Apolipoprotein E in Alzheimer's disease trajectories and next-generation clinical care pathway

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Abstract

Alzheimer's disease (AD) is a complex, progressive primary neurodegenerative disease. Since pivotal genetic studies in 1993, the epsilon 4 allele of Apolipoprotein E (*APOE ε4*) has remained the strongest single genome-wide associated risk variant in AD. Scientific advances in *APOE* biology, AD pathophysiology, and ApoE-targeted therapies have brought *APOE* to the forefront of research with potential translation into routine AD clinical care. This contemporary review will merge *APOE* research with the emerging AD clinical care pathway, and discuss *APOE* genetic risk as a conduit to genomic-based precision medicine in AD, including ApoE's influence in the ATX(N) biomarker framework of AD. We summarize the evidence for *APOE* as a significant modifier of AD clinical-biological trajectories. We then illustrate the utility of *APOE* testing and future of ApoE-targeted therapies in the next generation AD clinical-diagnostic pathway. With the emergence of new AD therapies, understanding how *APOE* modulates AD pathophysiology will become critical for personalized AD patient care.

Introduction

As the most common cause of dementia in later life, Alzheimer's disease (AD) is projected to affect 152.8 million people by 2050 worldwide[1]. Historically, AD has been diagnosed by clinical symptoms based on impaired memory, cognition, and function leading to loss of independence[1]. However, this symptom-based model does not incorporate the underlying pathophysiology of AD rooted in proteinopathy, characterized by the accumulation of soluble, bioreactive amyloid beta (Aβ) species aggregating into plaques and downstream hyperphosphorylated tau aggregation, gliosis, and subsequent regional neurodegeneration[2]. These converging pathophysiological processes precede clinical signs and symptoms by 20 to 30 years[3], supporting the conceptual evolution of AD from a purely clinical diagnosis to a clinical-biological diagnostic construct. One that includes asymptomatic preclinical stages with progressive underlying biological mechanisms[3]. This revision is depicted in the hypothesis-independent ATX(N) biomarker classification framework of AD, which is driving the development of biomarker-guided, pathway-based targeted therapies for AD[3]. As other components of AD pathophysiology are discovered, the ATX(N) system will continue to be extended and updated.

One key component of this framework is the genetic contribution to AD pathophysiology with the ε4 allele of the apolipoprotein E gene (*APOE ε4*) being the strongest single genomic risk variant in AD[4]. *APOE ε4* increases the lifetime risk of AD[5] and is associated with earlier disease onset in a dose-dependent manner[6], while *APOE ε2* is associated with decreased risk relative to *APOE ε3*[7]. The magnitude of the *APOE* risk is influenced by ethnicity and sex[7-9]. *APOE ε4* is also associated with increased risk of other proteinopathy-related neurodegenerative diseases,

including Dementia with Lewy Bodies (DLB), Parkinson's disease dementia (PDD), and TAR DNA-binding protein 43 (TDP-43) pathology in AD brains[4].

While the investigation of *APOE* in AD has previously been investigated mostly in parallel between basic science and clinical research, we propose these two lines will now converge with emerging therapeutics that target underlying AD pathophysiology. In this review, we begin with an overview of the biology of ApoE, then go onto illustrate this convergence by describing how *APOE* and its pathophysiology fit into the expanding ATX(N) biomarker framework of AD. We then discuss the role of *APOE* testing in the clinical care pathway and how potential APOE-targeted therapies may enhance the compendium of AD therapies in the future.

Biology of ApoE

Structure and function of ApoE

Human ApoE is a glycoprotein of 299 amino acids occupying the surface of specific lipoprotein particles where it binds to cholesterol and phospholipids (**Figure 1A)**. ApoE has two key domains: the N-terminal domain (NTD) which binds to low-density lipoprotein receptor (LDLR) and the C-terminal domain (CTD) which binds to the surface of lipoproteins[10] (**Figure 1A**). In the lipid-free state, the NTD comprises of a 4-helix bundle connected to the CTD lipid-binding residues and helices via a hinge helix, with seven intermolecular salt bridges stabilizing the secondary structure[10] (**Figure 1B-C**). There have been two proposed structural models of lipidated ApoE. One model suggests that upon ApoE lipidation, the NTD 4-helix structure stretches to expose its hydrophobic core while the CTD dissociates from its compact

conformation, with the CTD sitting on top of the exposed hydrophobic residues of the NTD forming a belt-like configuration and two ApoE belts dimerizing on the edge of lipid core to stabilize the lipid particle[10]. The second model suggests an open or compact hairpin structure formed by the helices, with ApoE dimers forming a lipid disc[10].

ApoE facilitates the cell-to-cell transport of lipoprotein particles and cellular uptake via interaction with LDLR and LDL-related protein 1 (LRP1)[4] (**Figure 1D**). Peripheral APOE is expressed primarily in the liver, as well as adipose tissue, kidneys, and adrenal glands whereby hepatic ApoE is involved in cholesterol metabolism without crossing the blood-brain barrier (BBB)[11] (**Figure 1D**). In the central nervous system (CNS), non-neuronal cells including astrocytes and reactive microglia produce ApoE[4]. Cholesterol and phospholipids are transferred to astrocyte-secreted APOE by the cell-surface ATP-binding cassette transporters ABCA1 and ABCG1, creating lipoprotein particles similar in size to HDL[4] (**Figure 1D**). The size of the APOE lipoprotein complex differs based on isoform, with APOE ε2 being the largest and APOE ε4 being the smallest due to differential transfer of cholesterol [10]. In addition to its role in lipid homeostasis, ApoE may also play a role in synaptic plasticity and cerebrovascular function, with potential crosstalk between peripheral and CNS ApoE in brain physiology[4].

APOE has two common polymorphisms, leading to three main ApoE proteoforms: *APOE ε2*, *APOE ε3*, and *APOE ε4*. These differ at two amino acid sites 112 (rs420358) and 158 (rs7412) whereby ApoE4 contains arginine on both positions, ApoE3 contains cysteine and arginine respectively, and ApoE2 has cysteine on both[10] (**Figure 1C**). These amino acid changes substantially alter the structure and function of ApoE[4]. The isoforms differ in their binding to LDLR, with stronger affinity for ApoE3 and ApoE4 and weaker affinity for ApoE2 [10] (**Figure 1D**) In

the periphery, decreased binding of ApoE2 to LDLR impairs clearance of lipoprotein particles, contributing to type III hyperlipoproteinemia[12], whereas enhanced binding of ApoE4 to very low density lipoprotein (VLDL) particles impairs the lipolytic processing of VLDL, resulting in proatherogenic changes[4] (**Figure 1D**). Recent studies showed ApoE4 exhibits conformational heterogeneity in both lipid-free and lipid-bound states[13], which may further affect its function in receptor binding. Despite recent progress in elucidating the structure of *APOE* isoforms and their physiological functions, it is still unknown how structural changes in these isoforms affect ApoE's role in lipid homeostasis and other physiological processes[4].

Genetics of *APOE* in AD

Alzheimer's disease (AD) can be subdivided into early-onset (EOAD) and late-onset (LOAD) based on age of onset, with EOAD cases developing symptoms before the age of 65[4]. A small percentage of EOAD cases are caused by familial autosomal dominant mutations (ADAD; autosomal dominant AD), while the more common LOAD is attributed to a combination of genetic susceptibility and environmental factors[4]. Pedigree-based genetic association studies identified three highly penetrant genes in *APP*, *PSEN1,* and *PSEN2* in ADAD[2]. LOAD is more common and polygenic, with several genetic risk factors now identified through large-scale genome-wide association studies (GWAS)[2].The *APOE ε4* allele on chromosome 19q13.2 was the first and most significant LOAD risk locus identified in AD[4].

Unlike ADAD mutations, *APOE* is not deterministic for AD with a small percentage of *ε4* homozygotes never developing the disease[14]. These individuals exhibit cognitive resilience despite *APOE ε4* status, with other genetic makeup, ethnicity, sex, general health, education, and other environmental factors possibly contributing to resilience[15,16]. Originally identified and

associated with a specific form of lipid disorder, *APOE* is also associated with dysbetaliproteinemia[17]. Of the three major allelic variants of *APOE*, *ε3* is the most common and *ε2* is the least common with *ε4* allele frequency differing among Caucasians, Japanese, Hispanic, and African American individuals[7,8] (**Figure 2B-C** and **Table 1 top panel right box**). The cumulative incidence of AD increases over age based on *APOE ε4* allele dosage (**Figure 2A)**[14], and there is a dose-dependent increase in the likelihood of AD development with each *ε4* allele[7,8](**Supplemental Figure 2D-E** and **Table 1 top panel**). In contrast, *APOE ε2* remains the strongest genetic protective factor against sporadic AD[7,8], with very few *APOE ε2/ε2* individuals developing AD up to age 90[14,18].

The ε4 allele appears to influence AD differently depending on the population, with Japanese having the greatest risk and Hispanics having the lowest[7,8] (**Figure 2D-E** and **Table 1 top panel**). While AD dementia is more prevalent among African Americans compared to Caucasians, African American *ε4* carriers paradoxically have lower AD neuropathological burden[19]. In a Chinese population, frequency of *ε3* was lower in AD patients than healthy controls[20], while cognitively unimpaired Japanese *ε4* carriers had steeper cognitive decline during aging[21]. *APOE* alleles also present different effect sizes across populations: a recent study with ~13,000 individuals showed that *APOE ε4* and *ε2* have a higher effect on Aβ burden in Caucasians, followed by African Americans and Asians[22]. Another *APOE* variant (rs5117) was specifically associated with brain amyloidosis in Caucasians and Asians but not African Americans[22]. These ethnic differences may be due to local ancestry of *APOE* rather than global ancestry or environmental factors[23].

APOE ε2 carriers have ~50% decreased risk for AD compared to *APOE ε3/ε3* with ethnic variability, with the strongest protective effect in non-Hispanic Whites[7,8] (**Figure 2D-E** and **Table 1 top panel**). *APOE ε2/ε2* individuals have a stronger protective effect for lifetime risk of AD when confirmed with neuropathology (though not stratified by ethnicity)[14]. *APOE ε2/ε4* individuals have an increased disease risk relative to the *APOE ε3/ε3* individuals, suggesting a dominant effect of *ε4* allele over *ε2* (**Table 1 top panel**)[7,8]. While there were no differences in AD risk between men and women with *APOE ε3/ε4* at later ages, female *APOE ε3/ε4* had decreased AD risk at younger ages (**Table 1 middle panel**)[9]. Women *ε4* carriers are also more likely to develop mild cognitive impairment (MCI), likely due to AD, compared to men, again at younger ages[9]. In contrast, *APOE ε2/ε3* has a greater protective effect in women compared to men[9] (**Table 1 bottom panel**).

The *ε4* allele also increases the risk of EOAD, particularly in homozygous individuals without a family history, and in *ε4 carriers* with a positive family history[24,25]. Similar to LOAD, *APOE ε4* decreases the age of disease onset for ADAD patients with *APP, PSEN1, or PSEN2* mutations, while *APOE ε2* has a delaying effect in *PSEN1* mutation carriers[4]. *APOE ε4* carriers were also seen with later onset of EOAD, suggesting other unknown variants may influence disease onset beyond *APOE*[26]. *APOE ε4* carriers in EOAD in general show faster decline in memory, executive, and processing speed domains[27]. Similarly, *APOE ε4* affects the age of onset and rate of cognitive decline in ADAD[4].

In addition to detrimental effects, there exist rare protective variants in the *APOE* gene, including the *APOE3*-Christchurch (p.R126S) mutation, the *APOE3*-Jacksonville (p.V236E) mutation, and the *APOE4-p.*R251G mutation[28]. Despite being a single case study of unknown

generalizability and mechanistic explanation, a previous study showed *APOE3*-Christchurch mutation in homozygous state to be associated with a 30-year delay in cognitive decline in one individual carrier with the *PSEN1* E280A mutation[29], with evidence of Aβ deposition but attenuated tau pathology and inflammation[30].Given this has occurred in a single individual, it remains to be ascertained if the protective effect is solely due to the Christchurch mutation or some other genetic change.

Genetic mechanisms of *APOE* variants towards AD pathophysiology so far escape straightforward labelling of loss of normal function vs. gain of toxic function. Most studies, including those using animal models, support the idea that ApoE3 and ApoE4 increase AD pathology in a dose-dependent fashion[31-33]. It is yet unclear whether ApoE2's protective effect is due to a loss of normal ApoE function or a gain of protective function. One may posit *APOE* variants as naturally occurring polymorphisms with pleiotropic effects on human diseases, with resulting protein isoform's effect on disease occurring independently of one another. This view would also account for background haplotype effects and ethnic differences and could begin to unravel possible epistasis and genetic interaction between rare variants.

Given *APOE*'s role in lipid metabolism and cardiovascular risk, several studies have investigated the relationship between multiple environmental factors that interact with *APOE* to modulate AD risk[34], where healthy diet was associated with a greater reduction of dementia risk in *APOE ε4* non-carriers than carriers[34]. Others gene-environment interaction analyses included pre-morbid education level, smoking, and physical activity and the increased risk of AD with the *ε4* allele, but the direction of this effect has been mixed[34].

AD and the ATX(N) Framework

Historically, AD diagnosis was based on clinical features, with post-mortem confirmation of Aβ plaques and tau neurofibrillary tangles needed for definitive diagnosis[35]. The discovery of *in vivo* biomarkers of the core pathophysiological alterations have better characterized the preclinical and prodromal phases of AD[35]. This led to an evolution of AD from a clinical to biological concept, creating a comprehensive biological research framework based on amyloid, tau, and neurodegeneration, known as the AT(N) Research Framework[3] (**Figure 3**). The AT(N) system has since been extended to define and stage AD across its entire spectrum[35], describing the temporal sequence of underlying pathological changes prior to the clinically symptomatic stages. This process begins with the early accumulation of soluble Aβ and subsequent aggregation into fibrillar plaques, followed by the hyperphosphorylation, fibrillization, and spreading of tau protein in neurofibrillary tangles, which is strongly associated with synaptic loss, gliosis, vascular abnormalities, and eventually neurodegeneration[2,3] (**Figure 3A**).

These pathological changes can be detected by core feasible biomarkers of each component of the AT(N) framework[3]: CSF Aβ42/40, Aβ PET, and some CSF phosphorylated tau species (p-tau181 or p-tau217) that are better correlated with Aβ pathology ("A")[36,37] ; tau PET scans for tau aggregates ("T") as they spread into the neocortex[3];
CSF total tau (t-tau), CSF/plasma neurofilament light chain (NfL), or plasma brain-derived tau (BD-tau) for neurodegeneration ("N")[3,38]. This framework allows incorporation of new biomarkers "X", including neuroimmune system dysfunction (GFAP, YKL-40, TREM2), synaptic dysfunction (neurogranin (Ng), SNAP-25, synaptotagmin), and vascular abnormalities (sPDGFRβ)[3]. Blood-

based biomarkers (BBBM) will offer a low-cost, more accessible and scalable approach compared to PET or CSF for some of these biomarkers, particularly Aβ and tau[39].

APOE and the ATX(N) Framework

While the risk relationship between *APOE* and AD is clear, more recent work has illustrated the relationship between *APOE* and AD biomarkers, suggesting a role for *APOE* for modulating different components of the ATX(N) system[40]. Based on evidence to date, we describe *APOE's* role in the clinical-biological continuum of AD whereby *APOE* genotype shifts the clinical-biological trajectories within the ATX(N) construct (**Figure 3A-D**). In the following sections, we consider how *APOE* might contribute to each component of the ATX(N) framework[3], including *APOE's* effect on biomarkers and underlying pathophysiology of Aβ ("A"), tau ("T"), neurodegeneration ("N"), vascular ("X"), and glia ("X").

<u>*APOE* in the AD clinical continuum</u>

APOE genotype affects the age of onset of LOAD symptoms with one *ε4* allele decreasing AD onset by ~3 years and two *ε4* alleles decreasing onset by ~9 years, and the *ε2* allele increasing onset[6,18] (**Figure 3D**). *APOE ε4* carriers have an increased risk of progression in cognitively unimpaired and MCI individuals to the next stage of AD continuum while *APOE2* carriers have a lower risk of progression[41,42], likely due to the earlier onset of Aβ pathology[43] .

The effect of *APOE* on rate of cognitive decline in AD is more complex and influenced by Aβ and tau pathology. Earlier studies with mixed results on *APOE's* modulation of rate of decline did not normalize for Aβ deposition[27,43-47] with newer studies showing the effect of *APOE* on rate

of decline is mediated by Aβ status[47,48] and downstream tau pathology[49]. Studies also show variability of on the type of cognitive test used, where *APOE* had no effect on rate of decline on mini-mental state examination (MMSE) when adjusting for Aβ status[47], while amyloid-positive *APOE ε4* carriers progressed faster when examined with the Clinical Dementia Rating Sum-of-Boxes (CDR-SB) scale[48]. These differential findings could be due to sensitivity of cognitive testing according to the disease stage and Aβ measurements. Further work in this area is required to dissect the contribution of Aβ vs other AD pathologies.

APOE's effect on disease development is influenced by sex where cognitively unimpaired *APOE ε4* women are more likely to progress to MCI and AD compared to men with the same genotype and conditions, particularly at earlier ages[9]. Female *APOE ε4* carriers undergo age-related cognitive decline faster than men across the AD continuum, likely due to underlying Aβ pathology[46,]. Only EOAD females with *ε4* allele showed accelerated cognitive decline compared to men[27].

<u>*APOE* and AD biomarkers in the ATX(N) Framework</u>

Amyloid Beta ("A")

Initial studies differentiating *APOE* status in AD demonstrated that onset of Aβ plaque formation is influenced by *APOE* genotype[2,4]. When visualized by PET, cognitively normal *ε4* carriers start to accumulate plaques much earlier than non-carriers[50-52], reaching high Aβ plaque density ~17-18 years earlier[51,52], while *APOE ε2* homozygotes develop plaques much later (**Figure 3B**). *APOE ε4* does not affect the rate of Aβ accumulation once plaques reach abnormally high levels[53], and *ε4* non-carriers eventually reach the same level of Aβ plaques as *ε4* carriers at later

ages based on Aβ imaging data[51] (**Figure 3B**). *ε2* remains protective against longitudinal Aβ accumulation, particularly in those without the *ε4* allele[52,53], confirmed by neuropathological studies[33,54]. CSF biomarker showed similar shifts where *APOE ε4* is associated with lower levels of CSF Aβ42[50], indicating earlier Aβ deposition in brain parenchyma[55]. Consistent with plasma Aβ42/40 and PET/CSF Aβ concordance[39], the predictive value for brain amyloid by plasma Aβ42/40 is increased when accounting for *APOE* status with age[56]. One of the BBBM assays includes an ApoE proteoform assay to detect ApoE peptides corresponding to *APOE* genotype[56], suggesting *APOE* testing could become a part of the BBBM battery.

Three potential mechanisms could explain how *APOE* genotype shifts the amyloid beta "A" curve in AD (**Figure 3B**): Aβ aggregation, Aβ clearance, and Aβ production/secretion. On aggregation of $A\beta^2$ (**Figure 4A**), there is an *APOE ε4>ε2>E3* effect on the onset and extent of Aβ deposition in animal models[31,32,57]. The *ε4* allele accelerates the initial seeding and formation of Aβ plaques but with little effect on amyloid accumulation after plaque deposition begins[58]. In humans, *APOE ε4* carriers have greater amounts of soluble Aβ oligomers[59,60] with *APOE ε4* in vitro increasing Aβ oligomerization[60], while *APOE ε2* and *ε3* inhibit the conversion of protofibrils into fibrils[61]. Co-injection of ApoE3 (but not ApoE4) with Aβ protofibrils to rodent brain *in vivo* attenuated the deposition of Aβ plaques[61]. ApoE's direct binding to Aβ *in vitro* is dependent on isoform, cellular source, lipidation status, and Aβ species[62], though *in vivo* physiological relevance remains unclear.

APOE affects both the degradation and clearance of Aβ that normally occurs through cellular and enzymatic degradation, BBB clearance, interstitial fluid (ISF) bulk flow clearance, and CSF absorption into the circulatory and lymphatic systems[4]. *APOE ε4* is less efficient at soluble Aβ

clearance from the ISF and cellular uptake and subsequent degradation in astrocytes, microglia, and neurons are all attenuated in ApoE4 [57] (**Figure 4B-C**). Aβ clearance via the BBB also occurs in an ApoE isoform-dependent manner. ApoE2 and E3 mediate Aβ clearance through both the LRP1 and VLDLR receptors at the BBB, whereas ApoE4 only utilizes VLDLR, leading to slower Aβ removal[63] (**Figure 4C inset**). LDLR also mediates BBB Aβ clearance, likely through indirect mechanisms such as uptake into astrocytes and neurons[64] (**Figure 4C inset**). ApoE4 is also less efficient at transporting Aβ across BBB-associated pericytes via LRP1[4,65].

APOE isoforms may affect the production and secretion of Aβ from proteolytic cleavage of the amyloid precursor protein (APP)[2]. ApoE stimulated *APP* transcription and Aβ production in an isoform-specific manner *in vitro*, with greater production with ε4[66]. While human iPSC neurons with *ε4/ ε4* increased Aβ secretion more than *ε3/ε3*[67], ApoE had no effect on transcriptional regulation of *APP* in mouse models[68] or on APP or APP C-terminal fragments in vivo[57]. *APOE* does inhibit ϒ-secretase cleavage of APP in an isoform dependent manner[69].

Tau ("T")

While initial PET studies found no evidence of a direct effect of *APOE ε4* on tau deposition, there was an Aβ-dependent effect on tau pathology[50,70] (**Figure 3C**). *APOE ε4* carriers have increased levels of CSF p-tau and plasma p-tau217/p-tau181, likely related to the earlier brain deposition of Aβ[50,55,71]. *APOE ε4* carriers have more tau tangles post-mortem but only in the presence of Aβ[33,72] while *APOE ε2* was associated with lower burden of Aβ-mediated tau pathology[72,73] (**Figure 3C**). *APOE ε4* also mediates amyloid-related tau spreading in individuals with lower Aβ levels[49]. More recent studies show *APOE ε4* may have an Aβ-independent effect

on tau deposition in the medial temporal lobe[74-76], an effect potentially mediated by sex[74-76] and microglial response to tau pathology[77]. *APOE* also influences tau biomarkers in CSF and plasma[37] independently of CSF Aβ42[71],

While human studies have shown *APOE* has primarily an Aβ-dependent effect on tau, non-clinical studies have demonstrated Aβ-independent effects of *APOE* on tau aggregation. *In vitro* and mouse model studies have shown *ε4* increases tau hyperphosphorylation and aggregation[48,78,79], causing neurodegeneration in the absence of Aβ as mediated by microglial activation[48,78,79] (**Figure 4B**). Gut microbiota may mediate gliosis in tauopathy mice, as a potential link between dietary factors and *APOE*-mediated neurodegeneration[80]. Both astrocyte- and neuronal-derived APOE ε4 may play an active role in tau-mediated gliosis and neurodegeneration (**Figure 4B**)[74,81]. A recent human study supported the role of microglial activation in *APOE ε4* carriers having Aβ-independent effects on tau accumulation[77].

Other Biomarkers ("X") – Glial dysfunction

APOE plays a critical role in modulating the neuroimmune system, particularly microglia and astrocytes, in AD[82,83]. Disease-associated microglia (DAM) or microglia of neurodegenerative phenotypes (MGnD) increase *APOE* expression[82] (**Figure 4B**). Lack of *APOE* expression attenuates the DAM signature in AD mouse models[48,84], while *APOE ε4* expression increases microglial DAM signature[67,85]. *APOE* deletion leads to decreased plaque-associated microgliosis[84], while *APOE ε4* reduces plaque coverage and compaction by microglia (**Figure 4B**)[86,87]. Several studies have also demonstrated a link between *APOE* and triggering receptor expressed on myeloid cells 2 (*TREM2*, another genetic risk factor in AD) in regulating microglial response to Aβ pathology[83]. Microglia

may contribute to co-deposition of ApoE in amyloid plaques as part of a TREM2-dependent response[88,89]. *APOE ε4* also impairs the ability of microglia to phagocytose and degrade extracellular Aβ (**Figure 4B**)[90,91].

APOE ε4 exacerbates tau-mediated neurodegeneration by increasing microglial activation, infiltration of activated CD4 and CD8 T cells, and expression of DAM-associated genes[48,79,92], while reduction of ApoE decreases microgliosis and tau-mediated neurodegeneration[93,94] (**Figure 4B**). *APOE ε4* expression can induce a reactive astrocyte signature *in vitro* and *in vivo*[48], promoting neuronal death *in vitro* and brain atrophy *in vivo*[48]. Indeed, removal of astrocytic ApoE4 reduced tau-mediated neurodegeneration, with decreased disease-associated gene signatures in microglia, neurons, and oligodendrocytes[79].

ApoE may further modulate glial cell dysfunction in AD through glial lipid metabolism (**Figure 4B**). Deletion of *APOE* or the *APOE ε4* isoform promotes an accumulation of lipids in astrocytes and microglia[95-97]. *APOE ε4* impairs cholesterol transport out of microglia and increases cholesterol synthesis in astrocytes[95,97], leading to pro-inflammatory signaling[96,97], impaired astrocytic and microglial function, and glia-mediated neurodegeneration[96,98] (**Figure 4B**). *APOE ε4* expression caused aberrant cholesterol accumulation in oligodendrocytes, resulting in reduced myelination[99], and microglia-mediated infiltrating T cells also affect tau-mediated neurodegeneration in ε4-expressing mice[92] (**Figure 4B**).

Based on these non-clinical data, human studies also investigated the link between *APOE* and neuroimmune biomarkers in AD, showing an association between plasma GFAP and *ε4* carrier status in individuals diagnosed with AD[100,101]. One study also found a link between soluble TREM2 in CSF and *APOE ε4* carriers[102].

Other Biomarkers ("X") – Vascular Dysfunction

APOE affects the cerebrovasculature, being a known risk factor for ischemic stroke, vascular dementia, and cerebral amyloid angiopathy (CAA) that results from Aβ deposition in blood vessel walls leading to rupture and intracerebral hemorrhage[4,103]. CAA frequently co-occurs with AD[104], with moderate-to-severe CAA pathology observed in almost 50% of AD cases[104]. *ε4* carriers have the highest risk of CAA due to higher Aβ deposition in vessels leading to microbleeds, while *ε2* carriers have a higher risk of hemorrhage from CAA if present, given vessels are more prone to rupture[103] (**Figure 3C**). *APOE ε4* carriers show changes in multiple vascular biomarkers, including decreased cerebral blood flow (CBF), increased BBB breakdown, more white matter intensities, evidence of CAA, and increased CSF sPDGFRβ (soluble platelet-derived growth factor receptor beta)[105,106].

APOE has Aβ-independent effects on the BBB and cerebral vasculature, including direct effects on the neurovascular unit (NVU: neurons, astrocytes, brain endothelial cells (BECs), mural cells (vascular smooth muscle cells and pericytes), and endothelium)[105]. Independent of Aβ or tau biomarker levels, *APOE ε4* carriers have BBB breakdown seen by MRI in the hippocampus and medial temporal lobe, with increased severity in those with cognitive impairment compared to cognitively unimpaired[106]. *APOE ε4* transgenic mice showed similar increase in cerebrovascular permeability, with structural and cellular alterations leading to basement membrane degradation and impaired BEC function (**Figure 4C**)[105]. *APOE ε4* in mice leads to early disruption in the BBB transcriptome, resulting in progressive BBB breakdown and loss of pericytes[107], likely due to

peripheral *APOE* where liver-expressed *APOE ε4* impairs the cerebrovasculature, leading to synaptic dysfunction and worsened cognition[108].

APOE's effect on neuroimmune signaling in the CNS may also have a direct effect on the NVU, particularly through signaling between astrocytes, BECs, perivascular macrophages, and pericytes affecting BBB function[105]. The Aβ-mediated effect of *APOE* on vascular dysfunction may be linked to Aβ clearance across the BBB in *APOE ε4*, with perivascular accumulation of Aβ[57,63,65] leading to CAA with vessel wall breakdown and hemorrhage (**Figure 4C**)[103]. Inactivating *APOE* in Aβ-transgenic mice prevented the formation of CAA and associated microhemorrhages[109], while expression of human *APOE ε4* resulted in redistribution of Aβ from plaques to the vessels forming CAA[110], and removing astrocytic *APOE ε4* shifted Aβ deposition from plaques to CAA[111]. *APOE ε4* plays a role in CAA-related inflammation (CAA-ri), which occurs due to infiltration of neuroimmune cells around CAA-positive vessels (**Figure 4C**). This effect is likely due to a spontaneous immune response to Aβ[103], resulting in anti-Aβ antibodies detected in the CSF that bind to the CAA[112], inducing an inflammatory response via microglia, perivascular macrophages, and astrocytes[105].

The effect of *APOE ε4* on CAA may explain the mechanism of increased risk of amyloid-related imaging abnormalities (ARIA) in *APOE ε4* carriers[113]. ARIA is a treatment-emergent imaging abnormality that occurs with the use of anti-Aβ monoclonal antibodies that bind to aggregated forms of Aβ, characterized by parenchymal edema and sulcal effusions (ARIA-E) or microhemorrhages and hemosiderin deposition (ARIA-H)[103,114]. While the mechanism causing ARIA is not fully known, it is thought to be due to binding of anti-Aβ antibodies to CAA, resulting in perivascular inflammation from microglia or perivascular macrophages, followed by increased

vascular permeability with disruption of vascular integrity[103]. *APOE ε4* carriers have a clear increase in the risk of ARIA as shown in recent trials of anti-Aβ monoclonal antibodies[113], which may be due to the increased CAA in *ε4* carriers, resulting in increased CAA-related inflammation and hemorrhage[103,114].

Neurodegeneration ("N")

APOE likely has an upstream effect on neurodegeneration via the amyloid cascade and brain's innate immune response[83]. ^{18}F-FDG-PET measures showed *APOE ε4* carriers had lower cerebral glucose metabolism, correlating with Aβ pathology, brain atrophy, and cognitive measures across multiple stages of AD[115]. Recent studies have also shown a correlation between *APOE* genotype and CSF and plasma NfL levels[40,116]. Synapse loss associated with subsequent neuronal loss was also considered under neurodegeneration markers ("N")[2]. *APOE* also indirectly influences synaptic loss and dysfunction prior to neuron loss[2] where *APOE ε4* carriers have increased CSF synaptic biomarker SNAP-25[117], increased neurotoxic Aβ oligomers at synapses with synapse loss[59], and loss of synaptic proteins leading to impaired synaptic transmission[4] (**Figure 4D**).

***APOE* and the AD clinical care pathway**

Preceding sections described *APOE's* influence on AD pathophysiology through its effects on each component of the ATX(N) framework, thus priming *APOE's* utility in the AD clinical care pathway. The emergence of new AD therapies will likely transform the field, with *APOE* playing a key role in this transformation. Here, we will outline how *APOE* can fit into the next-generation AD clinical care pathway through *APOE* testing and APOE-targeted therapies.

APOE testing in AD clinical care

Contexts of use for APOE testing in AD care pathway

Genetic testing is becoming more widely used in clinical medicine, particularly in oncology and now in neurology[118]. Current genetic testing can be used to determine an individual's risk for a disease pathophysiology, improve accuracy of diagnosis or prognosis of disease, or for treatment selection and monitoring[119,120]. Thus far, *APOE* status has been considered in the context of AD risk. Since *APOE* is not deterministic in AD etiology, the predictive value of *APOE* testing has been limited[118], with the American College of Medical Genetics and the National Society of Genetic Counselors recommending against *APOE* testing in routine clinical practice[121].

APOE testing has been available through direct-to-consumer (DTC) genetic testing[118,122], with general public interest in obtaining testing[122]. DTC testing has raised ethical concerns given many companies do not provide genetic counseling to disclose risk of testing (ethical, legal, financial, and family) or educate consumers on the implications of test results[118]. DTC tests based on microarray have appreciable false positive/negative rates[123], of which consumers may not be aware. *APOE* testing has also become common in AD clinical trials for AD therapies in early stages and prevention trials to enrich for participants who are more likely to develop AD[118], with clear protocols on genetic counseling and disclosure[119,122]. Given *APOE* is not deterministic for AD and other variants may affect progression to AD[14,23], clinicians should be cautious of interpreting of *APOE ε4* status alone for AD risk determination.

With the recent emergence of biomarker-guided, pathway-based targeted therapies for AD, the clinical utility of *APOE* testing is now set to expand beyond just risk prediction for AD. As some of these therapies now suggest including *APOE* testing as part of treatment prescription (https://www.accessdata.fda.gov/drugsatfda_docs/label/2023/761269Orig1s001lbl.pdf)[124], APOE testing will become more prevalent as these therapies become more widely available in the maturing AD clinical care pathway. There is no current consensus on how *APOE* testing should be used in AD clinical practice due to ethical considerations[120]. *APOE* testing may be used and qualified for multiple contexts-of-use (CoUs) in the next-generation AD care pathway[35], including initial evaluation and diagnosis of AD, treatment selection and monitoring, and possibly screening during healthy aging in the future. How the insights gained from genetic research may affect biomarker development and context-of-use will largely depend on widespread application of novel high-throughput technologies[125].

As biomarker-guided AD therapies are now becoming clinically available, *APOE* testing will first extend to treatment selection and monitoring. Growing evidence shows *APOE* genotype plays a role in the risks and benefits of new AD therapies[126], with variable risks and benefits in *APOE ε4* carriers[126], which may influence clinical decisions on which therapy is appropriate for *ε4* carriers versus non-carriers. Other AD therapies in development are specifically being tested in *APOE ε4* carriers, including ApoE-targeted therapies (see below)[23,127]. *APOE ε4* carrier status may also affect the treatment monitoring protocol, particularly regarding risks of adverse effects. *APOE* testing is now suggested for monoclonal antibodies targeting Aβ for ARIA risk monitoring (https://www.accessdata.fda.gov/drugsatfda_docs/label/2023/761269Orig1s001lbl.pdf)[124], which will likely increase the use of *APOE* testing in AD care.

While initial *APOE* testing in the AD care pathway may begin in the context of therapeutic decision-making, *APOE* could also be used during initial evaluation of AD with an AD specialist, specifically if done in conjunction with other AD biomarkers. One BBBM test already combines an ApoE proteoform assay with age and plasma Aβ42/40 levels to increase prediction of brain Aβ[56]. As BBBM tests become more widely used in clinical practice, they may also provide *APOE* status results, possibly circumventing the need for separate *APOE* testing. Use of *APOE* testing during this AD evaluation stage can help risk stratify individuals prior to initiating treatment with AD therapies. If ongoing AD prevention trials using therapies that stratify based on *APOE* show benefit[128], *APOE* testing may have clinical utility in this population. Furthermore, if studies investigating the effect of lifestyle modifications on cognition based on *APOE* genotype have success[129], then identifying *APOE* status early to initiate lifestyle changes may prove beneficial. The combination of *APOE* with the polygenic risk score (PRS) may better predict AD risk and provide clinical validity for genetic testing in non-clinical AD[130,131].

Considerations for APOE testing in clinical practice

The impending use of *APOE* testing in AD clinical care necessitates guidance for healthcare providers on how to use, interpret, and communicate *APOE* results in the context of the scientific evidence discussed in this review. *APOE* testing also comes with emotional, family, ethical, legal, and financial implications that should be considered prior to obtaining testing (**Text Box 1**).

A series of randomized controlled trials called the Risk Evaluation and Education for Alzheimer's disease (REVEAL) Study evaluated the impact of providing *APOE* testing to individuals with first-degree relatives with AD, particularly on stress, depression and anxiety, cognitive test

performance, and changes in health behavior[118]. The initial study showed no differences in depression or anxiety between those who received their *APOE* results and those who did not, although they found individuals who were *APOE ε4* positive had slightly higher levels of short-term distress compared to those who were *APOE ε4* negative[132]. Other studies found learning one's *APOE* results can affect perceived memory abilities and performance on cognitive tests, suggesting knowledge of *APOE* status may bias cognitive testing results[133]. *APOE* status disclosure led to changes in health behavior, including taking nutritional supplements and purchasing long-term care (LTC) insurance, particularly in *ε4* carriers[119,134,135].

Disclosing *APOE* status may impact family members, given the increased likelihood of family members also carrying *ε4* allele in those who test positive[120]. The potential risks for family members should be discussed with individuals before and after obtaining testing[119] (**Text Box 1**), particularly given many individuals bring family members to clinical visits. Most individuals chose to share their *APOE* testing with family members, although not all family members pursued testing thereafter[119]. Individuals who learn their *APOE* status also expressed concerns about stigma and discrimination particularly in the workplace, although the Genetic Information Nondiscrimination Act (GINA) passed in the United States in 2008 prohibits employers and insurance companies from using genetic information to make decisions on hiring or insurance coverage and premiums[118,119]. However, GINA does not cover life, disability, or LTC insurance, so insurers could increase LTC premiums or deny coverage based on *APOE* genotype, an important concern if *APOE* testing becomes more widely used[118]. Given the guidelines recommending against routine *APOE* testing, insurance companies typically do not cover the cost of testing (except for symptomatic individuals), affecting the accessibility of testing.

Given the ethical, legal, and financial implications surrounding *APOE* testing, appropriate protocols will be necessary for *APOE* testing and disclosure prior to widespread clinical use. These protocols can be developed from those in clinical trials and based on national guidelines developed in other countries (**Text Box 1**)[118,122]. When assessing an individual's *APOE* status in the clinical setting, clinicians can consider specific questions and how to discuss these issues with patients (**Text Box 1**). As *APOE* testing becomes more widely used, tools for discussing *APOE* results in the clinical setting can be developed as they have been for other diseases[136].

APOE and Precision Medicine (PM) in AD

APOE testing can be one of the first steps towards implementing PM in AD. The concept of PM has already become well-established in oncology, with genetic testing identifying risk for developing certain cancers, treatment selection, and monitoring[137]. PM in AD should embrace the P4 paradigm (predictive, preventive, personalized, and participatory), with *APOE* testing playing a role in predicting disease risk, early AD detection and intervention, tailoring treatments to individual patient characteristics, and providing patient-centered data collection and communication[137].

Whole-genome sequencing studies in AD have identified variants in other genes that modify *APOE's* effect on AD risk or influence similar pathways as *APOE*[23]. These studies highlight the importance of considering the entire genetic landscape of an individual in determining AD risk. Since the first GWAS studies in AD, at least 75 risk loci in addition to *APOE* have been associated with AD[138,139], which can be incorporated in the PRS to improve AD risk determination[130,131]. While the PRS alone performs worse than *APOE* in predicting AD risk, the

525 combination of PRS with *APOE* increases predictive value[130]. The PRS may offer an even more
526 predictive and personalized approach to AD in the future[130,131].
527
528 ApoE-targeted therapies
529 Given *APOE*'s role in multiple aspects of AhaD pathogenesis, one attractive option is
530 targeting ApoE itself for AD therapy. With the advent of AD treatments that target Aβ and tau,
531 ApoE could be a good therapeutic target as an adjuvant to these other treatments, including
532 using anti-ApoE antibodies to facilitate clearance of ApoE-Aβ complexes in plaques and CAA,
533 decreasing ApoE levels or switching *APOE* isoforms using gene therapy, and increasing ApoE
534 lipidation[23] (**Text Box 2**). Given *APOE*'s primary influence on Aβ pathophysiology and tau-
535 mediated gliosis, ApoE-targeted therapies may be used prior to or in conjunction with anti-Aβ
536 and anti-tau therapies. We will not review all of the potential ApoE-targeted therapies in
537 development here given a recent comprehensive review[23]; instead, **Text Box 2 and Figure 5**
538 highlight those ApoE-targeted therapies that may be used as an adjuvant to other emerging
539 therapies.
540 ApoE-targeted therapies may be used in combination with emerging anti-Aβ and anti-tau
541 therapies for improved therapeutic efficacy (reduction of aggregated Aβ, tau, or
542 neurodegeneration leading to improved cognition) and safety (reduction of CAA and associated
543 neuroinflammation). ApoE-targeted treatments may be used prior to or in parallel with these
544 other AD therapies to provide a synergistic effect (**Figure 5**). For example, anti-ApoE antibodies
545 that bind specifically to amyloid plaques and CAA could be used in early-stage AD to reduce Aβ
546 plaques and CAA to remove this pathology and reduce ARIA risk[140]. Similarly, *APOE* allele

switching from ε4 to ε2 prior to or in conjunction with Aβ-targeted treatments could mitigate the risk of ARIA in ε4 carriers[113,114], although careful monitoring for intracerebral hemorrhage is needed given increased risk of CAA-related hemorrhage with *APOE ε2*[103]. *APOE* ASOs may be effective in preclinical AD prior to the onset of plaques[141], but also later as they have been shown to decrease tau-mediated neurodegeneration[94]. In the future, a treatment targeting the *APOE ε4* allele may have the greatest utility for the prevention of AD by screening for and reducing the risk allele in the general population.

There are challenges to translating ApoE-targeted therapies into humans, particularly given the complex role *APOE* plays in AD pathophysiology, the differential effects of peripheral versus CNS ApoE, and the methods used to target CNS-specific ApoE[23] (**Figure 5**). Any ApoE-targeted treatment will need to evaluate its peripheral and central effects (**Figure 5**). Certain anti-ApoE antibodies have been shown to reduce serum cholesterol in *APOE ε4* and *ε2* transgenic mice, possibly providing beneficial peripheral as well as central effects[142]. However, switching from the ε4 to ε2 allele could have deleterious consequences in the periphery given the association with type III hyperlipoproteinemia[12] (**Figure 5**). Changing the balance of ε4 and ε2 or decreasing ε4 levels in the periphery could also increase the risk of hyperlipidemia, atherosclerosis, and cardiovascular events, while expression of *APOE ε2* in the CNS may have adverse consequences given the association of *ε2* with CAA-related intracerebral hemorrhage[103], primary tauopathy[143], and possibly glaucoma[144], necessitating monitoring for these events in future trials of these therapies.

For ApoE-therapies to move from research investigation into clinical practice, these challenges must be addressed in forthcoming clinical trials. Most ApoE therapies are still in the

non-clinical stage[23], so future human trials should be designed with consideration of the use of and timing with anti-Aβ monoclonal antibodies. These trials should also monitor for potential adverse events as described above, with attention to peripheral lipid metabolism, ARIA, and intracerebral hemorrhage. If these trials are successful, how ApoE treatments could be used for AD prevention at a population level will need to be evaluated. Recent advances in our understanding of the protective effects of *APOE ε2* and the role of rare *APOE* variants may pave the way for new therapeutic methods[28], such as a recent ApoE antibody mimicking the *APOE Christchurch* mutation (**Text Box 2**)[145]. Advances in gene therapy use from clinical trials to clinical practice will accelerate the use of these therapies in AD clinical care.

Conclusion

Thirty years of scientific and clinical research advances have demonstrated how *APOE* plays a central role in AD pathogenesis. With the transformation of AD into a clinical-biological construct via the ATX(N) biomarker framework, *APOE* can now be incorporated into this concept and moved from the research space into clinical practice. The *APOE* genotype has direct augmentative effects on biomarkers of core Aβ pathology, as well as indirect effects on tau and neurodegeneration biomarkers, with emerging evidence showing its role in vascular and glial biomarkers. More work is still needed to elucidate the mechanisms by which each of the isoforms contribute to each component of disease progression (**Text Box 3**). *APOE* testing is now being increasingly incorporated into multi-modal AD biomarker testing, including neuroimaging, CSF, and blood-based biomarkers, which can be used for earlier detection and AD diagnosis in the future.

591 The current ATX(N) framework does not account for the additional complexities of AD
592 onset, particularly the interplay of genes, biological determinants, and environmental factors[137].
593 This complexity can best be explained by a systems theory approach, using a combination of
594 systems biology, systems neurophysiology, and quantitative systems pharmacology to provide a
595 thorough conceptual framework to understand AD processes[137]. Recent scientific progress in the
596 "omics" of AD are beginning to provide the basis for future liquid biopsy capturing heterogeneity
597 and individual variability in underlying biology and clinical manifestations, which can be used to
598 expand the ATX(N) framework and move toward a PM model of AD[137].

599 *APOE* testing is primed to transition into the next-generation AD clinical care pathway,
600 where it may be used for initial evaluation of AD with other biomarkers, treatment selection and
601 monitoring of emerging AD therapies, and possible screening during healthy aging. As new AD
602 therapeutics are brought to market, the role of APOE status on disease antecedents, detection,
603 efficacy and safety responsivity will manifest under real world conditions where longitudinal data
604 will become highly informative to ultimate treatment selection. More work needs to be done to
605 determine how the *APOE* genotype affects the risks and benefits of emerging therapies prior to
606 its clinical use, and more data from early intervention trials in AD are needed to determine the
607 clinical utility and validity of early *APOE* screening during healthy aging. With the rapid progress
608 in genomics and epigenomics in AD, the addition of other genetic and epigenetic risk factors with
609 *APOE* will help identify biologically defined subgroups of the heterogeneous AD population to
610 tailor biomarker-guided individual treatment plans. As larger and more comprehensive lifestyle
611 modification studies in AD, such as the FINGER trial, are conducted[129], we may find specific
612 interventions benefit genetic subgroups, and lead to more personalized and participatory AD

613 care [137]. Thus, *APOE* will be an important initiating element for the future healthcare practice of
614 PM in AD, hopefully transforming practice in other prevalent neurodegenerative and neurological
615 diseases.
616
617
618
619
620 **Table and Figure Legends**
621 **Figure 1: Structure and function of ApoE in the periphery and CNS**
622 A) Linear structure of ApoE protein showing N-terminal domain (red; 1-167 amino acids), LDLR
623 receptor binding domain (yellow; 136-150 amino acids), hinge region (black; 167-206 amino
624 acids), and C-terminal domain (blue; 206-299 amino acids) reprinted from Chen et al with
625 permission[10] (Copyright @ 2020 Elsevier Inc.). ApoE isoforms are differentiated by positions 112
626 and 158. B) Full-length 3D structure of ApoE3 by NMR (PDB:217b) reprinted from Chen et al
627 with permission[10] (Copyright @ 2020 Elsevier Inc.) demonstrating folding and interaction
628 between N-terminal, hinge, and C-terminal domains with color-coding as in part A. C) The
629 amino acid substitutions between ApoE isoforms are shown in 3D structure. D) Diagram shows
630 the varied functions of ApoE in the periphery and CNS. *Left*: ApoE is produced primarily by the
631 liver in the periphery, where it is involved in cholesterol metabolism, with ApoE2 and E3
632 binding HDL particles and ApoE4 binding VLDL particles. Decreased binding of ApoE2 to LDLR
633 impairs clearance of lipoprotein particles while ApoE4 binding VLDL leads to downregulation of
634 LDLR and increased plasma cholesterol. *Right*: ApoE does not cross the BBB, but is produced

primarily by astrocytes in the CNS, which transfer cholesterol to the ApoE protein via ABCA1/ABCG1 receptors. The size of ApoE lipoprotein decreases from E2 to E3 to E4 due to the differential transfer of cholesterol. ApoE is then taken up by neurons via the LDLR and LRP1 receptors, with preferential uptake of ApoE2 and E3 by LRP1 and E4 by LDLR.

Figure 2: *APOE* genotype and the risk of AD

A) Lifetime risk of AD based on age and genotype adapted from Reiman et al[14] (Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/) B) *APOE* allele frequencies in different populations[8]. C) *APOE* genotype frequencies in different populations[7]. D) Odds ratio (OR) for AD in different ethnic populations based on *APOE* allele[7]. E) Odds ratio (OR) for AD in different ethnic populations based on *APOE* genotype[7].

Figure 3: Effect of *APOE* on AD biomarkers in AT(N) Framework

A) Hypothetical biomarker-based model of AD pathophysiology demonstrating the AT(N) framework, with initial changes in amyloid beta (A) leading to downstream effects on tau (T) and neurodegeneration and related synaptic changes (N) leading to cognitive decline and decrease in clinical function (adapted from Hampel et al[2] and Jack et al[146]). Aβ can be detected by Aβ PET or CSF Aβ42/40, with many p-tau species correlating with plaque load as well. Tau can be detected by tau PET and neurodegeneration can be detected by MRI, CSF t-tau or CSF/plasma NfL. B) Effect of *APOE ε4* and *APOE ε2* on amyloid beta (A) biomarkers compared to *APOE ε3* as baseline. C) Effect of *APOE ε4* and *APOE ε2* on tau (T) biomarkers compared to

APOE ε3 as baseline. D) Downstream effect of *APOE ε4* and *APOE ε2* on cognition following changes in AD pathophysiology within AT(N) framework

Figure 4: Relationship between ApoE and underlying AD pathophysiology

A) *APOE* and Aβ: *APOE,* particularly *APOE ε4*, promotes aggregation of Aβ from monomer to intermediate oligomers/protofibrils to fibrils that compose Aβ plaques. Aβ aggregation leads to downstream effects on gliosis, vascular, and synaptic dysfunction. Inset: Differential clearance of Aβ aggregates at the blood-brain barrier (BBB), with decreased perivascular drainage in *ε4* compared to *ε3* carriers. *APOE ε2* and *ε3* mediate Aβ clearance through both the LRP1 and VLDLR receptors, while *APOE ε4* switches Aβ clearance from LRP1 to solely VLDLR. B) *APOE* and Gliosis: (1) Microglia interact with Aβ plaques near ApoE co-deposition and *APOE ε4* impairs microglial phagocytosis and degradation of Aβ aggregates. *APOE ε4* changes microglial transcriptomic signature to a pro-inflammatory state, which coupled with tau aggregation, leads to neurodegeneration in tau mouse models. Reactive microglia also interact with infiltrating T cells to facilitate tau-mediated neurodegeneration. (2) Both LRP1 and LDLR are involved in Aβ uptake into astrocytes. ApoE4 competes with Aβ for uptake into astrocytes via LRP1, resulting in decreased Aβ uptake. *APOE ε4* astrocytes become more reactive, leading to increased tau aggregation and neurodegeneration. (3) *APOE ε4* impairs cholesterol transport out of microglia, increases cholesterol synthesis in astrocytes, and increases cholesterol synthesis and intracellular storage in oligodendrocytes, leading to glia-mediated neurodegeneration and demyelination. *APOE ε4* mediates the interaction between microglia, astrocytes, and glial cells in these pathways. C) *APOE* and Vascular Dysfunction: *APOE ε4*

carriers have increased CAA, leading to BBB leakiness that can cause hemorrhage and changes in the inflammatory milieu leading to CAA-ri. Changes in pericyte-astrocyte signaling may underlie these downstream effects. *APOE ε4* independently affects many components of cerebrovascular function, including direct effects on the neurovascular unit (not shown). Inset: Aβ is less efficiently cleared at the BBB in *ε4* carriers, with ApoE2 and E3 mediating Aβ clearance through both the LRP1 and VLDLR receptors, while ApoE4 only utilizes VLDLR. LDLR also mediates Aβ clearance at the BBB, likely through uptake into astrocytes, with ApoE3 and ApoE4 having much stronger binding affinity to LDLR compared to ApoE2. There is impaired perivascular drainage of Aβ with ApoE4, resulting in Aβ accumulation in periarterial spaces, leading to CAA. D) *APOE* and synaptic dysfunction: *APOE ε4* carriers have increased accumulation of neurotoxic Aβ oligomers that interact with ApoE at synapses, with increased synapse loss around plaques. *APOE ε4* expression also resulted in decreased spine density and loss of synaptic proteins, leading to impaired LTP and synaptic transmission.

Figure 5: Overview of ApoE-targeted therapies

Schematic demonstrating three major ApoE-targeting therapies: anti-ApoE antibodies, ApoE ASOs, and *APOE* allele switching. The figure summarizes the mechanism of action, effect on AD pathology, treatment timing, effects of peripheral vs. central administration, and potential challenges of translating each of these therapies into the clinic.

Table 1: *APOE* genotype/allele frequencies, odds ratio (OR), and lifetime risk for AD by ethnicity and sex

Top panel: Population-based studies demonstrating *APOE* genotype and allele frequencies in different ethnicities with associated odds ratio (OR) for AD[7,8]. Middle panel: Results using Alzheimer's disease-normal cognition data set showing lifetime risk for AD based on genotype and sex[5]. Bottom panel: Results using Rochester incidence rates showing OR for AD based on genotype and sex[9]

Text Boxes

Text Box 1: Considerations and discussion points for clinicians and patients/caregivers prior to *APOE* testing

(1) Patient characteristics to consider for *APOE* testing:

a. Symptomatic individuals considering biomarker-guided targeted therapies[118]

b. Given limited predictive value at this time, guidelines do not recommend testing asymptomatic individuals unless for enrollment in preventative clinical trial. In the future, if preventative trials show benefit in this population, they may be considered for testing[118]

c. Particular attention to individuals who already exhibit cognitive symptoms for genetic counseling[118,120]

(2) Genetic counseling pre- and post-testing

a. Genetic counseling should be ideally conducted by trained healthcare providers or genetic counselors[118]

b. Testing not recommended for those with psychiatric disorders that may interfere with comprehension of potential benefits and harms of *APOE* testing, particularly those patients for whom *APOE* disclosure may trigger suicidal ideation[118,122]

c. Genetic risk assessments should be patient-centered and consider sex and ethnic diversity given different risk estimates in different populations.

d. Future research studies investigating the impact of genotyping results should be more ethnically, socioeconomically, and culturally diverse.

e. Discussion of benefits/risks should be inclusive of all stakeholders, including family members who may be affected

(3) Education of HCPs and patients/caregivers on *APOE* testing

a. Use visual aids for education of individual risk during counseling, such as age/sex/ethnicity specific incidence curves for *APOE*[118,120]

b. Develop take-home educational materials to reinforce the knowledge and provide strategies for coping with risk[118]

c. Consider medical, ethnic, and socioeconomic factors that may impact understanding of genetic testing results[118]

(4) Equitable accessibility to tests,

a. Guidelines need to be updated for risk-assessment *APOE* testing prior to biomarker-guided, targeted therapy use

b. Insurance companies should cover *APOE* testing prior to the administration of biomarker-guided targeted therapies[118]

744 c. Additional legal protections needed to prevent LTC insurance discrimination and
745 other stigma associated with *APOE* genotype[118]
746 (5) Questions for clinicians to consider prior to testing[118]
747 a. Is the patient eligible for a biomarker-guided targeted therapy? Will *APOE* genotype
748 affect treatment choice or monitoring?
749 b. Is the patient experiencing symptoms of AD and can they comprehend the
750 information to make an informed decision?
751 c. Is the patient psychologically able to cope with test results?
752 d. Does the patient have all eligible insurance coverage, including health, life, and LTC?
753 e. Does the patient plan to discuss test results with family members who may have
754 increased risk of carrying *ε4* allele?
755 (6) Talking points for clinicians to discussion *APOE* testing with patients and care-
756 partners[136]*:*
757 a. Use plain language, provide only key information, keep discussion interactive,
758 use visual aids
759 b. AD is caused by multiple factors, both genetic and environmental, some of which
760 are not known yet. Whether or not this gene variant is present, other factors can
761 also influence the chance of developing the disease.
762 c. This testing can look for one of the gene variants involved in AD, and can help
763 make decisions for starting a particular treatment.
764 Text Box 2: ApoE-targeted therapies
765 *Anti-ApoE antibodies*

One therapeutic approach to *APOE* has focused on removing ApoE/Aβ complexes using anti-ApoE antibodies. The anti-human ApoE antibody HAE-4 reduces insoluble Aβ and plaques by preferentially binding to non-lipidated ApoE present in Aβ plaques and CAA without affecting other physiological forms of ApoE[140]. This antibody decreased CAA in mice and rescued CAA-induced cerebrovascular dysfunction, while anti-Aβ antibodies can exacerbate CAA and related microhemorrhages[140]. Using anti-ApoE antibodies alone or in conjunction with anti-Aβ antibodies may offer the possibility of removing Aβ from brain parenchyma and CAA with less risk of ARIA if similar effects are seen in humans. Removing ApoE/Aβ complexes may mitigate downstream Aβ-mediated tau seeding and spreading as shown in one study, suggesting that targeting this interaction can have effects on other AD pathophysiology[147]. A recent anti-ApoE antibody mimicked the *APOE-Christchurch* mutation by reducing ApoE-HSPG interaction and ameliorating tau pathology in mice[145], providing a novel approach combining genetics and antibodies for an ApoE-targeted therapy.

APOE gene therapy

Another therapeutic approach for ApoE has been using gene therapy to switch *APOE* isoforms from *ε4* to the protective *ε2* allele[23]. Viral gene delivery of *APOE ε2* in AD mouse models reduced oligomeric Aβ and plaque formation[23,148,149], and is now being tested in human clinical trials[23]. Switching *ε4* carriers to *ε2* could be used prior to the initiation of anti-Aβ or other therapies to allow for better efficacy and safety profiles of these treatments. Gene therapy using antisense oligonucleotides (ASOs) is also being used to lower the overall levels of *APOE ε4*[23]. ASOs lowering

APOE ε4 levels reduce Aβ plaque deposition, but only if used prior to the onset of Aβ pathology[141]. *APOE ε4* reduction using ASOs can also mitigate tau aggregation and tau-associated gliosis and neurodegeneration[94].

Text Box 3: Key outstanding questions regarding *APOE* in the field

APOE and AD clinical progression

1. What are the biological and environmental factors contributing to *APOE's* sex and ethnic differences in AD?
2. How does *APOE* modulate clinical progression based on sex and ethnicity?
3. What are the mechanisms underlying the gene-environment interactions with *APOE* in AD?

AD biomarkers and pathophysiology

1. How does APOE contribute to Aβ aggregation and the role of APOE lipidation in this process?
2. What are the mechanisms by which APOE contributes to tau aggregation and gliosis independent of Aβ?
3. How does *APOE* influence Aβ-dependent and Aβ-independent mechanisms of tau aggregation?
4. What are the exact mechanisms underlying *APOE*'s role in CAA and CAA-ri? How does the relationship between CAA and parenchymal Aβ in AD play a role in this process?

5. How does *APOE ε2* confer a protective effect for AD but increases the risk of hemorrhage from CAA?
6. What role does *APOE ε2* play in tau aggregation and tau-related mechanisms of AD pathogenesis?
7. How does the interplay of *APOE*, tau aggregation, and glial cells contribute to neurodegeneration in AD?
8. How does *APOE* genotype influence new AD biomarkers ("X")?
9. How can *APOE* genotyping be used in combination with other AD biomarker testing to improve early AD diagnosis?

APOE in clinical practice

1. How can *APOE* be used in conjunction with other genetic factors (i.e., PRS) to better identify risk for AD?
2. How does *APOE* influence the efficacy and safety of emerging biomarker-guided targeted therapies?
3. What are the best practices to implementing *APOE* testing into the current AD clinical care pathway?
4. Will early *APOE* screening during healthy aging lead to lifestyle interventions that possible provide clinical utility?
5. How can emerging *APOE*-targeted therapies be used in combination with other biomarker-guided targeted therapies to provide better efficacy and safety for AD patients?

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Competing Interests

Dr. Hampel is an employee of Eisai Inc. He serves as Reviewing Editor for the Journal Alzheimer's & Dementia.

He is inventor of 11 patents and has received no royalties:

- In Vitro Multiparameter Determination Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Patent Number: 8916388
- In Vitro Procedure for Diagnosis and Early Diagnosis of Neurodegenerative Diseases Patent Number: 8298784
- Neurodegenerative Markers for Psychiatric Conditions Publication Number: 20120196300
- In Vitro Multiparameter Determination Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Publication Number: 20100062463
- In Vitro Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Publication Number: 20100035286
- In Vitro Procedure for Diagnosis and Early Diagnosis of Neurodegenerative Diseases Publication Number: 20090263822

• In Vitro Method for The Diagnosis of Neurodegenerative Diseases Patent Number: 7547553

• CSF Diagnostic in Vitro Method for Diagnosis of Dementias and Neuroinflammatory Diseases Publication Number: 20080206797

• In Vitro Method for The Diagnosis of Neurodegenerative Diseases Publication Number: 20080199966

• Neurodegenerative Markers for Psychiatric Conditions Publication Number: 20080131921

• Method for diagnosis of dementias and neuroinflammatory diseases based on an increased level of procalcitonin in cerebrospinal fluid: Publication number: United States Patent 10921330

Dr. Holtzman co-founded, has equity in, and is on the scientific advisory board of C2N Diagnostics. He is on the scientific advisory board of Denali, Cajal Neuroscience, and Genentech and consults for Alector. He is an inventor on US patent application US-20190270794-A1 "Anti-APOE antibodies". Anti-APOE antibodies and this patent application have been licensed by Washington University the NextCure.

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