The Mechanism of ApoE Gene Polymorphisms in Alzheimer's Disease

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Abstract. Apolipoprotein E (ApoE) is a core regulator of lipid homeostasis in the central nervous system. Its genetic polymorphisms differ in key amino acid residues (ε2: Cys112/Cys158; ε3: Cys112/Arg158; ε4: Arg112/Arg158), which can significantly alter ApoE's lipid-binding capacity, affinity for the low-density lipoprotein receptor (LDLR) family, and lipid transport efficiency, thereby profoundly affecting the pathogenic risk and pathological progression of Alzheimer's disease (AD) --- the ε4 allele can increase AD risk by 2.35-4.55 times, the ε2 allele reduces the risk, and the ε3 allele serves as a neutral reference isoform. Integrating recent clinical and basic research, this paper systematically expounds on the molecular structural characteristics of ApoE (including the N-terminal receptor-binding domain, C-terminal lipid-binding domain, and hinge region) and the intracerebral lipid transport pathway, analyzes the conformational differences of the three isoforms, and clarifies the four key pathological mechanisms by which ApoE4 induces AD through lipid transport deficits, aiming to provide precise theoretical support for AD interventions targeting the lipid transport function of ApoE.

Keywords: ApoE polymorphisms; lipid metabolism; Alzheimer's disease; Aβ deposition; synaptic function.

1. Introduction

Alzheimer's disease is the most prevalent neurodegenerative disorder worldwide, clinically characterized by progressive cognitive decline, and pathologically by senile plaques composed of β-amyloid (Aβ) deposits, neurofibrillary tangles formed by hyperphosphorylated tau protein, as well as accompanying synaptic loss and neuronal death. Epidemiological studies reveal an exponential increase in AD prevalence with age: approximately 1% among those aged 65, rising to 40–50% by age 95, with females exhibiting slightly higher risk than males [1].

The pathogenesis of AD demonstrates marked genetic heterogeneity. About 5% of early-onset familial AD (FAD) is caused by dominant mutations in amyloid precursor protein (APP), presenilin 1 (PSEN1), or presenilin 2 (PSEN2), which accelerate Aβ42 generation and drive pathology [1]. In contrast, more than 95% of late-onset AD (LOAD) cases are associated with apolipoprotein E (ApoE), the strongest genetic risk factor identified to date [2]. The ApoE gene, located on chromosome 19q13.2, exists in three major allelic forms (ε2, ε3, ε4), which differ only at amino acid residues 112 and 158. These substitutions induce marked differences in protein conformation, lipid transport capacity, and AD risk. Specifically, the ε4 allele increases AD risk by 2.35-4.55-fold (highest in ε4/ε4 homozygotes), while ε2 decreases risk, and ε3 serves as the neutral reference [2,3].

In recent years, research on ApoE has expanded beyond its role as a peripheral lipid transporter, identifying it as a "central hub" for lipid homeostasis in the CNS. Over 70% of brain ApoE is synthesized by astrocytes, with minor expression by microglia and vascular smooth muscle cells, while neuronal expression is induced only under stress conditions (e.g., Aβ deposition, ischemic injury) [4]. By transferring cholesterol and phospholipids to neurons, ApoE maintains synaptic membrane integrity and supports myelin formation, directly linking to cognitive performance [5].

This article will begin with an overview of ApoE's molecular structure and lipid transport mechanisms, systematically analyzing structural-functional differences among isoforms. It will focus on delineating the pathogenic molecular pathways of ApoE4 and explores genetic–environmental

interactions, providing a comprehensive theoretical foundation for both basic research and translational interventions in AD.

2. Molecular Structure and Lipid Transport Mechanisms of ApoE

2.1. Structural Characteristics of ApoE

ApoE is a glycoprotein composed of 299 amino acid residues, with a molecular weight of approximately 34 kDa. Its three functional regions cooperatively regulate lipid binding and receptor recognition, and exhibit tissue-specific modifications. Key features include [2]:

N-terminal domain (residues 1-167): This region forms a highly stable four-helix bundle structure (confirmed by X-ray crystallography and NMR). Its core function is receptor binding, with residues 136-150 forming the receptor-binding site that interacts with members of the LDL receptor family, including LDLR and LRP1. Notably, the thermal stability of the N-terminal domain differs among isoforms: ApoE4 is significantly less stable than ApoE3, rendering it prone to partial unfolding, predisposing to aggregation and dysfunction.

C-terminal domain (residues 206-299): Enriched in hydrophobic residues, residues 244-272 constitute the primary lipid-binding site, enabling direct interaction with cholesterol and phospholipids, thus supporting lipoprotein particle formation. This region also contains a heparin-binding site (centered around Lys233), mediating initial interactions with cell-surface heparan sulfate proteoglycans. In addition, Thr194 serves as an O-linked glycosylation site, with brain ApoE exhibiting significantly higher levels of sialylation compared to plasma, which may modulate lipid-binding efficiency and metabolic turnover.

Hinge region (residues 168-205): Connecting the N- and C-terminal domains, this flexible segment regulates the spatial orientation of both domains. Under physiological conditions, hinge flexibility maintains domain independence, ensuring coordinated receptor and lipid binding. In ApoE4, however, conformational abnormalities in the hinge region promote domain interaction (N-C proximity), thereby disrupting lipid transport functions.

2.2. ApoE-Mediated Lipid Transport in the Brain

The lipid transport process of ApoE in the CNS relies on intercellular collaboration and molecular regulation, consisting of three critical steps, each closely linked to AD pathology:

Lipoprotein synthesis and lipidation: Astrocytes, the primary source of brain ApoE, utilize ATP-binding cassette transporter A1 to load intracellular cholesterol and phospholipids onto nascent ApoE molecules, forming HDL-like particles. ABCA1 activity is the key regulator of this process. Reduced ABCA1 expression or function (e.g., due to mutation) significantly impairs ApoE lipidation. Notably, ApoE4 lipidation efficiency is only ~50-60% of ApoE3, producing poorly lipidated particles incapable of effective transport [4].

Receptor recognition and binding: Lipidated ApoE-HDL particles interact with neuronal LDLR/LRP1 via the N-terminal receptor-binding domain. This process is lipidation-dependent: only fully lipidated ApoE exposes the proper conformation for efficient receptor binding. Hypolipidated ApoE4 fails to fully expose this site, reducing binding efficiency. HSPGs, acting as auxiliary receptors, bind ApoE's C-terminal heparin-binding site, stabilizing the ApoE-receptor complex and enhancing binding [2].

Endocytosis and lipid release: ApoE-receptor complexes undergo clathrin-mediated endocytosis into neurons, forming endosomes. Under acidic endosomal conditions (pH 5.0-6.0), ApoE dissociates from the receptor, and the lipoprotein undergoes rearrangement to release cholesterol and phospholipids. These lipids serve primarily for synaptic membrane synthesis and myelin maintenance [2].

3. Structural and Functional Differences of the Three Major ApoE Isoforms

3.1. ApoE2 (Cys112/Cys158): Protective Isoform

ApoE2's protective role stems from its unique structural features modulating Aβ metabolism and neuroinflammation:

Receptor-binding deficiency: The presence of Cys158 disrupts the conserved Arg158–Asp154 electrostatic interaction present in ApoE3/4, altering electrostatic distribution in the receptor-binding region. Consequently, LDLR affinity is reduced to only 1-2% of ApoE3 [2]. While this defect predisposes to type III hyperlipoproteinemia peripherally, in the brain it may enhance compensatory Aβ clearance.

Enhanced Aβ clearance: Despite lower lipid transport efficiency, ApoE2 binds Aβ more strongly than other isoforms. Its C-terminal domain forms stable soluble complexes with Aβ, facilitating clearance via LRP1/HSPG-mediated microglial uptake [6]. Furthermore, ApoE2 suppresses microglial polarization to pro-inflammatory M1, reducing secretion of TNF-α and IL-1β, thereby mitigating neuroinflammation.

Population-level protection: Epidemiological studies show ε2 carriers have half the AD risk of non-carriers. By age 85, the cumulative incidence of AD among ε2/ε2 individuals is only ~5%, with cerebrospinal fluid Aβ42 levels significantly lower than ε3/ε3 individuals, underscoring its protective role [1].

3.2. ApoE3 (Cys112/Arg158): Neutral Isoform

ApoE3, the most common isoform (~77% frequency), maintains structural and functional balance:Structural stability: Arg158 forms a stable salt bridge with Asp154, preventing the N-C domain interaction unique to ApoE4. Additionally, Arg61 remains buried within the N-terminal helix bundle, avoiding abnormal domain interactions [2]. This enables ApoE3 to maintain plasticity: the C-terminal domain remains folded in the lipid-free state but opens upon lipidation to expose binding sites, ensuring efficient lipidation.

Lipid transport efficiency: ApoE3 strongly favors HDL-like particles. Following ABCA1-mediated lipidation, ApoE3 efficiently forms cholesterol-rich HDL-like particles, which deliver cholesterol to neurons via LDLR/LRP1. ApoE3-mediated cholesterol uptake is ~40-50% higher than ApoE4, sufficiently supporting synaptic repair and myelin maintenance [2].

Neutral AD risk: ApoE3 maintains intermediate efficiency in Aβ clearance and lipid transport, neither increasing nor reducing AD risk, thus serving as the functional baseline for AD research [1]. Population studies show ε3/ε3 homozygotes (60-70% prevalence) have a cumulative AD risk of ~15% by age 85 [1].

3.3. ApoE4 (Arg112/Arg158): Pathogenic Isoform

ApoE4 is the major genetic risk factor for AD. The Arg112 substitution drives conformational rearrangements that impair lipid transport: Domain interaction: Arg112 forms a new salt bridge with Glu109, altering N-terminal electrostatics. Conformational changes expose Arg61, which forms a long-range salt bridge with Glu255 in the C-terminal domain, promoting domain interaction. FRET and EPR studies confirm reduced Arg61-Glu255 distance in ApoE4 compared to ApoE3, validating structural abnormality [2].

Lipid-binding defects: Domain interaction reduces accessibility of the C-terminal lipid-binding site, lowering cholesterol and phospholipid affinity by 30-40%. ApoE4 preferentially associates with VLDL/LDL rather than HDL-like particles, resulting in hypolipidated, cholesterol-poor lipoproteins (~50% cholesterol content of ApoE3 particles). Neuronal cholesterol uptake mediated by ApoE4 is 15-20% lower than ApoE3. Astrocytic ApoE4 promotes formation of large, low-density Aβ fibril plaques, co-localizes with Aβ, and enhances dose-dependent fibrillization. Additionally, ApoE4 drives microglia toward disease-associated phenotypes (DAM), upregulating CD68 and cathepsin D, exacerbating neuroinflammation [2,3,7].

Protein instability and aggregation: Structural rearrangements yield a compact lipid-free conformation, yet reduce overall thermostability with melting temperature 5-8°C lower than ApoE3. ApoE4 is more susceptible to proteolytic cleavage, generating toxic fragments. Exposed hydrophobic surfaces promote self-oligomerization into dimers, tetramers, and larger aggregates, which impair lipid transport and nucleate Aβ aggregation, accelerating plaque formation [2].

4. Molecular Mechanisms by Which ApoE4 Induces AD via Lipid Transport Deficits

4.1. Impaired Lipidation Suppresses Aβ Clearance

Under physiological conditions, ApoE binds Aβ to form soluble complexes, mediating receptor-dependent clearance. ApoE4 lipidation defects disrupt this balance: Reduced ApoE4-Aβ complex stability: Lipidation enhances ApoE-Aβ binding. Fully lipidated ApoE (e.g., ApoE3) binds Aβ42 stably (Kd ≈ 20 nM), while hypolipidated ApoE4 exhibits 60% lower affinity, forming complexes prone to dissociation in acidic lysosomes. CSF from ApoE4 carriers shows 35% lower soluble ApoE-Aβ complexes and 2-fold higher Aβ oligomer levels compared to ApoE3 carriers [5,8].

Decreased receptor recognition: Domain interaction reduces exposure of the receptor-binding site, limiting ApoE4-Aβ complex recognition by neuronal/microglial LRP1. In vitro, ApoE4-Aβ binding to LRP1 is only 30% of ApoE3-Aβ, impairing uptake. ApoE4 also competes with Aβ for LRP1, further suppressing clearance [2].

Accelerated Aβ aggregation: Reduced clearance prolongs Aβ half-life in interstitial fluid, promoting oligomerization and fibrillization. In APP transgenic mice lacking ABCA1, Aβ deposition is 2.3-fold higher in ApoE4 mice than ApoE3, with denser plaques [8].

4.2. Neuronal Cholesterol Imbalance Drives Tau Pathology

Tau hyperphosphorylation represents another AD hallmark: Cholesterol deficiency: ApoE4-mediated transport deficits lower neuronal membrane cholesterol by 15-20% compared to ApoE3, disrupting lipid raft integrity. Lipid rafts anchor kinases such as GSK-3β, enabling its activation [3].

Tau kinase activation: Cholesterol loss activates GSK-3β, which phosphorylates tau at Ser396, Thr231, among others. Hyperphosphorylated tau detaches from microtubules, aggregates into neurofibrillary tangles, disrupting cytoskeleton and axonal transport [9].

Microglial lipid dysregulation: ApoE4 suppresses microglial ABCA1, inducing lipid droplet accumulation(↑ triglycerides 1.8-fold). Lipid-laden microglia release TNF-α and IL-1β, which activate neuronal CDK5, further promoting tau pathology [9].

4.3. Lipid Toxicity Activates Neuroinflammation

ApoE4-induced lipid dysregulation triggers chronic inflammation: Aberrant microglial sensing: Microglia detect ApoE lipoproteins via LRP1. ApoE4-lipoproteins destabilize LRP1 signaling, failing to activate PI3K–Akt, but instead triggering NF-κB, elevating TNF-α, IL-1β, IL-18 [9].

Lipid toxic molecules: Oxidized cholesterol species (e.g., 7β-hydroxycholesterol) accumulate in ApoE4 neurons, activate microglial NLRP3 inflammasomes, and boost IL-1β maturation. In ApoE4-targeted replacement mice, 7β-hydroxycholesterol is 2.1-fold higher and NLRP3 expression 3-fold higher than ApoE3 [9].

Microglial phenotype imbalance: ApoE4 inhibits M2 polarization, lowering Arg1/CD206 by 50%, while upregulating M1 markers (iNOS/CD16) 3-fold. This reduces Aβ phagocytosis by 40% and sustains inflammation [9]. NF-κB activation further elevates TNF-α and IL-1β secretion 3-fold, activating astrocytes to release complement C3, amplifying neuronal injury [10].

4.4. Synaptic Lipid Alterations Impair Function

Synaptic loss is the strongest correlate of cognitive decline: Cholesterol deficiency: Reduced neuronal cholesterol lowers synaptic membrane cholesterol by 18-25%, diminishing presynaptic vesicle release efficiency (30-50%) and reducing postsynaptic NMDA receptor clustering (25%). Electrophysiology shows ApoE4 mice exhibit 40% lower NMDA receptor-mediated LTP than ApoE3, correlating with cognitive impairment [7].

Disrupted lipid rafts: Lipid raft disassembly decreases insulin-degrading enzyme (IDE) activity by 30%, hindering Aβ degradation. PI3K-Akt signaling is impaired (↓50% Akt phosphorylation), reducing dendritic spine density (20%) and synaptic plasticity [9].

5. Conclusion

This study systematically delineates how ApoE polymorphisms regulate lipid metabolism and drive AD pathology. Residue substitutions at positions 112 and 158 alter electrostatics and domain conformations, yielding isoform-specific differences in lipid binding, receptor affinity, and transport. ApoE2 enhances Aβ clearance and suppresses inflammation, ApoE3 maintains neutral function, while ApoE4's lipidation deficits constitute the primary genetic risk for AD. The pathogenic mechanism of ApoE4 has multiple targets. Suppressed Aβ clearance, exacerbated tau pathology, chronic neuroinflammation, and synaptic dysfunction converge into a vicious "lipid dysregulation-pathology" cycle.

Future research can be carried out in the following directions:

Targeting ApoE lipidation: ABCA1 agonists improve ApoE4 lipidation, reducing Aβ deposition by 30% in APP mice.

Structural correction of ApoE4: Small molecules stabilizing ApoE4 domain orientation may restore lipid-binding.

Multifactorial interventions: Combining cognitive training (upregulating ABCA1) and dietary modulation (Mediterranean diet) with ApoE genotype-informed strategies may yield personalized prevention. Multi-modal imaging (fMRI/PET) and genetics (ApoE + polygenic risk scores) may refine early prediction.

These approaches may shift AD from "incurable" to "preventable and treatable."

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