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ApoE cascade hypothesis in the pathogenesis of Alzheimer's disease and related dementias

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Summary

The ε4 allele of the *apolipoprotein E* gene (*APOE4*) is a strong genetic risk factor for Alzheimer's disease (AD) and several other neurodegenerative conditions including Lewy body dementia (LBD). The three *APOE* alleles encode protein isoforms which differ from one another only at amino acid positions 112 and 158; apoE2 (C112, C158), apoE3 (C112, R158), and apoE4 (R112, R158). Despite progress, it remains unclear how these small amino acid differences in apoE sequence among the three isoforms lead to profound effects on aging and disease-related pathways. Here, we propose a novel "ApoE Cascade Hypothesis" in AD and age-related cognitive decline that the biochemical and biophysical properties of apoE impact a cascade of events at the cellular and systems levels ultimately impacting aging-related pathogenic conditions including AD. As such, apoE-targeted therapeutic interventions are predicted to be more effective by addressing the biochemical phase of the cascade.

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Author Contributions

Y.A.M. and T.K. led the initial drafting of the manuscript, figures and edited the manuscript. N.Z., C-C.L., A.J.Y., A.M.G., and D.M.H co-edited the manuscript. GB supervised the writing and edited the manuscript. All authors have read and agreed on the final manuscript.

Declaration of Interests

GB consults for SciNeuro and Lexeo, has consulted for Vida Ventures, AbbVie, E-Scape, and Eisai, is on the scientific advisory board of Kisbee Therapeutics, and serves as a Co-Editor-in-Chief for Molecular Neurodegeneration. DMH is as an inventor on a patent licensed by Washington University to C2N Diagnostics on the therapeutic use of anti-tau antibodies. DMH co-founded and is on the scientific advisory board of C2N Diagnostics. C2N Diagnostics has licensed certain anti-tau antibodies to AbbVie for therapeutic development. DMH is on the scientific advisory board of Denali, Genentech, and Cajal Neurosciences and consults for Eli Lilly. DMH receives sponsored research agreements to Washington University from NextCure, C2N Diagnostics, Yumanity, Eli Lilly, and Novartis. AMG is on the scientific advisory board of Genentech and has consulted for Cognition Therapeutics and AbbVie. Other authors declare no competing interests.

In brief:

In this review, Martens et al. propose a novel "ApoE Cascade Hypothesis" that the biochemical and biophysical properties of apoE impact a cascade of events at the cellular and systems levels ultimately leading to Alzheimer's disease and age-related cognitive decline.

Introduction

The ε4 allele of the *apolipoprotein E* gene (*APOE4*) vastly increases the risk for Alzheimer's disease (AD) compared to the more common *APOE3* allele, while *APOE2* is protective (Corder et al., 1993; Corder et al., 1994; Farrer et al., 1997; Saunders et al., 1993). *APOE4* not only increases the risk but also lowers the age at onset of AD in a dose-dependent manner (Corder et al., 1993; Sando et al., 2008). In addition to AD, *APOE4* is also associated with the risk for age-related cognitive decline in non-demented individuals as well as other neurodegenerative conditions such as Lewy body dementia (LBD) and TDP-43 pathology in AD (Bras et al., 2014; Dhana et al., 2021; Guerreiro et al., 2018; Tsuang et al., 2013; Wennberg et al., 2018; Yang et al., 2018). Despite these strong genetic associations, the molecular pathobiology underlying the differential effects of the three apoE isoforms remains puzzling.

AD is a progressive neurodegenerative disease neuropathologically characterized by the deposition of amyloid-β (Aβ) cleaved from amyloid precursor protein (APP) as senile plaques and hyperphosphorylated tau as neurofibrillary tangles in the brain (Alzheimer's Association, 2021). Given that Aβ accumulation appears to precede the onset of other AD phenotypes such as neocortical tauopathy and cognitive impairment; Aβ may contribute to diverse pathways related to the disease onset and progression. As such, the "amyloid cascade hypothesis" has long been considered central to the pathogenesis of AD (Hardy and Higgins, 1992). Indeed, this hypothesis is well supported by genetic evidence from autosomal dominant AD (ADAD) cases in which mutations in *APP, PSEN1*, or *PSEN2* are causatively involved in AD development by increasing APP amyloidogenic processing and Aβ production or its seeding propensity. However, with >99% of AD cases being sporadic (Bekris et al., 2010) where a variety of mixed pathologies are present in AD brains, the linearity and broader relevance of the "amyloid cascade hypothesis" is at times challenged. Further, the therapeutic efficacy of various Aβ-targeting approaches on cognitive decline during the symptomatic phase of AD are limited despite effectiveness in reducing brain Aβ deposition (Knopman et al., 2021). Other hypotheses such as the "cellular phase of AD" or a consideration of the other elements both downstream but also independent of Aβ (Musiek and Holtzman, 2015) have been proposed to link broader pathways impacted in AD, integrating both the effect of pathological tau as well as other brain cell types in particular astrocytes, microglia, oligodendrocytes, and vascular cells.

How apoE pathobiology fits into these existing hypotheses represents an opportunity for exploring therapeutic avenues targeting apoE in AD and related dementias. Interestingly, a recent case report showed that carrying two copies of the *APOE3* p.R136S referred to as *APOE3* Christchurch mutation is linked with preserved cognition at a much later age than expected despite high brain amyloid levels due to FAD-linked *PSEN1* p.E280A mutation

(Arboleda-Velasquez et al., 2019). This *APOE* polymorphism is located in the receptor binding region of apoE and has been suggested to be protective by reducing apoE binding to the heparan sulfate proteoglycan (HSPG) (Arboleda-Velasquez et al., 2019). We have also recently reported that a rare apoE3 variant, *APOE3* p.V236E referred to as *APOE3* Jacksonville variant, reduces amyloid plaques and neuronal damage by preventing apoE self-oligomerization and promoting lipid metabolism (Liu et al., 2021). These studies support the notion that changes in the biochemical properties of apoE such as receptor binding, oligomerization, and lipid metabolism have differential impacts on cellular functions which manifest as phenotypic changes leading to eventual effects on disease onset. Mounting evidence has demonstrated the importance of apoE in the pathogenesis of AD and age-related cognitive decline (Frisoni et al., 2022); however, there has not been a central hypothesis that links the apoE isoform-related molecular events to the cellular changes and eventually to the disease manifestation of AD. Herein, based on accumulating evidence from biochemical, cellular, animal, and human studies, we propose a novel "ApoE Cascade Hypothesis" in AD and age-related cognitive decline that the biochemical and biophysical properties of apoE initiate a cascade of events at the cellular and systems levels ultimately impacting aging-related pathogenic conditions including AD (Figure 1), thus preventative or therapeutic interventions are likely to be more effective by targeting the apoE biochemical phase of the cascade.

Overview of ApoE Cascade Hypothesis

This cascade starts from the biochemical and biophysical properties of apoE including apoE structure, lipidation, oligomerization, protein levels, and receptor binding (Figure 1). *APOE* genotype or rare variants, epigenetics, posttranslational modifications, aging, sex, and lifestyle can all affect this phase. These biochemical/biophysical differences are then propagated to functional effects on cellular homeostasis including events known to be differentially impacted by apoE isoforms such as cellular stress (autophagy, mitochondria stress, ER stress), endosomal-lysosomal trafficking, and lipid metabolism. Some of these cellular effects can be either cell autonomous in cells expressing abundant apoE (astrocytes and reactive microglia in the brain, hepatocytes and macrophages in the periphery, and vascular mural cells interfacing the periphery with the brain), or non-cell autonomous (e.g., by binding to apoE receptors on neurons which themselves express little apoE). These cellular effects lead to systems level phenotypes highlighted by neuroinflammation, vascular dysfunction, neuropathology, synaptic loss, and neurodegeneration, leading to age-related cognitive decline and other aging-related pathological conditions such as AD. In simpler terms, the qualitative and/or quantitative changes of apoE depending on its isoforms and other modifications during aging trigger the cascade of pathogenic events leading to cognitive impairment and dementia. Biological events associated with aging such as oxidative stress, cellular senescence, chronic inflammation, glial activation, and lifestyle including sleep pattern, diet, and activity level can contribute to the cascade both in an apoE-dependent, through the biochemical phase, and in an apoE-independent manner by directly impacting cellular and phenotypic phases.

In this review, we will focus on discussing the roles of apoE in the development of age-related cognitive decline and AD as they relate to this ApoE Cascade Hypothesis.

Biochemical phase in the ApoE Cascade Hypothesis

ApoE is a 299-amino acid glycoprotein composed of the N-terminal domain (residues 1–167), a hinge region (residues 168–205), and the C-terminal domain (residues 206–299) (Chen et al., 2021) (Figure 2: apoE3 amino acid sequence shown). While the receptor-binding region (residues 136–150) is within the N-terminal four helix bundle, the lipid-binding region (residues 244-272) is in the C-terminal domain. By interacting with cellular membranes through ABC transporters, apoE incorporates membrane lipids and forms lipoprotein particles. Subsequently, the lipidated apoE particles transport and distribute lipids from cell to cell through binding to cell-surface apoE receptors including the low-density lipoprotein receptor (LDLR), the LDLR-related protein 1 (LRP1), and HSPGs (Herz and Bock, 2002; Herz and Chen, 2006; Wahrle et al., 2004). The isoform-dependent biochemical and biophysical properties of apoE to interact with lipids and receptors are intimately linked to its functions in health and disease (Huang and Mahley, 2014; Yamazaki et al., 2019).

ApoE structure, lipidation, and receptor binding.

NMR structural studies indicate that the structured helix regions of apoE3 are bound by several unstructured intrinsically disordered regions (IDRs) and smaller flexible regions (Chen et al., 2011; Frieden et al., 2017). The relatively unstable structural feature could allow apoE to be incorporated in different sizes of lipid particles with diverse compositions. While the pocket between the part of Helix 3 in the N-terminal helical bundle (residues 88-104) and the C-terminal domain (residues 251-266), brought together by several salt bridges, is possibly the initial lipid binding site (Frieden et al., 2017), the structural differences in apoE isoforms may differently influence the lipid recognition and the curvature of lipoprotein particles. Helix 4 in the N-terminal helical bundle contains a receptor-binding region enriched in positively charged Lys and Arg residues, providing the binding site to negatively charged moieties in apoE receptors (Chen et al., 2021). Although several models regarding the conformational changes of the N-terminal domain are hypothesized in the lipid-bound form of apoE (Chen et al., 2021; Hatters et al., 2006a), the receptor-binding region is likely undocked upon lipid-binding, increasing the accessibility to apoE receptors and enabling efficient cellular lipid delivery.

The three major apoE isoforms encoded by each corresponding *APOE* allele differ in two amino acid residues at positions 112 and 158 (apoE2: Cys112/Cys158; apoE3: Cys112/Arg158; apoE4: Arg112/Arg158) (Mahley and Rall, 2000). *In vitro* lipid efflux assays for cholesterol or phospholipids showed the superior role of apoE2 to apoE3 and apoE4 as a lipid acceptor (Michikawa et al., 2000). In cerebrospinal fluid (CSF), the size of apoE/lipoprotein particles is apoE isoform-dependent following the order of apoE2>apoE3>apoE4 (Heinsinger et al., 2016; Lanfranco et al., 2020). The same apoE isoform-dependent apoE/lipoprotein particle size in mouse brains has also been reported supporting the notion that apoE2 as a better lipid transporter (Hu et al., 2015). Since residue 112 is connected to the lipid-binding site of Helix 3 and residue 158 is located behind the lipid-binding domain (Chen et al., 2011; Frieden et al., 2017), these amino acid differences could substantially impact the apoE properties in forming lipoprotein particles and receptor binding. In the

periphery, apoE2 and apoE3 bind preferentially to HDL while apoE4 binds to VLDL, and this is thought to be due to the presence of the Arg residue at amino acid position 112 leading to altered domain interaction between the N- and C-terminal domains (Weisgraber, 1990). In addition, Cys158 in apoE2 alters the conformation of the positively charged receptor binding domain, thus reducing its affinity for the LDLR (Mahley et al., 2009). This reduced affinity of apoE2 to LDLR results in decreased clearance of triglyceride-rich lipoprotein particles and increases the risk of developing Type III hyperlipoproteinemia in small group of apoE2 homozygous individuals (Mahley et al., 1999).

ApoE protein levels.

In plasma, apoE concentrations are isoform-dependent where apoE2 is higher and apoE4 is lower compared to apoE3 (Rasmussen et al., 2015). For the apoE levels in the CNS, the result is mixed depending on the quantification methods used. ELISA measurements revealed that apoE2-TR mice display highest levels of apoE in brain parenchyma, CSF, and ISF followed by apoE3-TR mice, then apoE4-TR mice (Riddell et al., 2008; Shinohara et al., 2016; Ulrich et al., 2013). However, using stable isotope amino acid labeling and mass spectrometry, Wildsmith et al found no isoform-dependent differences in apoE levels and its turnover rate between apoE3 and apoE4 in CSF of young cognitively normal individuals as well as apoE-TR mice (Wildsmith et al., 2012). The same study also reported that the level of apoE2 appears to be higher. The lack of isoform-dependent difference in apoE levels in CSF was later confirmed in another study in non-AD and AD subjects (Martinez-Morillo et al., 2014). More recently, a study using induced pluripotent stem cell (iPSC)-derived astrocytes and cerebral organoids showed apoE4 being associated with higher apoE levels compared to apoE3 (Lin et al., 2018) while another study did not find such a difference in iPSC-derived cerebral organoids (Zhao et al., 2020a). The reason for these discrepancies is not entirely clear, but one possibility is that the structural differences among the apoE isoforms affect epitope presentation leading to different apoE concentrations by ELISA. Moreover, the isoform-dependent apoE levels may be due to differences in receptor binding ability, structural stability, and oligomerization propensity.

ApoE oligomerization.

As lipid-free forms of apolipoproteins are not conformationally stable in general, they possess misfolding and self-oligomerization propensities (Hatters and Howlett, 2002). While the apoE C-terminal domain is predominantly assembled as coiled-coil dimeric or tetrameric species *in vitro* (Choy et al., 2003), the full-length apoE is prone to form soluble protofilament-like amyloid fibrils with a high α-helical conformation in an isoform-dependent manner (apoE4 > apoE3 > apoE2) (Hatters et al., 2006b). The greater apoE4 aggregation propensity is also seen in human brains (Liu et al., 2021). Additionally, the isoform-dependent propensity of apoE to form amorphous aggregates is hindered by lipidation *in vitro* (Hubin et al., 2019).

While strong evidence from human clinical and animal model studies suggests that a major mechanism by which *APOE4* increases the risk of AD is by driving earlier and more abundant amyloid pathology in the brain (Christensen et al., 2010; Koffie, 2012; Kok, 2009; Liu et al., 2017a; Morris, 2010; Polvikoski, 1995; Reiman et al., 2009; Schmechel,

1993; Tiraboschi, 2004), in the absence of an *APOE4* allele, any change that affects the biochemical and biophysical properties of apoE will have a greater cascading impact to the subsequent phases.

ApoE posttranslational modifications.

Posttranslational modification of proteins is a well-known phenomenon that affects protein structure and dynamics (Mann and Jensen, 2003). The differential posttranslational modifications of apoE isoforms also play an important role in modulating its function (Figure 2). A number of posttranslational modifications of apoE have been reported including glycation (Shuvaev et al., 1999), glycosylation (Flowers et al., 2020; Ke et al., 2020; Lee et al., 2010), phosphorylation (Jaros et al., 2012; Raftery et al., 2005), and oxidation (Jolivalt et al., 1996; Miyata and Smith, 1996; Strittmatter et al., 1993). In clinical studies, plasma levels of posttranslationally modified apoE (glycosylation, methylation, demethylation, and dihydroxylation) have been reported to increase in breast cancer patients (Uen et al., 2015), and the increased apoE citrullination is observed in the synovial fluid of rheumatoid arthritis patients (van Beers et al., 2013). Aging also impacts protein biochemical properties and functions through posttranslational modifications including oxidation and glycation (Santos and Lindner, 2017). Further studies should refine how aging-, apoE isoform-, and disease status-dependent changes in posttranslational modifications impact the structural and biochemical features of apoE under physiological and pathological conditions.

ApoE epigenetic modifications.

Epigenetic modification is another example of modifiers that can alter the biochemical phase of the ApoE Cascade Hypothesis. Tulloch et al. reported increased DNA methylation of *APOE* 3’ DNA in postmortem AD brains compared to control brains in a tissue- and *APOE* genotype-specific manner (Tulloch et al., 2018). The increased *APOE* DNA methylation has been shown to negatively correlate with total *APOE* mRNA levels (Lee et al., 2020), which may result in reduced apoE protein levels. Other epigenetic modifications of *APOE* such as chromatin remodeling and noncoding RNA have been reported (Yu and Foraker, 2015), but further investigation is needed to examine their impact on apoE protein levels.

Cellular phase of ApoE Cascade Hypothesis

The disruption of biochemical and biophysical properties of apoE such as misfolding and self-assembly of apoE (“structure” and “oligomerization”), decreased binding to lipids (“lipidation”), and decreased production or increased degradation of apoE (“protein levels”) can negatively impact apoE-dependent cellular functions in a cell-type specific manner leading to the second phase of the ApoE Cascade Hypothesis called the “cellular phase”.

ApoE is mainly produced by astrocytes, reactive microglia, vascular mural cells, and choroid plexus cells in the brain (Kang et al., 2018; Xu et al., 2006). Under stress conditions, neurons display an enhanced lipid metabolism accompanied by apoE production, perhaps to repair damaged membrane (Najm et al., 2019). Since excess intracellular lipid accumulation can cause cellular stress, apoE may also play a predominant role

in transporting lipids from intracellular to extracellular space. However, lipids are also important for cellular homeostasis. Subcellular organelle membranes consist primarily of lipids, dividing/proliferating or damaged cells may require sufficient lipid supplies for membrane remodeling or repair. The binding of apoE/lipoprotein particles with cell surface apoE receptors and subsequent endocytosis are essential mechanisms for cell-to-cell lipid distribution in the brain (Figure 3). Therefore, the “ApoE Cascade Hypothesis” predicts that disruption of apoE-mediated cellular lipid homeostasis initiates a pathogenic cascade that contributes to AD-related cellular dysfunction. In addition to apoE isoforms, biochemical properties, and concentrations, brain cell type-specific apoE metabolism and functions through cell-autonomous and non-cell-autonomous mechanisms may also significantly modulate the cellular phase of AD and age-related cognitive decline (De Strooper and Karran, 2016). As such, there is a strong need to define how apoE properties and quantity affect each brain cell type, and how they are involved in the disease phenotypes at the cellular level.

ApoE and intracellular trafficking dysregulation.

As the enlargement of endosomes is often detected as a cytopathological hallmark in early stages of AD, endosomal-lysosomal dysregulation is one of the central pathways in the cellular phase of AD pathogenesis (Nixon, 2005; Small and Petsko, 2020; Van Acker et al., 2019). Intriguingly, this phenotype appears to be exacerbated by *APOE4* both in the brains of AD patients (Cataldo et al., 2000) and aged *APOE*-targeted replacement (TR) mice (Nuriel et al., 2017), independently of the amyloid pathology. A transcriptomics study has revealed that genes involved in endosomal-lysosomal pathways are enriched in the brains of apoE4-TR mice compared to apoE3-TR mice (Nuriel et al., 2017). Chen and colleagues previously reported that apoE4 reduces cell surface levels of apoER2, a neuronal signaling receptor for Reelin and apoE, as well as glutamate receptors by sequestering them in the endocytic compartments, thereby reducing synaptic activity (Chen et al., 2010; Lane-Donovan et al., 2014). The low pH environment of endosomes induces structurally labile apoE4 to form a molten globule which leads to reduced cell surface apoER2 expression due to dysregulation of endosomal intracellular trafficking (Xian et al., 2018). Our group found that apoE4 also suppresses cell surface insulin receptor (IR) and impairs IR trafficking by aggregating and retaining IR in the early endosomes (Zhao et al., 2017). As a result, the downstream signaling and the effects of insulin-induced glycolysis and mitochondrial respiration are significantly suppressed by apoE4. Altogether, these studies suggest that apoE4 may suppress various signaling cascades by impairing the trafficking of cell surface receptors.

While phosphoinositides contribute to vesicular transport by regulating vesicular budding, membrane fusion and cytoskeleton dynamics (De Craene et al., 2017), brain levels of phosphoinositol biphosphate (PIP2) are also decreased in *APOE4* carriers regardless of AD stage (Zhu et al., 2015). Overexpression of phosphatidylinositol binding clathrin assembly protein (PICALM) restores endocytic defects caused by *APOE4* in iPSC-derived astrocytes (Narayan et al., 2020). Thus, defining the link between apoE and phosphoinositide metabolism may provide important clues to uncover the pathogenic mechanism contributing to impaired vesicle trafficking in AD. In addition, altered apoE

solubility and membrane association under acidic conditions in lysosomes may directly destabilize the vesicle membranes and cause lysosomal leakage (Van Acker et al., 2019). Since phospholipid asymmetries in the endomembrane system likely trigger exocytic/endocytic vesicle budding (Huijbregts et al., 2000), it is also possible that apoE-mediated membrane lipid modifications indirectly influence the cellular trafficking.

ApoE and cellular stress.

Whereas mitochondria and ER are essential organelles in maintaining cellular homeostasis, the dysregulation of the ER-mitochondria axis has been implicated in the pathogenesis of several age-related neurodegenerative diseases including AD (Filadi et al., 2017; Swerdlow et al., 2014; Wang et al., 2020). Of note, apoE is also involved in ER stress and mitochondrial dysfunction in AD pathogenesis (Dose et al., 2016). In apoE-TR mice, apoE4 increases eukaryotic initiation factor-2α (eIF2α) phosphorylation which indicates aggravated ER stress responses in the brain (Machlovi et al., 2022; Segev et al., 2013). The mitochondrial dynamics such as fusion and fission is also altered in the presence of apoE4, which is accompanied by impaired mitophagy in mouse brains (Simonovitch et al., 2019). While the misfolding of non-lipidated apoE may lead to those cellular stress responses, the domain-domain interaction in apoE4 and fragmented apoE are also possibly involved in this mechanism (Dose et al., 2016). In addition, apoE4 facilitates the physical interaction between mitochondria and ER through mitochondria-associated ER membranes (MAMs) (Tambini et al., 2016). Indeed, the critical roles of MAMs have been increasingly recognized in regulating proper cellular functions including calcium signaling and energy homeostasis (Eysert et al., 2020; Veeresh et al., 2019), and apoE may differently regulate ER-mitochondria functions through MAM formation depending on apoE isoforms or biochemical properties. Furthermore, ER stress and mitochondrial dysregulation are also associated with the formation of lipid droplets, which have been shown to accumulate in different brain cell types including neurons, astrocytes and microglia during aging and AD (Ralhan et al., 2021). Supporting the biochemical to cellular cascade, reduced receptor binding of apoE4 to LRP1 (biochemical phase) has been shown to upregulate cyclophilin A expression leading to increased cellular stress (cellular phase) through NF-κB pathway activation in pericytes (Bell et al., 2012). This can result in neuroinflammation and vascular dysfunction (phenotypic phase), which precede age-related cognitive impairment and AD.

ApoE and lipid dysregulation.

Lipid droplets contain non-polar lipids such as triglycerides and cholesterol esters, and are often considered as organelles budded from the ER and associated with other organelles. They regulate cellular metabolism and buffer lipotoxicity (Olzmann and Carvalho, 2019) but can also be pathogenic when dysregulated. Interestingly, apoE4 is associated with greater lipid droplet formation in astrocytes compared to apoE3 (Sienski et al., 2021); however, it is suppressed by apoE4 in neurons (Qi et al., 2021). The study by Qi and colleagues (Qi et al., 2021) highlights an effect of apoE4 impacted by its structure and protein levels (biochemical phase) in decreasing sequestration of fatty acid into lipid droplets in neurons, and in reducing its transport to astrocytes. As such, apoE4 is associated with decreased fatty acid degradation and lipid accumulation, leading to lipid dysregulation and accumulation of lipid droplets in astrocytes and increased mitochondrial stress (cellular phase). A similar

finding of impaired lipid transport from neurons to astrocytes by apoE4 has also been reported using a drosophila model (Liu et al., 2017b). The impaired lipid metabolism can lead to synaptic dysfunction and neurodegeneration in neuropathology-dependent and independent manner (Chew et al., 2020) (phenotypic phase).

Interestingly, glial lipid metabolism appears to be most affected by apoE. Significant alterations were found in cholesterol esters and other lipids predominantly in microglia and to some extent in astrocytes with little change in whole brain in *Apoe*-KO mice (Nugent et al., 2020). This suggests a focus on apoE effects in glial lipid metabolism will likely provide important insights into apoE-related pathways in the normal brain, aging, and in AD. Thus, cell autonomous or non-autonomous apoE functions in lipid metabolism and cellular stress responses might differ depending on brain cell types during the cellular phase but converge to impact phenotypic outcomes.

Phenotypic phase of ApoE Cascade Hypothesis

While neurodegeneration is fundamental in the phenotypic phase of AD and age-related cognitive decline (De Strooper and Karran, 2016), apoE-mediated lipid metabolism and cellular dysregulation undoubtedly participate in the pathogenic process through both neuropathology-dependent and independent pathways as already described. In AD brains, apoE and Aβ frequently co-deposit in amyloid plaques (Cho et al., 2001). ApoE deficiency in mice vastly reduces brain Aβ deposition as fibril plaques and cerebral amyloid angiopathy (CAA) (Bales et al., 1997; Holtzman et al., 2000; Kim et al., 2011), suggesting that apoE promotes aggregation and fibrillization of Aβ in AD and CAA. When apoE is hyperlipidated upon ABCA1 overexpression, Aβ deposition is significantly reduced (Wahrle et al., 2008). ApoE2 is reported to be hyperlipidated compared to apoE3 and apoE4 in human CSF (Heinsinger et al., 2016) and in culture medium of immortalized astrocytes derived from apoE-TR mice (Morikawa et al., 2005) supporting the notion that increased lipidation of apoE protects against AD by reducing Aβ deposition.

Of note, *APOE* genotype has also been associated with the occurrence and severities of diverse neuropathologies including tau, α-synuclein, and TDP-43 in addition to Aβ (Belloy et al., 2019). Histological studies found colocalization of apoE with neurofibrillary tangles in AD brains (Benzing and Mufson, 1995; Richey et al., 1995), whereas apoE3 likely has a greater binding affinity than apoE4 to non-phosphorylated tau and prevents its phosphorylation *in vitro* (Hoe et al., 2006; Strittmatter et al., 1994). ApoE fragments are also detected within Lewy bodies in the brains of Parkinson's disease patients (Rohn and Mack, 2018). Supporting this, deletion of apoE has been shown to increase α-synuclein solubility in Syn^{A30P} transgenic mice (Gallardo et al., 2008). Together, these lines of evidence indicate that apoE is involved in the development of various neuropathologies by impacting protein aggregation and deposition in an isoform-dependent manner. Further studies should define how apoE biochemical properties contribute to protein aggregation during aging and in aging-related pathological conditions. In addition, emerging evidence indicates that apoE produced by microglia or produced by other cells that act on microglia impacts the immune response in the brain during aging and AD (Guerreiro, 2018; Shi et al., 2019; Shi et al., 2017). In fact, *APOE* is ranked as one of the highest disease-associated

microglia (DAM) genes, which are associated with aging, amyloid, and tau (Deczkowska, 2018; Krasemann, 2017; Rangaraju, 2018; Song and Colonna, 2018; Ulrich, 2018). ApoE along with phospholipids have been demonstrated as ligands for the triggering receptor expressed on myeloid cells 2 (TREM2), which is also a strong AD risk gene expressed in microglia (Atagi, 2015; Bailey et al., 2015; Wang et al., 2015; Yeh et al., 2016). Thus, apoE is a critical factor regulating AD-related neuroinflammation, although how microglia-expressed apoE influences the pathologies compared to astrocytic apoE still needs further investigation. Other common phenotypes in AD are the disturbances of cerebrovascular integrity and function. *APOE4* is also a strong genetic risk factor for multiple vascular conditions including hypercholesterolemia, atherosclerosis, vascular cognitive impairment, and cerebral amyloid angiopathy (Davidson, 2006; Rannikmae, 2014; Shinohara, 2016; Sun, 2015). The presence of *APOE4* is associated with increased severity of white matter hyperintensities, accelerated pericyte degeneration, and compromised blood-brain barrier integrity (Halliday, 2013; 2016; Schilling, 2013; Sudre, 2017). Thus, a better understanding of the biology and pathobiology regarding how apoE isoforms produced by different brain cell types and their biochemical properties impact cerebrovascular functions will provide new insights in AD-related phenotypes.

Targeting apoE-initiated cascade events in the disease process.

The ApoE Cascade Hypothesis proposed here should guide the design of novel therapeutic strategies against age-related cognitive decline and AD. The pharmacological, genetic, or lifestyle interventions that alter the biochemical and biophysical properties of apoE (biochemical phase) will lead to changes in subsequent phases of this cascade. A study by Xian et al. reported that the low pH environment of endosome induces structurally labile apoE4 to form a molten globule (biochemical Phase) leading to reduced cell surface apoER2 level (cellular Phase) (Xian et al., 2018). Pharmacological reduction of the pH in endosomes by inhibiting NHE6 reverses the apoE4-mediated endosomal dysfunction and restores synaptic function. In a follow up study by the same group, deletion of NHE6 reduces apoE-mediated amyloid plaque buildup in an animal model of AD (phenotypic phase) (Pohlkamp et al., 2021).

Interventions to increase lipidation, reduce oligomerization, increase or decrease the protein levels or receptor binding of apoE depending on apoE isoforms, as well as the use of structural correctors are all strategies that are being or should be investigated for treating AD and age-related cognitive decline (Chen et al., 2012; Liao et al., 2018; Tai et al., 2014; Zhao et al., 2014). Some of the strategies show promising results against amyloid pathology (Xiong et al., 2021), but others need future optimization to reduce potential toxic side effects (Tai et al., 2014). Due to its complex biology and pathobiology of apoE, future interventions may benefit from the use of bi-functional molecules or those that enable cell type-specific delivery of drugs. Target engagement of therapeutic interventions to alter the biochemical and biophysical properties of apoE, followed by the investigation of their impact on the cellular phase of apoE cascade should be validated using *in vitro* or *in vivo* assays (Hughes et al., 2011). The validation of their effects on the phenotypic phase will require more complex model systems such as animal models and human iPSC-derived cerebral organoids (Park et al., 2021; Singh and Seed, 2021).

ApoE Cascade Hypothesis in other age-related disorders.

The biochemical properties of apoE can also cascade down to cause age-related disorders. *APOE4* has been found to be a genetic risk factor for Lewy body dementia (LBD). Using animal models and human iPSC models, our group have demonstrated a pathogenic role of *APOE4* in exacerbating α-synuclein pathology independent of amyloid (Davis et al., 2020; Zhao et al., 2020a; Zhao et al., 2020b). Our group has also reported an association between the *APOE2* genotype and risk of tauopathies such as progressive supranuclear palsy and corticobasal degeneration (Zhao et al., 2018). These findings suggest that *APOE2* status may influence the risk and progression of primary tauopathy. ApoE2 and apoE4 increases the risk of cardiovascular diseases through different mechanisms. The low affinity of apoE2 for LDLR leads to reduced clearance of triglyceride-rich VLDL which is prone to form atherosclerotic plaques while the preference of apoE4 for VLDL is associated with higher plasma LDL cholesterol which results in increased coronary heart disease risk (Mahley, 2016). While apoE2 is protective against AD and AD-related neuropathologies, it is a risk factor for age-related macular degeneration (AMD) (Thakkinstian et al., 2006). The main pathological features of AMD is the formation of lipid-rich drusen, yellow deposits under the retina (Wang et al., 2010). A study by Levy et al. reveals that subretinal mononuclear phagocytes from apoE2-TR mice exhibit increased subretinal inflammation, promoting choroidal neovascularization in subretinal space (Levy et al., 2015). Although the exact mechanism of how apoE2 increases the risk of AMD remains unknown, disruption of lipid metabolism in retina cells has been suggested in an animal model (Saadane et al., 2018). Altogether, this evidence supports the generalizability of the ApoE Cascade Hypothesis to decipher pathogenic mechanisms of other apoE-related conditions.

Concluding remarks

How apoE isoforms, differing only by a single amino acid from one another, have such profound effects on the risk of AD and related dementias, has been puzzling the apoE field for almost three decades (Yamazaki et al., 2019). To this end, we propose a potential “butterfly effect” of apoE on AD and age-related cognitive decline referred to as “ApoE Cascade Hypothesis”; a collection of differences in structural and biochemical properties depending on apoE isoforms, posttranslational modifications, and/or altered apoE expression initiate a cascade of events at the cellular and systems levels during aging, thus driving AD-related pathogenic conditions (Figure 1). Whereas most proteins encoded by AD risk genes have been shown to impact lipid metabolism, immune response, or membrane trafficking (Kanekiyo and Bu, 2014), apoE is involved in all three pathways. Furthermore, apoE has been shown to contribute to the development of amyloid pathology (A), tauopathy (T), and neurodegeneration (N), collectively known as ATN classification, as well as neuroinflammation and cerebrovascular dysfunction at the presymptomatic stage of AD. Therefore, it is reasonable to hypothesize that apoE triggers multifaceted pathways in AD. Although *APOE4* is the strongest genetic risk factor for AD impacting 50-70% of all cases, it is not a causative gene (Corder et al., 1993; Corder et al., 1994; Farrer et al., 1997; Saunders et al., 1993). Moreover, it is still not entirely clear how cell type, disease status, and apoE isoform collectively or individually modulate the biochemical phase of apoE. Interestingly, recent work from our group revealed that the apoE lipoprotein particle sizes

are affected by both cell type (astrocytes vs. microglia) and apoE isoform (Huynh et al., 2019). There is a dire need to further address these critical gaps in knowledge to better design mechanism-based therapeutic strategies. Thus, secondary modifiers such as age, sex, and other genetic/epigenetic or environmental factors may accelerate or decelerate the apoE cascade in AD and age-related cognitive decline.

Although the field has learned so much about the ways apoE contributes to AD, much work is still needed to further support or strengthen the “ApoE Cascade Hypothesis”. First, the limited structural information for lipid-bound apoE produced by different brain cell types and the differential effects of apoE isoforms call for focused efforts in addressing apoE structural properties related to lipid association and protein oligomerization. Second, much of the information on apoE is derived from the detrimental effects of apoE4, whereas the field can gain greater insights by understanding the protective mechanisms of apoE2, as well as the rare apoE3-Christchurch (Arboleda-Velasquez et al., 2019) and apoE3-Jacksonville (Liu et al., 2021) variants. Third, there is increasing evidence suggesting a contributing role of peripheral apoE, thus understanding how apoE isoforms expressed by the liver and macrophages represents an opportunity for greater appreciation on how peripheral system impacts the brain and AD. Despite the need of more knowledge, we believe that the “ApoE Cascade Hypothesis” can guide the design of therapeutic strategies for AD and related dementias by targeting early events such as apoE structure, apoE concentration, posttranslational modifications, oligomerization, receptor binding, and/or lipidation.

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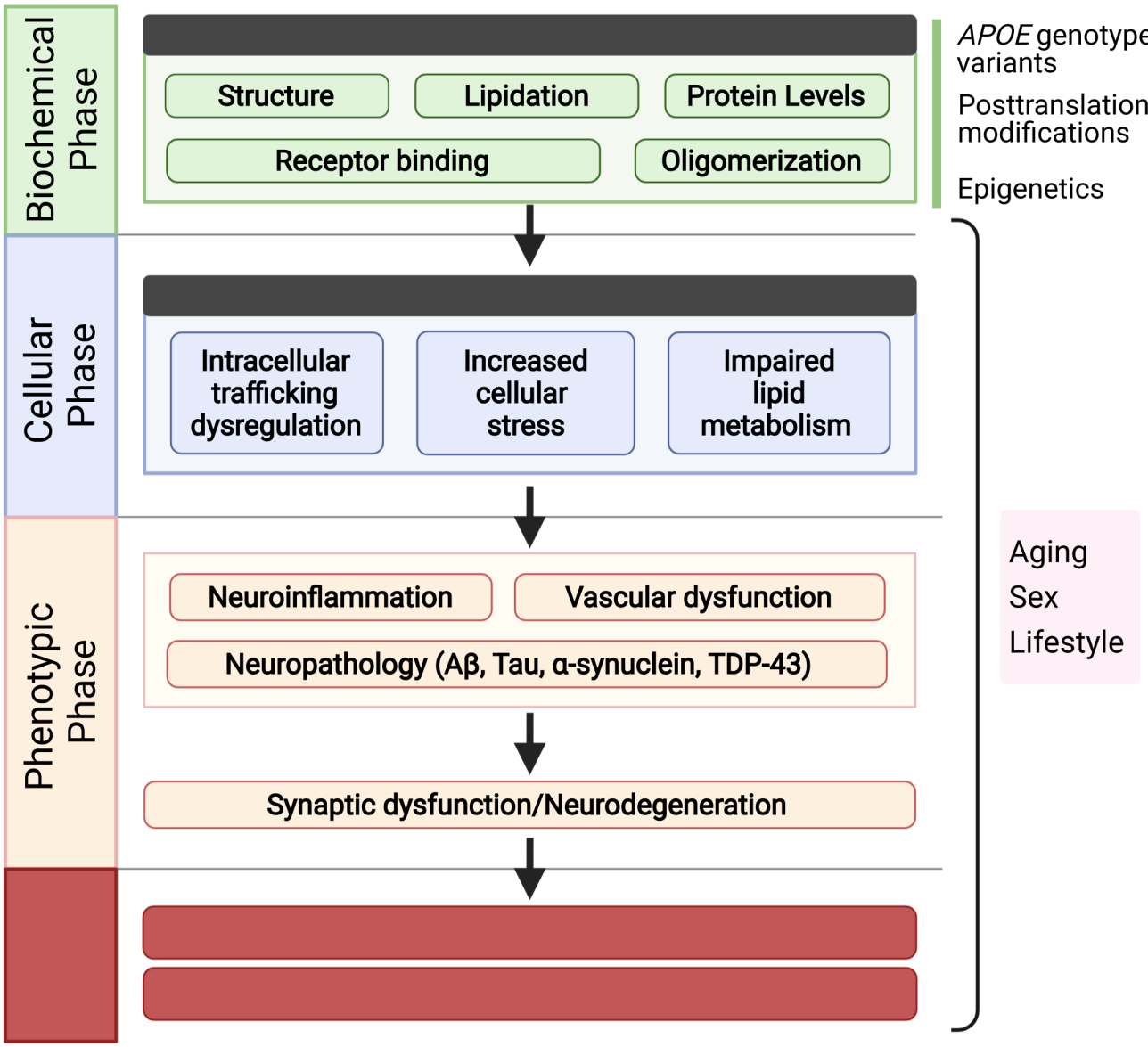

Figure 1. ApoE cascade hypothesis.

The cascade starts from the different biochemical and biophysical properties including apoE structure, lipidation, protein levels, receptor binding, and oligomerization. These biochemical/biophysical differences are then propagated to functional effects on cellular homeostasis including cellular stress, endosomal-lysosomal trafficking, as well as lipid dysregulation. Not depicted here, some of these cellular effects can be either cell autonomous in cells expressing abundant apoE (astrocytes and microglia in the brain, hepatocytes and macrophages in the periphery, and vascular mural cells interfacing the periphery with the brain), or non-cell autonomous (e.g., secreted apoE from one cell type binding to apoE receptors on another including neurons). These cellular effects are further relayed to trackable phenotypes at the systems level highlighted by neuroinflammation, vascular dysfunction, and neuropathologies, leading to synaptic dysfunction/loss, neurodegeneration, and eventual age-related cognitive decline and AD.

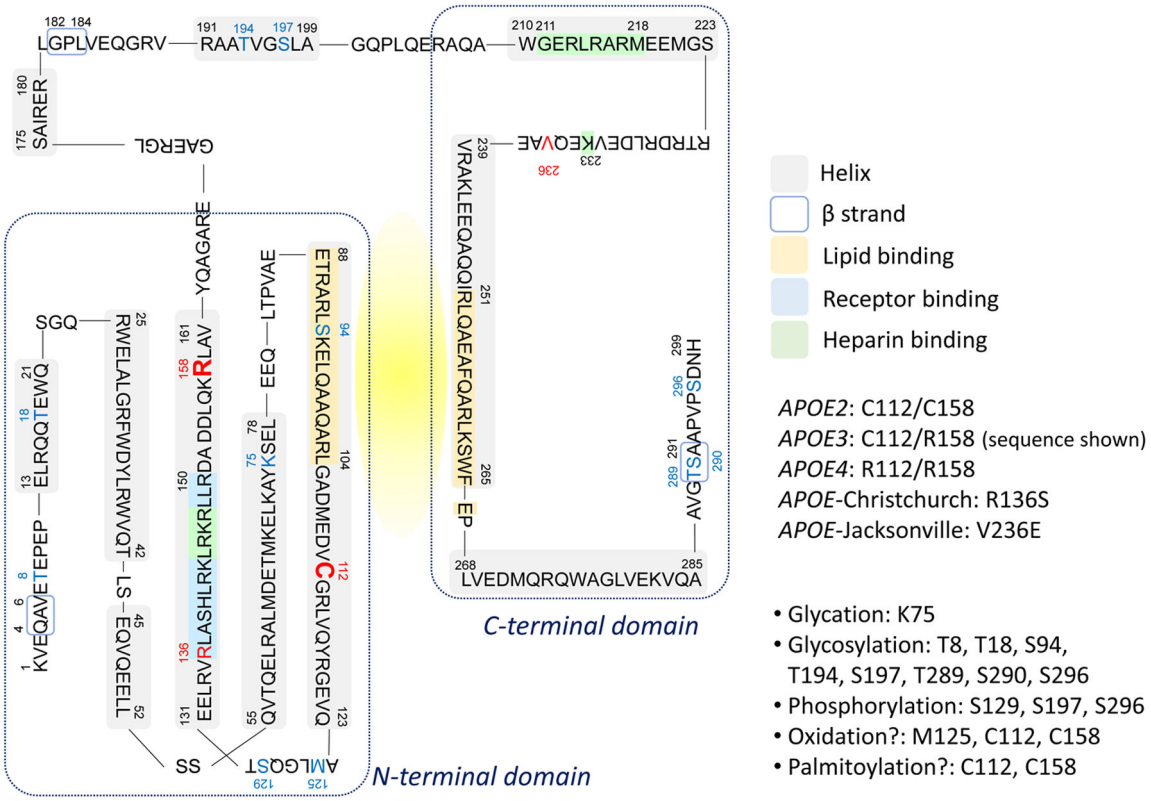

Figure 2. ApoE amino acid sequence and potential post translational modification sites.

Amino acid sequence of apoE3 is depicted. Key functional regions and residues that differ among apoE isoforms and variants, as well as known or potential posttranslational modification sites are marked.

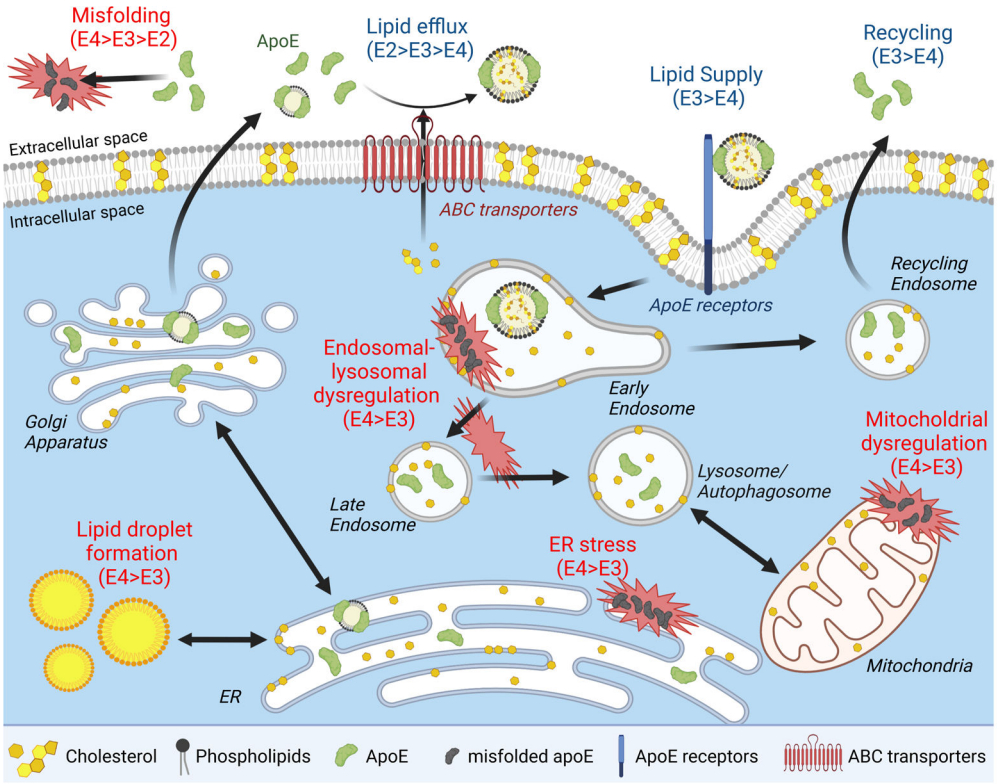

Figure 3. ApoE and cellular homeostasis.

ApoE traffics through the secretory pathway as a non-lipidated or lipidated protein into the extracellular space. ABC transporters load membrane and traffic intracellular lipids onto apoE to produce nascent apoE/lipoprotein particles. ApoE-containing lipid particles can undergo further lipid modifications and are taken up by various cells through receptor-mediated endocytosis by binding to apoE receptors. This process supplies cells with diverse lipids including phospholipids and cholesterols necessary to maintain cellular homeostasis and support synaptic integrity and plasticity. The endocytosed particles and their components are transported to lysosome/autophagosome through late endosomes or recycled back to the extracellular space through recycling endosomes. ApoE isoforms impact cellular homeostasis by differentially modulating membrane trafficking, ER stress, and mitochondria function due to their individual effects on protein homeostasis, aggregation, and lipid metabolism.