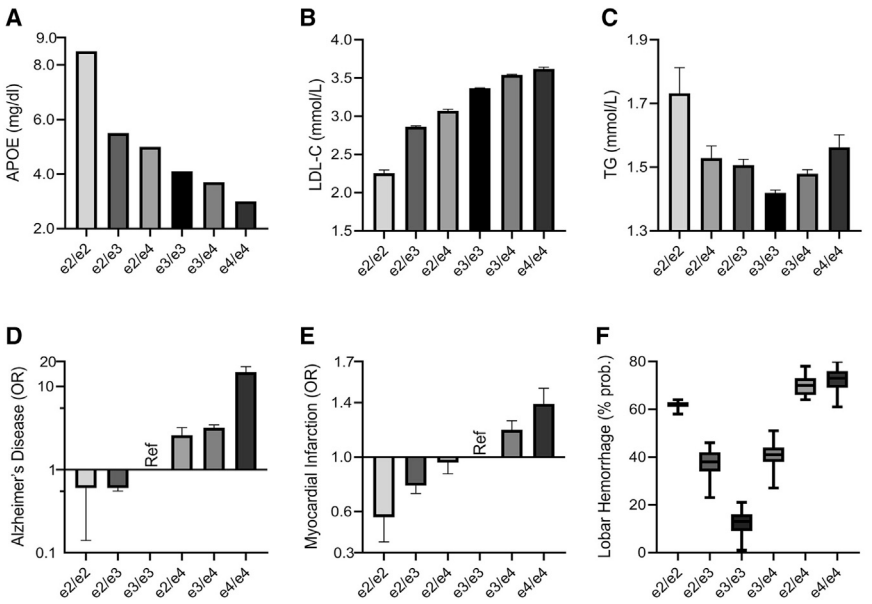

Figure 4. Parametric Associations of *APOE* Alleles with Alzheimer's Disease, Cardiovascular Traits, and Cerebrovascular Pathology

(A–E) Across all six combination of *APOE* alleles, clear parametric decreases can be observed for plasma APOE levels (A) and increases for low density lipoprotein cholesterol (LDL-C) levels [mean ± SEM] (B), while TG levels are marked by a U-shape (geometric mean ± SEM) (C), indicating risk for hypertriglyceridemia in *APOE2* and *APOE4* carriers (Rasmussen, 2016). The parametric association of *APOE* genotypes with lipid traits is also reflected in the association with risk, indicated by OR ± SE, for AD (pathologically confirmed, Caucasian subset; Farrer et al., 1997) (D) and myocardial infarction (MI) (Wang et al., 2015) (E).

(F) While the *APOE*-related risk for AD follows *APOE2* > *APOE3* > *APOE4*, cerebral amyloid angiopathy (CAA) and related risk for intracerebral lobar hemorrhage display a U-shape (% probability of cases versus controls; median ± min-max), indicating increased risk for both *APOE2* and *APOE4* carriers (Biffi et al., 2010).

In (C), (D), and (F), the position of the *APOE* (2/4) genotype is shifted to better indicate the combined effect of the two detrimental *APOE* alleles. (A) is a graphical representation, and (B) and (C) are adaptations, with permission, from Elsevier © Rasmussen, K. Atherosceloris. 255, 145–155 (2016). (D) is a visual adaptation of data, with permission, from American Medical Association © Farrer, L. et al. JAMA. 278, 1349–1356 (1997). (E) is a visual adaptation of data, under CC BY, from Wang, Y. et al. FEBS open bio. 5, 852–858 (2015). (F) is a visual adaptation, with permission, from John Wiley and Sons © Biffi, A. et al. Ann. Neurol. 68, 934–943 (2010).

does not (Burwick et al., 2006; Pinholt et al., 2006). This conclusion is best reflected in a very large study aptly titled "Closing the case of APOE in MS: No association with disease risk in over 29 000 subjects." (Lill et al., 2012a). As with post-stroke cognitive decline, it is also reasonable to ask whether *APOE4* could affect the likelihood of developing dementia due to MS. This has been looked at by a number of investigators, but these study sample sizes are uniformly small (ranging from 50 to 500 patients), and the results are fairly heterogeneous (Carmona et al., 2011; Ghaffar and Feinstein, 2010; Ghaffar et al., 2010; Koutsis et al., 2007; Shi et al., 2011). This question of cognitive decline in MS is particularly compelling in some ways because this patient population skews considerably younger than the other neurodegenerative disorders and any APOE4 effects in this age range would likely be wholly unrelated to concomitant AD or vascular pathology. Cognitive impairment in MS is a growing sub-field and, as MS-specific cognitive assessments become finer-tuned, the potential role of *APOE4* will be worth revisiting in a large *n* study (Sumowski et al., 2018).

The genetic advances in frontotemporal lobar degeneration (FTLD) and ALS over the last decade have been astounding (DeJesus-Hernandez et al., 2011; Renton et al., 2011). On the fringe of these major advances, there is waning interest in the role of *APOE* in these two often, but not always, related disorders. The verdict is in for ALS. A large meta-analysis (4k cases and 10k controls) in 2014 found no association between ALS risk and *APOE* genotypes (Govone et al., 2014). The story in FTLD is less straightforward. This is due, in part, to FTLD being more challenging to diagnose accurately *in vivo* compared to ALS. In addition, FTLD is now commonly sub-divided into the two main neuropathological subtypes of tau-positive or TDP-43 positive (with still more esoteric branching points below that), whereas ALS is, with the exception of rare SOD1 and FUS mutations, almost always due to TDP-43 pathology (van Es et al., 2017; Neary et al., 2005; Taylor et al., 2016). Furthermore, a clinical diagnosis of FTLD will not uncommonly be corrected at autopsy with a gold-standard pathologic diagnosis of AD. This confound, in combination with the younger age at onset in FTLD patients, will bias studies toward finding an *APOE* effect if the FTLD phenotype is not defined pathologically. A recent, multisite study of FTLD, for example, found that roughly 12% of clinically diagnosed behavioral variant frontotemporal dementia cases have AD pathology (Perry et al., 2017). Keeping these caveats in mind, three meta-analyses done over the last 20 years have come up with some divergent findings. The first found that *APOE2* increased the risk of FTLD but found no effect of *APOE4*, while the two more recent (and larger) studies found no effect of *APOE2* but did show that *APOE4* increased risk of FTLD (Rubino et al., 2013; Su et al., 2017; Verpillat et al., 2002). The effect size, reflected in the odds ratio (OR), for a single *APOE4* allele is considerably smaller in these two positive studies of FTLD (varying from 1.6 to 1.8) than in AD (ranging from 2 to 4). The studies collated across these meta-analyses have a mixture of clinically defined and autopsy-confirmed phenotypes and co-mingle TDP-43 and tau pathology. The international frontotemporal dementia consortium recently completed a study that examined the role of *APOE* (and other loci) in the three main FTLD clinical subtypes (Mishra et al., 2017). Only 3% of cases were autopsy confirmed. They found that *APOE4* increased the risk of the behavioral, progressive non-fluent aphasia, and semantic dementia variants. The finding was strongest in the behavioral variant group (which has the largest sample size), but across all three clinical subtypes, the ORs here were, again, modest (from 1.3 to 1.4). It should be noted that the two language variants of FTLD are probably more likely than the behavioral variant to be caused by AD pathology at autopsy. Importantly, they also

looked at frontotemporal dementia with motor neuron disease (the most reliable diagnosis given that these patients have ALS, which allows for a highly accurate clinical diagnosis) and found no association with *APOE*4 even in the homozygous state. A recent autopsy study of roughly 1,000 subjects from the Religious Orders Study and Memory and Aging Project (ROSMAP) has made a compelling case that *APOE*4 drives TDP-43 pathology even after covarying for co-morbid AD or alpha-synuclein pathology, though with a moderate OR hovering around 1.5 in different models that were run (Yang et al., 2018). This suggests that the finding of an *APOE*4 link to FTLD might be driven by the TDP-43 cases, but this runs counter to the lack of an association in ALS and frontotemporal dementia with ALS (known TDP-43opathies). The potential role of APOE4 in FTLD remains an open question to be settled soon, we suspect, once the question is asked in the growing samples of pathology-confirmed and pathology-stratified FTLD cases. As in MS, even if APOE does not impact the risk of developing FTLD it is worth considering whether it might affect the subsequent course of disease as has been suggested in both human and mouse studies (Agosta et al., 2009; Shi et al., 2017).

The strongest case for a role of *APOE* in non-AD neurodegenerative diseases can be made in dementia with Lewy bodies (DLB). Even more so than FTLD, the clinical diagnosis of DLB is often confounded with AD (McKeith et al., 2017). More challenging still, there is a high degree of overlap between AD and DLB pathology at post-mortem (Robinson et al., 2018; Spires-Jones et al., 2017). This is due, in part, to the strong correlation of age with each disease such that dual AD-DLB pathology is particularly prevalent in patients who die in their 80s and 90s (Spires-Jones et al., 2017). Owing to these challenges, the only means of determining a clear link between DLB and *APOE* would be a study done with autopsy-confirmed diagnoses. Tsuang and colleagues undertook such a daunting task and characterized subjects as pure AD (n = 244), mixed AD-DLB (n = 224), pure DLB (n = 91), pure Parkinson's disease dementia (n = 81), and 269 controls without significant AD or Lewy body pathology (Tsuang et al., 2013). In this multisite, tour de force the authors showed, convincingly, that *APOE*4 increases risk not just for AD (OR = 9.9), but also for mixed AD-DLB (OR = 12.6), pure DLB (OR = 6.1), and Parkinson's disease dementia (PDD) (OR = 3.1). Another subsequent study showed that patients with both AD and Lewy body pathology (n = 215) had a higher frequency of at least one *APOE*4 allele compared to just AD pathology (n = 316) (Chung et al., 2015). Despite this strong link to DLB, *APOE* has not clearly been associated with other synucleinopathies such as multiple system atrophy (MSA) or Parkinson's disease (PD). MSA is relatively rare, and to date studies examining a link between *APOE* and disease risk or age at onset have been negative (Cairns et al., 1997; Morris et al., 2000, 2001). In PD, an earlier meta-analysis suggested that *APOE*2 may be associated with increased risk (Huang et al., 2004a), but subsequent work further updated this meta-analysis and suggested that no clear significant associations could be determined (Williams-Gray et al., 2009). Since then, as is the case for other neurodegenerative disorders, PD has been marked by advances in genetic association analyses, culminating in the creation of the PDGene database and two large-scale meta-analyses that could not establish *APOE* as a significant risk locus for PD (Lill et al., 2012b; Nalls et al., 2014). As in MS, there remains the important possibility that while *APOE* may not increase risk for PD, it may increase the risk for dementia in PD (Aarsland et al., 2017; Collins and Williams-Gray, 2016; Monsell et al., 2014). This touches on the ongoing controversy of whether PDD and DLB are two distinct entities or one in the same thing. The authors of the current review are firmly among the lumpers, as opposed to the splitters, in viewing PDD and DLB as slightly distinct clinical disorders that merge into a single phenomenon at pathology (Jellinger and Korczyn, 2018; Langston et al., 2015). Viewed in this light, it seems likely that future studies with pathology-confirmed diagnoses will expand on the work of Tsuang et al. to show that *APOE*4 increases risk for PDD and DLB, in part via AD-related co-pathology, but independently as well. This association has solid basic science support as well with work in a PD mouse model showing a pronounced upregulation of APOE in response to alpha-synuclein pathology (Gallardo et al., 2008).

Longevity

Unsurprisingly—perhaps, given the strong effect on cardiovascular disease, stroke, and at least two age-related neurodegenerative disorders—*APOE* has consistently been reported as one of the two most robust genetic loci that associate with longevity across populations (Shadyab and LaCroix, 2015; Slagboom et al., 2018). The other locus contains the transcription factor *FOXO3*, a member of the forkhead box O family, which is an important regulator of homeostasis and response to cellular stress (Eijkelenboom and Burgering, 2013). Clearly, longevity is a highly pleiotropic trait, influenced by many lifestyle and environmental factors that contribute to the aging process (Christensen et al., 2006). In addition to these environmental factors, there appears to be a substantial genetic component to longevity as well, with heritability in twin studies estimated at about 25% (Herskind et al., 1996). It did not take long after the discoveries of *APOE* as a risk factor for cardiovascular disease and AD, before the research community established a link between *APOE* and aging (Smith, 2002). Subsequent work on a Danish cohort of centenarians suggested that *APOE*4 exerts increasing influence on mortality across aging (Jacobsen et al., 2010). A meta-analysis of centenarian studies (4k centenarians and 7k younger controls) determined that *APOE*2 increased (OR = 1.3) and *APOE*4 decreased (OR = 0.6) the probability of longevity (Deelen et al., 2011). On the other hand, a large meta-analysis of GWAS of aging in the general population, from the CHARGE consortium (n = 25k), did not find any significant effects at the *APOE* locus (Walter et al., 2011). The mean age at death in this study was lower, however, compared to the prior two studies, due to the focus on participants from the general population rather than centenarians. This suggested that the analysis by Walter and colleagues lacked the power to determine an association with longevity or that *APOE* may play a more important role particularly for very old age. Follow-up analysis on particularly long-lived subjects (>90 years of age, 6k cases versus 4k younger controls) of the CHARGE consortium did, indeed, identify a significant association with the *APOE* locus (Broer et al., 2015). The largest, to date, meta-GWAS on longevity (>90 years old, 21k cases and 77k younger controls) also established the *APOE* LD block as the most strongly associated genetic locus.

Several other meta-analyses have consistently reproduced this finding and established the association of *APOE2* with increased, and *APOE4* with decreased, longevity (Garatachea et al., 2014, 2015; Revelas et al., 2018; Sebastiani et al., 2013). In terms of the size of these effects, the most recent meta-analysis found an OR of 0.4 for *APOE4* and of 1.4 for *APOE2* (Revelas et al., 2018). Using parental lifespan as a proxy for longevity, several recent, very large studies from the UK Biobank and Lifespan projects have also found a significant effect at the *APOE* locus (Joshi et al., 2016, 2017; Pilling et al., 2016, 2017).

A Spectrum of APOE Function?

Across the disorders described above, where there is a clear association with *APOE*, a few distinct patterns emerge (Figure 3). The first is that, in AD (as in CAD, LDL-C levels, longevity, etc.), there tends to be a stepwise increase in risk from *APOE2* to *APOE3* to *APOE4*. While this pattern cannot definitively support a toxic gain- versus loss-of-function mechanism for APOE4 in AD, in our view, it tips the balance somewhat toward a loss of function. Combined with data from biomarker studies in humans, it seems that *APOE4* carriers are more prone to amyloid deposition and AD than *APOE3* carriers, who in turn are more prone than *APOE2* carriers. If the increased risk of AD in *APOE4* carriers were due to a gain of function in APOE4, we would need a separate explanation for the protective effect of APOE2 compared to APOE3. It seems more straightforward, instead, to invoke a spectrum of decreasing protein function from APOE2 to APOE3 to APOE4. This spectrum perspective is not universally applicable, however, as illustrated by APOE's binding to the LDL receptor, which is roughly equivalent between APOE3 and APOE4 but severely defective for APOE2 (Huang, 2010; Huang and Mahley, 2014). A second point to make is that there are at least a couple replicable examples in non-AD disorders of a U-shaped curve across the six genotypes in which *APOE2* and *APOE4* carriers are both at increased risk for disease. This serves as a reminder that the general pattern of *APOE2* "better" than *APOE3* "better" than *APOE4* does not always hold. A final point to make is that the step up in AD risk from the *APOE* (3/4) to (4/4) genotype is probably not linear but, rather, quadratic. This is important when considering the essentially required step of co-varying for *APOE4* dose in AD genetic and biomarker studies. It is arguable that rather than simply co-varying for the number of *APOE4* alleles, as is commonly done in genetic association analyses, it may be better to use all six genotypes weighted by their specific ORs, as derived from a large-sample AD meta-analysis (Darst et al., 2017).

Gleaning Biological Insights into APOE from Statistical Interactions

Cell and animal model experiments in *APOE* biology have, inconsistencies notwithstanding, provided some crucial insights into potential mechanisms of AD pathogenesis. These approaches have several obvious advantages pertaining to experimental control, precision, and reproducibility that we cannot hope to enjoy in human studies of *APOE* and AD (which are inherently noisy, time-consuming, and costly). On the other hand, human studies have the decided advantage of real-world applicability in that we are studying the disease we want to cure in the organism in which we want to cure it. While our capacity to alter biological variables in human studies is limited, we can profit from looking at natural independent variables such as ancestral background, sex, education, medications, and environmental exposures and how they affect the dependent variable of *APOE*-related risk for AD. Here, we will focus specifically on two such independent variables: ancestral background and sex, which appear to interact strongly with *APOE* genotype to affect AD risk. Leveraging these interactions has the potential to reveal critical new biological insights into *APOE*-related AD pathogenesis.

APOE and Ancestral Background

Genetic association studies provide essential information about the association of *APOE* polymorphisms with a given trait. Examining the evolutionary genetics and geographical distribution of *APOE* alleles may provide further insights into the diverse molecular functions of this protein. The evolutionary shifts in the *APOE* genotypes are reflected in the wide range of genotype frequencies encountered today across different ethnic backgrounds. Considering the evolutionary changes in *APOE*, together with the variance in genotypes across modern-day ethnicities, will help make sense of the remarkable differences in *APOE*-related AD risk between different ancestral backgrounds.

Evolutionarily speaking, ancient predecessors of all apolipoproteins already existed in very early eukaryotes and have individually evolved with increasing phyla, orders, and species (Babin et al., 1999). In vertebrates, APOE is widely expressed in fish, reptiles, and mammals (Duggan and Callard, 2001). Consistent evidence from primates and early human DNA sequencing indicates that *APOE4* is the ancestral allele and was later substituted by *APOE3* during human evolution through selective adaptation (Hanlon and Rubinsztein, 1995; Hixson et al., 1988; McIntosh et al., 2012). *APOE2* represents the most recent variation at the locus, with an approximate age of 80,000 years (Fullerton et al., 2000). As reviewed in detail by Huebbe and Rimbach, several hypotheses have been formulated about the functional reasons and potential selective pressures contributing to the evolution and the global distribution of human *APOE* alleles (Huebbe and Rimbach, 2017). Adaptations in APOE function likely reflect selection for early-life survival and effective reproduction within a particular environment (e.g., protection against high infection pressure; van Exel et al., 2017), rather than for late-life traits like AD that are more common to modern-day affluent societies. Although these selective pressures have acted over the course of millennia of human evolution, by shedding light on ethnogeographically specific, early-life survival demands, they may provide useful information to understand and establish research lines aiming to counteract the deleterious effect of *APOE4* in late-onset traits. Notably, the ancestral background of *APOE* is also relevant to provide context for translational research in rodent models. *APOE* is strongly conserved between rodents and humans (78% and 70% homology at the cDNA and protein level, respectively). Both rats and mice have the same amino acid as the human *APOE4* allele at position 112, confirming the ancestral origin of this allele (Rajavashisth et al., 1985). Despite this, mouse APOE functionally behaves more like human APOE3 in that it does not display the APOE4 domain interaction (as mice have a threonine rather than an arginine at the critical salt bridge position 61) (Raffai et al., 2001). Both *in vitro* and *in vivo*, *humanized* mouse

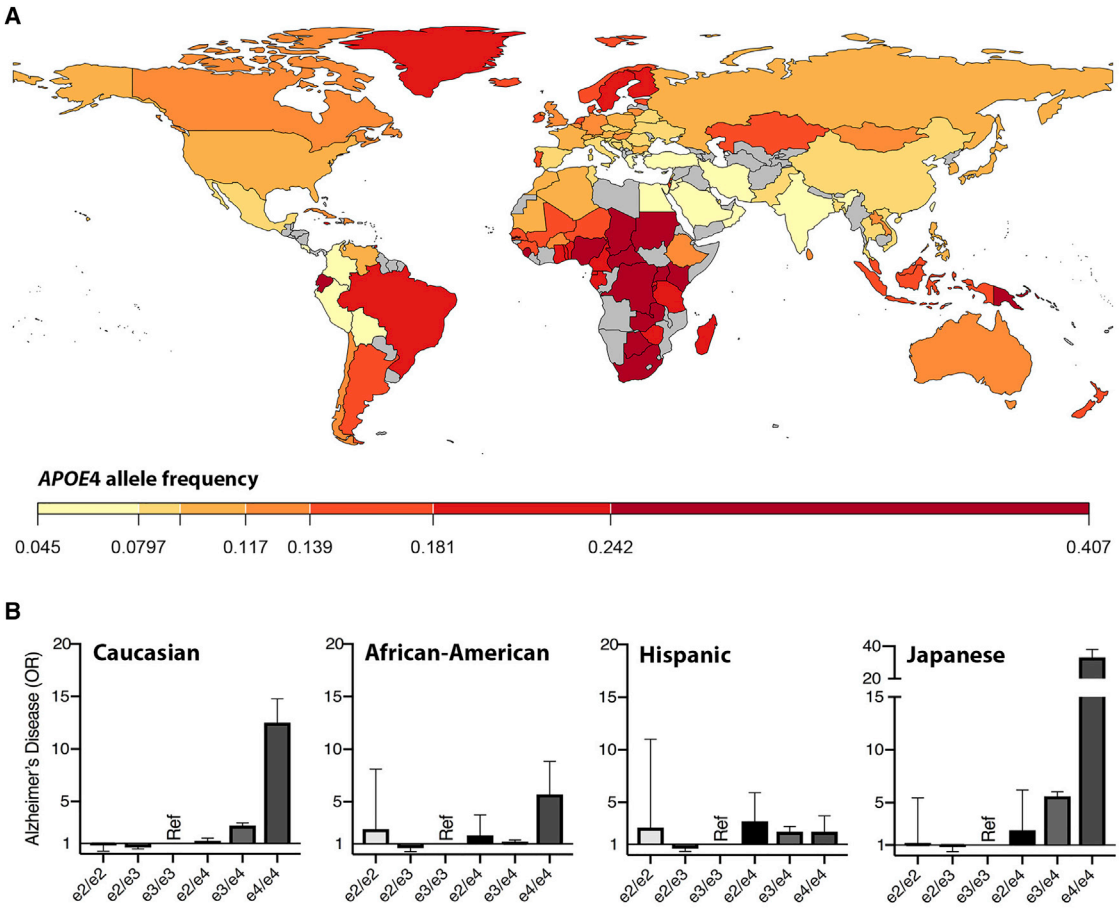

Figure 5. Geographical Differences in *APOE*4 Frequency and Ethnic Risk Mitigation

(A) Worldwide *APOE*4 allele frequency (for methods and references, see Methods S1).

(B) *APOE*-related risk for AD (clinically defined, OR ± SE) across all six *APOE* genotypes, for Caucasians (left), African-Americans (second from left), Hispanics (second from right), and Japanese (right) patient groups (Farrer et al., 1997). (B) is a visual adaptation of data, with permission, from American Medical Association © Farrer, L. et al. JAMA. 278, 1349–1356 (1997).

apoE (Arg-61), like human APOE4, binds preferentially to VLDL. In contrast, wild-type mouse *apoE* (Thr-61) displays an APOE3-like preference for HDL (Raffai et al., 2001). Clearly, these differences will be critical when studying AD pathology on a mouse *APOE* background compared to human *APOE* knockin models (which is why the latter approach is generally preferred).

The world-wide distribution of human *APOE* alleles varies considerably. *APOE*3 is the most common in all the human populations, but at frequencies ranging from 85% (Asia) to 69% (Africa) (Corbo and Scacchi, 1999; Singh et al., 2006). *APOE*4 allele frequency is negatively correlated with *APOE*3 allele frequency ($r = -0.97$), and it is enriched in indigenous populations of Central Africa (40%), Oceania (37%), and Australia (26%) (Corbo and Scacchi, 1999). The distribution across Europe and Asia follows an apparent north-to-south gradient, with the low *APOE*4 frequency in the Mediterranean area or South China (<10%) and increasing in more northern regions (up to 25%) (Egert et al., 2012; Hu et al., 2011). *APOE*2 is the least common major allele with a general worldwide frequency of 7.3%, and it is absent in many indigenous populations, without showing any apparent geographical trend (Corbo and Scacchi, 1999; Singh et al., 2006). Some factors that may contribute to these geographical distributions are adaptation to climate extremes and infection load (for review, cf. Huebbe and Rimbach, 2017). The wide geographical variability of *APOE*4 distribution can be appreciated in Figure 5A.

The three *APOE* polymorphisms demonstrate remarkable, ethnicity-specific differences in the risk for AD and other late-onset diseases. This point can be illustrated by examining the role of *APOE* genotypes in three common diseases—AD, lobar hemorrhage, and CAD—across the three most studied populations (African, East-Asian, and European). East-Asians seem to be the most vulnerable to the *APOE*4 effect in all three of these diseases (Bennet et al., 2007; Farrer et al., 1997; Tzourio et al., 2008; Zhang et al., 2014). A similar trend has been reported in regard to longevity in East-Asians versus Europeans (Garatachea et al., 2014). Conversely, despite the increased risk for AD in African-Americans (Hendrie et al., 2014) compared to European Americans, the association of *APOE*4 with AD is relatively weak

in African-Americans and only observable at homozygosity in Africans (Farrer et al., 1997; Hendrie et al., 2014). An early illustration of AD risk differences based on *APOE* genotype and ethnicity is shown in Figure 5B (Farrer et al., 1997). Similarly, *APOE* alleles appear to have no effect on lobar hemorrhage in African-Americans (Sawyer et al., 2018) or on CAD in Afro-Carribeans (Larifla et al., 2017). Recent efforts further demonstrate that the continuum of African ancestry in admixed African-Americans impacts the effect of *APOE*4 on AD and cognitive decline (Deters et al., 2018; Rajabli et al., 2018), such that *APOE*4 confers greater risk with decreasing percent of African ancestry. Rajabli et al. (2018) point out that this ethnicity-specific effect may be due to local ancestry at the *APOE* locus. The controversial debate on AD risk due to *APOE cis* elements was initiated shortly after the link between *APOE*4 and AD was established in 1993 (Lambert et al., 1997; Templeton, 1995). While three main promoter SNPs have since been identified, they have inconsistently been associated with AD risk (Rasmussen et al., 2015; Xiao et al., 2017; Xin et al., 2010). These discrepancies may in part be due to differing ancestries across study cohorts.

The evolutionary shifts in *APOE* genotype provide a window on to the pleiotropic role of this protein and may shed light on its potential role not just in cholesterol metabolism, but in other functions as disparate as bone metabolism and innate immunity (Dieckmann et al., 2013; Gale et al., 2014). Elucidating the role of the three *APOE* genotypes in these other physiological functions should, in turn, enhance our understanding of potential mechanisms related to AD risk. The wide range of *APOE*-related risk across ancestral backgrounds is a potentially very rich biological vein to mine. The *APOE*-by-ancestral background interaction is likely hiding critical gene-gene interactions that will reveal novel protein interactions and potential drug targets. Aside from the biological insights to be gleaned, with the move toward personalized medicine, the strong variability in *APOE*-related risk across ancestral backgrounds is critical for clinicians to factor into their assessment of a patient's risk of developing AD.

APOE and Sex

Just 1 year after the initial studies linking *APOE*4 to AD, a paper by Payami and colleagues found that the *APOE*4-asociated increased risk for AD was seen mainly in women (Payami et al., 1994). The plot thickened over the next 2 years when the Duke group could not replicate this increased risk in women (Corder et al., 1995a) and, in a separate paper, pointed out, rightly, that the question is complicated by the competing risks in *APOE*4 carriers of death and AD (Corder et al., 1995b). Payami and colleagues returned the following year with a follow-up paper redemonstrating the *APOE*-by-sex interaction in a large sample of cases and controls (Payami et al., 1996). The dust settled in 1997 when Farrer and colleagues published the first, definitive meta-analysis on the role of *APOE* in AD, examining the *APOE*2 and *APOE*4 effects in 5k+ cases and 8k+ controls (Farrer et al., 1997). This landmark 1997 paper not only replicated the main effects of *APOE*4 and *APOE*2, but strengthened, considerably, the case for the *APOE*-by-sex interaction. Figure 2 in the Farrer meta-analysis is reprinted here as Figure 6A and demonstrates that among Caucasians, the OR of AD in *APOE* (3/4) heterozygotes (compared to *APOE* [3/3] homozygotes) hovers around 3- to 4-fold in women between the ages of 50–80, whereas the OR in *APOE* (3/4) men hardly moves above 1. The OR in *APOE* (4/4) leaps to 10 and above for men and women, but even among the homozygotes there appears to be a slightly greater effect in women. For reasons that remain obscure (to these authors in any case), this prominent *APOE*-by-sex interaction did not initially receive a great deal of research attention and, to this day, is still not considered very often in the clinical setting. Some small studies in the late 90s did replicate this *APOE*-by-sex interaction (Breitner et al., 1999; Bretsky et al., 1999) but overall the importance of this interaction seemed not to take hold for another 10–15 years.

Interest in sex-based differences in disease has grown considerably over the last several years as reflected by, and in part due to, the NIH's insistence on considering the role of sex as a biological variable (NIH, 2015). We undertook a review of the interaction between sex and *APOE* 5 years ago that covered animal model work and human studies (Ungar et al., 2014). One of the most notable findings, expanded upon in that review, pertained to mouse models of AD. Historically, mouse model studies in AD and most disorders have tended to ignore female mice so as not to have to contend with the prominent effects of the estrus cycle. *ApoE*4 mouse models stood out in this regard in that, from the earliest studies, investigators found that the AD-relevant effects of these *apoE* mouse models were more obvious in female mice and so tended to focus on them (Raber et al., 1998). In other words, the *APOE*-by-sex interaction was found to play a prominent role in *apoE* mouse models and, happily, in the same direction (increasing *APOE*4 effects in females).

Regarding work in humans, the last 5 years have seen a number of studies published supporting the hypothesis that the *APOE*4 risk of AD is greater in women than in men. Our group, moving beyond the case-control analysis approach, used longitudinal data to examine the *APOE*-by-sex interaction on the risk of converting from healthy aging to cognitive impairment. As shown in Figure 6B, the *APOE*4-related increase in risk of conversion was significantly greater in women than in men (Altmann et al., 2014). This study then examined the same interaction on spinal fluid levels of beta-amyloid and tau (biomarkers of AD) and found that the *APOE*4 allele increased the risk for abnormal amyloid in men and women equally, but an *APOE*4 effect on tau was more prominent in women (Figure 6C). Revisiting the case-control approach, Neu et al. recently undertook a massive meta-analysis (combined cases and controls >50k) and found that, in the 65–75 age range, the *APOE*4 effect was significantly stronger in women than in men (Neu et al., 2017). A recent study by Hohman and colleagues has replicated, in a larger sample, our spinal fluid biomarker findings showing that the *APOE*4 effect on beta-amyloid is strong and similar in men and women, but that the *APOE*4 effect on tau is greater in women (Hohman et al., 2018). The spinal fluid data from our study and that of Hohman and colleagues can be interpreted in (at least) two ways. It is possible that the *APOE*4 effect on amyloid is equivalent between men and women and that, for a given amount of amyloid pathology, *APOE*4 has a greater effect on tau pathology in women. Alternatively, it may be that the greater *APOE*4 effect on tau pathology in women is indirect and due to the fact that *APOE*4 drives amyloid pathology at an earlier age in women than in

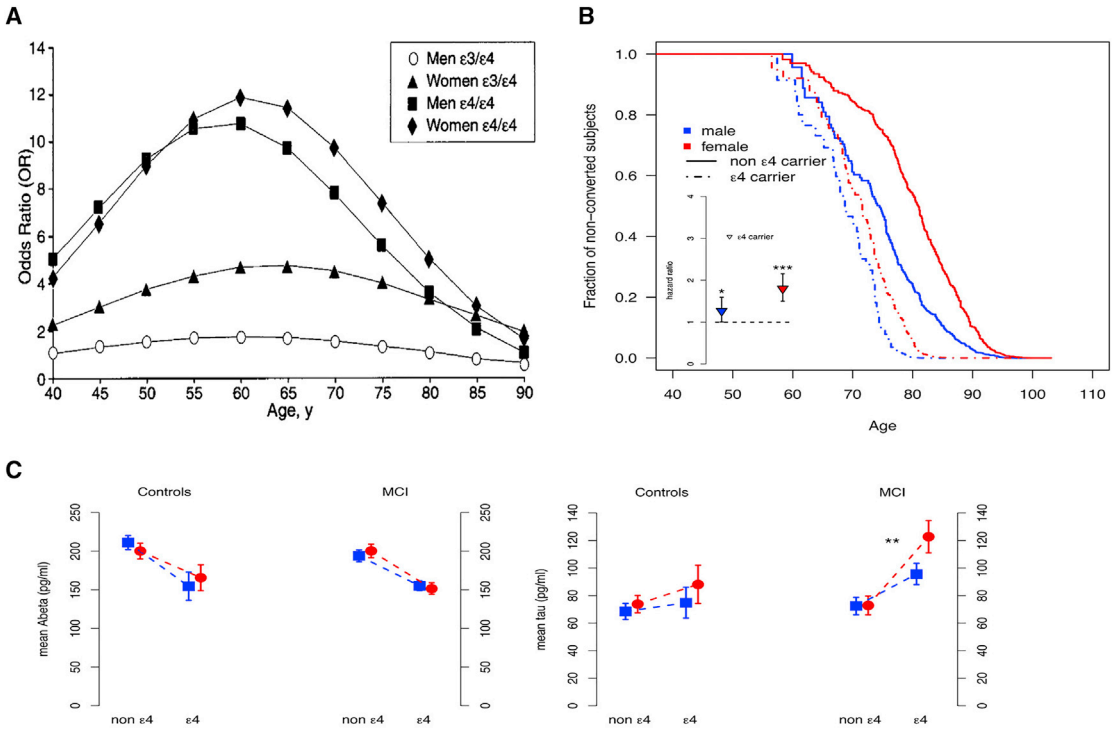

Figure 6. Sex Interacts with *APOE* to Affect Risk of Alzheimer's Disease, Clinical Decline, and Biomarker Levels
(A) Risk for AD based on *APOE* genotype, stratified by male and female sex (Farrer et al., 1997).
(B) Risk of clinical decline, defined as conversion from healthy controls to mild cognitive impairment (MCI) or AD, across the age range, stratified by sex. Inset shows the hazard ratio for conversion as determined for each sex independently, marking higher risk in women (Altmann et al., 2014). *p < 0.05; ***p < 0.001.
(C) In MCI patients, the *APOE*4 allele's effect on increasing tau levels was significantly greater in women than in men. (Analysis was adjusted for age and education; blue squares correspond to men and red circles to women.) Panels display levels of CSF Abeta (left) and tau (right). **p < 0.01.
(A) is a reprint, with permission, from American Medical Association © Farrer, L. et al. JAMA. 278, 1349–1356 (1997). (B) and (C) are reprints, with permission, from John Wiley and Sons © Altmann, A. et al. Ann. Neurol. 75, 563–573 (2014).

men. This latter possibility strikes us as more likely and cannot be resolved until more biomarker data are acquired in men and women in their early 50s when, as shown in Figure 2, amyloid pathology first becomes detectable in *APOE*4 carriers (Morris et al., 2010).

Two decades of research in humans (and mouse models) support the hypothesis that the *APOE*4 effect on AD risk is greater in women than in men. Investigators have only begun to scratch the surface of why this might be the case. Some evidence suggests that APOE expression is impacted by estradiol (Lambert et al., 2004; Levin-Allerhand et al., 2001; Srivastava et al., 2008; Stone et al., 1997; Struble et al., 2003). In this age of enormous AD GWAS meta-analyses, the role of the X chromosome variants remains essentially unexplored (note that the Manhattan plots in all the large AD GWAS papers only show chromosomes 1–22) (Lambert et al., 2013; Marioni et al., 2018). In addition to these hormonal and genetic possibilities, there are myriad environmental factors that likely play into the *APOE*-by-sex interaction, including, but not limited to, sex-based differences in education, head trauma, and all-cause mortality.

Gain or Loss of Function?

A quarter century after the initial studies linking *APOE*4 to AD, the most fundamental question remains unanswered: is *APOE*4 intrinsically bad or just not as good as *APOE*3 (which is not as good as *APOE*2)? This question looms particularly large now that clinical neuroscience is making remarkable headway with gene-targeting therapies. Prior animal and cell work has contributed substantially to this issue and stimulated the development of various therapies aimed at *APOE* (Huang and Mucke, 2012; Michaelson, 2014; Yu et al., 2014; Zhao et al., 2018b). Some of these studies pointed out that the mere presence of *APOE*, regardless of isoform, may drive AD pathology, suggesting it may even be better to not have *APOE* at all (Bien-Ly et al., 2012; Shi et al., 2017; Huynh et al., 2017b; Kim et al., 2011). Clearly, investigating this condition in human studies is highly complex due to the rarity of APOE-deficient patients and the lack of critical experimental controls in human genetic research. Nonetheless, in the first descriptions of APOE-deficient patients, no clinical symptoms referable to the nervous system were evident (Ghiselli et al., 1981; Lohse et al., 1992), which motivated the authors' conclusion that APOE may be of minimal physiological importance beyond its role in peripheral lipid metabolism (Lohse et al., 1992). Critical limitations to these early case reports include the fact that they appeared before the link between *APOE* and AD had been formed and the patients described are generally quite young when considering the possibility of AD-relevant phenotypes.

A more recent and far more compelling case report that touches on this critical question also highlights the many challenges inherent in human *APOE* studies. Mak and colleagues have described a 40-year-old African-American patient with severe hyperlipidemia due to compound heterozygosity for ablative, frameshift mutations in *APOE* (Mak et al., 2014). In addition to a cardiovascular assessment, the patient agreed to spinal fluid analysis, which showed normal levels of amyloid and tau. Structural brain imaging was reportedly normal and without evidence of vascular disease. The patient's cognitive assessment was complicated in that he had a history of presumed dyslexia but also showed substantial deficits across multiple non-language domains, including memory and visuospatial skills, though these deficits were felt to be long-standing and stable. In a single, potentially invaluable case study, with the capability to inform the field as to whether the lack of *APOE* increases the risk of AD, we are faced with all the challenges related to *APOE* research. The patient is about 10 years younger than when one begins to see amyloid abnormalities in healthy controls with *APOE*4 (Morris et al., 2010; Figure 2). He has an ancestral background that likely mitigates the effects of APOE dysfunction on AD risk (Deters et al., 2018; Farrer et al., 1997; Hendrie et al., 2014; Figure 5B). His cognitive impairment could reflect an important role of *APOE* in neural development or could reflect amyloid-independent effects of *APOE* and prodromal AD pathogenesis (Conejero-Goldberg et al., 2011; Shi and Holtzman, 2018; Yu et al., 2014). It is also pointed out, in subsequent scientific correspondence related to the cognitive impairment (Malloy et al., 2015), that the patient was "from a socially deprived environment and had profound disadvantages during childhood," raising the complex issue of gene-environment interactions. It would be most informative, of course, to repeat this assessment when the patient is 80, but he is at high risk of premature CAD and, in fact, already has some suggestive findings on stress testing, which will likely limit his longevity (Bennet et al., 2007; Joshi et al., 2017; Slagboom et al., 2018). If he were assessed at 75 or 80, were cognitively stable, and showed no evidence for abnormal amyloid, one could conclude that *APOE* deficiency does not drive AD pathogenesis. This would be concordant with recent human IPSC-derived neuron work showing that *APOE*-null cell phenotypes were similar to *APOE* (3/3) cell phenotypes (Wang et al., 2018). Such an outcome would provide good, albeit indirect, evidence that it would be safe, from a brain standpoint at least, to try knocking down *APOE*4 to prevent or slow AD in *APOE*4 carriers. From a systemic standpoint, however, given that peripheral APOE deficiency causes a profound dyslipidemia and increases risk for CAD (Marais et al., 2014), one would need either to consider CSF-specific approaches (intrathecal ASO-based therapy for example) or to monitor and correct any treatment-associated dyslipidemia. On the other hand, it is, in our view, equally likely that if he were reassessed at 50, he might then have evidence of amyloid abnormalities on spinal fluid assessment and perhaps some worsening of his cognitive impairment. This outcome would, of course, suggest that APOE deficiency is as bad, or worse, than *APOE*4 homozygosity and that, perhaps, *APOE*4's AD-related risk is due to reduced function that could be mitigated by increasing its expression.

The Path Forward

We do not intend to end this review, however, on a note of hopeless complexity. On the contrary, in many ways, the case study described above points the way forward and showcases the remarkable tools now at our disposal to solve the *APOE*-AD puzzle. *APOE* is pleiotropic and as neurologists and neuroscientists we will do well to collaborate closely with colleagues in cardiovascular research and lipid metabolism who have an additional decade of experience studying *APOE*. The ever-widening availability of next-generation sequencing and AD biomarkers means that these sorts of rare case studies will slowly expand into rare case series. A quick look at the exome data available in the AD sequencing project, for example, revealed two healthy heterozygous carriers of *APOE* loss-of-function mutations both above 85 years old and one with the *APOE* (3/4) genotype. At least two centers, including ours, are building a cohort of deeply phenotyped and whole-genome-sequenced, "protected" or "resilient" *APOE*4 carriers who remain cognitively healthy into their 80s and beyond. The expectation is that people with this extreme phenotype harbor rare, protective genetic variants that will offer critical insights into *APOE*4-related pathogenesis and, in turn, present novel targets for drug development. Additional promising approaches will explore the biology underlying the prominent *APOE*-by-ancestry and *APOE*-by-sex interactions on AD risk. Further, while not the focus of this review, there is no question that increasingly sophisticated cell-based and animal-model *APOE* research will continue to complement human studies. The best approaches will continue to be those that move back and forth between human findings and experimental models as the latter are critical in helping to validate and characterize (or refute and discard) candidate genetic variants. There is, undoubtedly, work to be done, but the tools are now at hand to take full advantage of the *APOE* lever and create a drug that eradicates AD.

SUPPLEMENTAL INFORMATION

Supplemental Information includes supplemental text and can be found with this article online at https://doi.org/10.1016/j.neuron.2019.01.056.

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