Apolipoprotein E: structure determines function, from atherosclerosis to Alzheimer’s disease to AIDS

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Abstract  Apolipoprotein (apo) E has roles beyond lipoprotein metabolism. The detrimental effects of apoE4 in cardiovascular, neurological, and infectious diseases correlate with its structural features (e.g., domain interaction) that distinguish it from apoE3 and apoE2. Structure/function studies revealed that apoE2 is severely defective in LDL receptor binding because of a structural difference that alters the receptor binding region and helped unravel the mechanism of type III hyperlipoproteinemia. ApoE4 is the major genetic risk factor for Alzheimer’s disease and sets the stage for neuropathological disorders precipitated by genetic, metabolic, and environmental stressors. ApoE also influences neuropathological disorders precipitated by genetic, metabolic, and environmental stressors. ApoE also influences the receptor family, including the LDL-receptor-related protein, and gives rise to three homozygous and three heterozygous phenotypes. The nomenclature arose by consensus among key investigators (7).

Utermann, Hees, and Steinmetz (8) recognized differences in the apoE isoform patterns that distinguished normolipidemic subjects from patients with the genetic lipid disorder type III hyperlipoproteinemia (HLP) (dysbeta-lipoproteinemia). Studies by Zannis, Breslow, Havel, and others (9, 10) helped unravel the isoform pattern. Ultimately, apoE2 was associated with type III HLP. Gladstone investigators elucidated the structural basis for the polymorphism of apoE and showed that apoE isoforms differ at two sites: apoE3 has Cys-112 and Arg-158, whereas apoE4 has arginines at both sites, and apoE2 has cysteines (11, 12). They also determined the structure of apoE mRNA and the 3.6-kb gene encoding apoE and elucidated the regulatory elements that control its expression (13–15).

Plasma apoE (~40–70 μg/ml) arises primarily from hepatic synthesis (>75%). The second most common site of synthesis is the brain (16). Although astrocytes produce a large proportion of cerebrospinal fluid apoE (~3–5 μg/ml), neurons synthesize apoE when stressed (17). Macrophages and other cell types also synthesize apoE (1, 3, 16).

Initially, apoE was shown to be involved in lipid transport and cardiovascular disease (1–3). It is the critical ligand in the plasma clearance of triglyceride- and cholesterol-rich lipoproteins (chylomicron remnants, VLDL, intermediate density lipoproteins, and a subclass of HDL). After Goldstein and Brown (18) identified the LDL receptor, Gladstone investigators showed that apoE is the major ligand (1, 3, 19, 20). It is also the ligand for other members of the LDL receptor family, including the LDL-receptor-related protein, which contributes to remnant lipoprotein clearance. ApoE binds to heparan sulfate proteoglycans (HSPG), which also clear apoE-containing remnant lipoproteins (3, 21). Studies of type III HLP were pivotal in elucidating the roles of...

Historical Background

Structural differences in apolipoprotein (apo) E isoforms impact cardiovascular, neurological, and infectious diseases (1–4). Discovered in the 1970s, this 34-kDa, 299-amino-acid protein was identified in triglyceride-rich lipoproteins and induced by cholesterol feeding in animal models and humans (1, 3, 5, 6). The three common isoforms (apoE2, apoE3, and apoE4) are encoded by a gene on chromosome 19. The three alleles differ in their frequencies: ε4 (15–20%), ε3 (65–70%), and ε2 (5–10%).

Supplementary key words  cholesterol • neurodegeneration • HIV • coronary heart disease • LDL receptor • dysbeta-lipoproteinemia • heparan sulfate proteoglycans • infectious diseases • evolution

HISTORICAL BACKGROUND

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Abbreviations: Aβ, amyloid β; AD, Alzheimer’s disease; apo, apolipoprotein; HLP, hyperlipoproteinemia; HSPG, heparan sulfate proteoglycans; HSV, herpes simplex virus.

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apoE in remnant lipoprotein metabolism and atherosclerosis (3, 22).

ApoE also has a key role in neurobiology and Alzheimer’s disease (AD) (4). It is critical for lipid transport in the brain and contributes to neuronal maintenance and repair. ApoE4 is the major genetic risk factor for AD, and 60–80% of AD patients have at least one apoE4 allele. ApoE also modulates susceptibility to infectious disease and possibly immunoregulation (1, 3). ApoE4 enhances the infectivity of HIV in vitro and hastens progression to AIDS and death in HIV-positive subjects (23).

**ApoE isoforms: structural differences predict function**

Structural analyses provided insight into the mechanisms of apoE’s involvement in cardiovascular, neurological, and infectious diseases (1–4). ApoE has two structural domains separated by a hinge region (Fig. 1A). The N-terminal domain (amino acids 1–191) contains the receptor binding region (amino acids 134–150 and Arg-172) (1–3, 24) and forms a four-helix antiparallel bundle (3, 25). The C-terminal domain (amino acids 225–299) contains the major lipid binding region (amino acids 244–272) (2, 3). The amino acid differences among the isoforms profoundly affect their structures and roles in disease.

**ApoE AND CARDIOVASCULAR DISEASE**

ApoE2 and apoE4 increase the number of atherogenic lipoproteins and accelerate atherogenesis (1, 3, 6). Understanding structural differences in apoE isoforms helped establish the molecular mechanism responsible for the associated pathology. First, the altered structure and impaired function of the receptor binding region of apoE2 increase triglyceride and cholesterol levels caused by delayed clearance of hepatic and intestinal remnant lipoproteins (β-VLDL), resulting in type III HLP (3, 22). Cys-158 in apoE2 affects the receptor binding region by altering salt bridges and lowering the positive potential (25). Second, the increase in plasma cholesterol, LDL, and apoB associated with apoE4 appears to reflect the influence of Arg-112 (1–3). Arg-112 alters the lipid binding region of apoE4 and changes the lipid binding preference from small phospholipid-rich HDL (apoE2 and apoE3) to large triglyceride-rich VLDL (apoE4). This difference is due to apoE4 domain interaction, in which the N- and C-terminal domains interact, resulting in a more compact structure.

**ApoE2: insights into the lipoprotein receptor and HSPG binding and type III HLP**

The receptor and proteoglycan binding regions were identified through structural studies of apoE2 and its role in type III HLP (3, 22). Before the structure was delineated, arginine and lysine basic residues were shown to be critical for binding to the LDL receptor (26, 27). In addition, binding to phospholipids was required for high-affinity binding activity (20). Mutagenesis studies identified the critical basic residues within the 134–150 region of apoE as well as Arg-172 (24, 28). Modeling of apoE into the molecular envelope of apoE bound to phospholipid revealed why lipid binding is required for high-affinity binding to LDL receptors (29). To fit the molecular envelope, apoE folded into a helical horseshoe, bringing the critical residues for binding, amino acids 134–150 and Arg-172, into close proximity (Fig. 1B).

ApoE2, which is severely defective in LDL receptor binding activity (<2% LDL receptor binding activity compared with apoE3), differs structurally from apoE3 and apoE4, which bind avidly to LDL receptors. In apoE3, the 134–150 region is largely solvent exposed and forms a 20Å field of positive potential, likely available for receptor binding. In apoE2, the presence of Cys-158 rather than arginine alters the conformation and size of the positive potential (3, 25).

Residue 158 lies outside the 134–150 region, so how does it influence binding activity? In apoE3, Arg-158 forms a salt bridge with Asp-154. However, in apoE2, Cys-158 disrupts the salt bridge, causing Asp-154 to interact with Arg-150 (30) (Fig. 2A). This alters the size of the positively charged domain and disrupts LDL receptor binding.
ApoE4: insights into lipid binding, domain interaction, and atherosclerosis

The C-terminal domain of apoE (amino acids 225–299) is predicted to form amphipathic α-helices (2). These helices are responsible for lipid binding in different apolipoproteins, and amino acids 244–272 constitute the lipid binding region of apoE (2, 3). ApoE3 and apoE2 preferentially bind to small, phospholipid-rich HDL, whereas apoE4 binds to large, triglyceride-rich VLDL. The difference is determined by how the N- and C-terminal domains interact (32). X-ray crystallographic analyses of apoE3 and apoE4 revealed differences in side chain orientation and rearrangements of salt bridges. In apoE4, Arg-112 forms a salt bridge with Glu-109 and causes the Arg-61 side chain to extend away from the four-helix bundle. In apoE3, this side chain is buried (25) (Fig. 2B).

The orientation of Arg-61 in apoE4 promotes domain interaction by interacting with Glu-255 within the lipid binding region, causing apoE4 to have a more compact conformation than apoE3 (Fig. 1A) (32, 33). Domain interaction is an important structural property of apoE4 (34, 35) that may be responsible for several of its pathogenic effects. ApoE3 and apoE2 are less likely to demonstrate domain interaction. Mutation of Arg-61 to threonine, or Glu-255 to alanine, abolishes domain interaction, causing the mutated apoE4 to function similarly to apoE3 (36). Domain interaction appears to alter the lipid binding region and, thus, lipoprotein preference. ApoE4 with Thr-61 corrects not only lipid binding preference but also several of the detrimental effects of apoE4 in neurobiology (4).

In most animals, including great apes, apoE has Arg-112 (like human apoE4) but has threonine at a site equivalent to Arg-61 in human apoE (2). Lacking domain interaction, it behaves like apoE3. Gene targeting to replace Thr-61 with arginine in mouse Apoe (37) produced a model of domain interaction (Arg-61 apoE mouse) that does not display other apoE4 structural properties. It established that domain interaction was responsible for the preference of apoE4 for VLDL.

ApoE4 increases plasma LDL levels and risk for atherosclerosis and is overrepresented in hyperlipidemic and cardiovascular disease patients (3). Because apoE4 binds preferentially to VLDL and remnants, it may accelerate their clearance, leading to downregulation of LDL receptors and increased LDL levels. Alternatively, remnants could compete for LDL receptors, retarding LDL clearance.

ApoE: roles in regulating VLDL production, lipolysis, and triglyceride levels

Plasma apoE levels are determinants of triglyceride-rich lipoprotein metabolism and explain 20–40% of variability in triglyceride levels in humans. Overexpression and accumulation of apoE appear to cause hypertriglyceridemia by stimulating VLDL triglyceride production and impairing VLDL lipolysis (38). In apoE-deficient mouse hepatocytes, VLDL triglyceride secretion is impaired, suggesting a physiological role for apoE in VLDL assembly secretion (39). In fact, hepatic overexpression of apoE stimulates VLDL production, increasing VLDL triglyceride secretion.
in transgenic mice and transfected hepatocytes and in hypertriglyceridemic patients. ApoE inhibits lipolysis in vitro, and plasma from hypertriglyceridemic patients has decreased lipoprotein lipase cofactor activity for apoCII, suggesting that the apoE:apoCII ratio in VLDL is critical for VLDL lipolysis by lipoprotein lipase (38). Thus, optimal expression of apoE is crucial for normal metabolism of triglyceride-rich lipoproteins. Too little apoE impairs plasma clearance of triglyceride-rich lipoproteins and their remnants. Too much apoE stimulates hepatic VLDL triglyceride production and impairs lipolysis, leading to hypertriglyceridemia.

ApoE AND NEURODEGENERATIVE DISORDERS

ApoE is the major known genetic risk factor for AD (40) and has a potential role in other neurological diseases and traumatic brain injury (4). In AD, apoE4 contributes to neuropathology by interacting with the amyloid pathway to modulate amyloid β (Aβ) peptide synthesis or clearance. ApoE4 can also cause neuropathology directly (4). ApoE4 sets the stage for “second hits” to precipitate neuropathology. These second hits may be genetic (α-synuclein in Parkinson’s disease, SOD-1 in amyotrophic lateral sclerosis, and presenilin in AD), metabolic (ischemia and oxidative stress), or environmental (aging, inflammation, and central nervous system trauma). The neuropathologic effects of apoE4 are mediated by its structural features (e.g., domain interaction) (4).

ApoE4 increases of Aβ production

In cultured neuronal cells expressing amyloid precursor protein, exogenous apoE4 enhances Aβ production more than apoE3 (36). ApoE4 with Thr-61, which lacks domain interaction, behaves like apoE3. When domain interaction is blocked by a small molecule that docks with apoE near Arg-61, apoE4 no longer enhances Aβ production (36). Thus, altering a structural feature of apoE4 also converts its biological activity to one resembling apoE3.

ApoE4 destabilizes membranes and increases apoptotic cell death

In neuronal cells cultured with Aβ1-42, exogenous apoE4 causes lysosomal leakage and apoptosis, whereas apoE3 is protective (41, 42). ApoE4 causes greater membrane disruption than apoE3; and apoE4 with Thr-61, lacking domain interaction, behaves much more like apoE3. A small-molecule structure corrector also blocked the detrimental effects of apoE4. These studies provide proof of concept that such effects can be prevented with a structure corrector that blocks domain interaction and converts apoE4 to a molecule that is structurally and functionally similar to apoE3 (4).

ApoE synthesis by neurons leads to neuropathology

ApoE synthesis by neurons can be induced by various stressors or stimuli (17, 43), possibly including age, oxidative stress, trauma, Aβ deposition, and ischemia. Neuronal expression of apoE is likely induced to protect the cells from noxious stimuli or to repair damage, possibly by redistributing lipids. However, there is a marked difference in how apoE3 and apoE4 are handled by neurons. ApoE4 synthesized by neurons undergoes proteolytic cleavage to a much greater extent than apoE3 (44). The resulting fragments with C-terminal truncations escape the secretory pathway and enter the cytosol. Most are neurotoxic (45). Similar apoE4 fragments are seen in the brains of transgenic mice expressing apoE4 in neurons (NSE-apoE) and in AD brains but not in transgenic mice expressing apoE4 in astrocytes. The initial fragment is produced by a chymotrypsin-like serine protease that primarily cleaves apoE at amino acids 268 and 272. The fragments accumulate with age in NSE-apoE4 mice, reaching a peak concentration at 6–8 months of age when these mice exhibit apoE4-associated neuropathology (reduced synaptophysin and microtubule-associated protein 2 immunoreactivity) and impaired learning and memory (water maze tests) (44).

The susceptibility of apoE4 to neuron-specific proteolysis is mediated by domain interaction. ApoE4 with Thr-61 is resistant to proteolysis by mouse brain lysate as the source of the cleaving enzyme. Here again, a structure corrector might protect apoE4 from proteolysis. Inhibition of the apoE-cleaving enzyme might be an additional therapeutic strategy (4).

In cultured neurons, the apoE fragments are primarily localized to mitochondria and to a lesser extent to filamentous neurofibrillary-tangle-like structures composed of phosphorylated tau (46). We suspect that the mitochondrial dysfunction characteristic of neurons synthesizing apoE4 is related to the translocation of the fragments to the mitochondria. The cytoskeletal abnormalities associated with apoE4 might relate to fragment localization within the filamentous inclusions. Again, the pathology can be correlated with the apoE structure (4). Studies of truncated and mutated apoE4 forms expressed in cultured neurons demonstrated how the apoE4 fragments lacking the C-terminal 27 amino acids escape the secretory pathway, translocate into the cytosol, interact with mitochondria, and cause neurotoxicity (46). The minimal structure of apoE required for these detrimental effects includes the receptor binding region (amino acids 134–150) and the lipid binding region (amino acids 244–272).

Translocation of the fragments is mediated by positively charged amino acids in the receptor binding region (46). This sequence resembles the protein translocation domain of many viral proteins. Mutation of critical lysines or arginines in this domain abolishes translocation of the fragments to the cytosol.

The hydrophobic lipid binding region is responsible for mitochondrial targeting and neurotoxicity (46). Mutation of conserved residues in this region blocks the interaction with mitochondria. Mitochondrial dysfunction can profoundly alter neurobiology in many ways. For example, mitochondria are important in synaptogenesis, and their localization to the site of synaptic spine formation is essential. Neuronal cells incubated with apoE4 (especially the 29-kDa
fragment) have fewer mitochondria located to the spines and many fewer spines than neurons incubated with apoE3 (47). These findings suggest an additional therapeutic strategy: preventing the interaction between apoE fragments and mitochondria with so-called mitochondrial protectors (4).

ApoE, INFECTIOUS DISEASE, AND IMMUNOREGREULATION

ApoE also plays a role in susceptibility to infection (1, 3), including malaria (48) and bacterial infections (49, 50). However, isoform specificity has not been explored.

Evidence suggests that apoE modulates viral infections, and there are isoform-specific effects related to herpes simplex virus (HSV) and HIV. HSV infection is associated with increased risk of AD, and apoE4 is overrepresented in HSV-infected subjects (51). In HIV-positive patients, apoE homozygosity hastens progression to AIDS and death and increases susceptibility to opportunistic organisms (23). Cultured cells are more susceptible to infection with HIV in the presence of apoE4 than apoE3, reflecting enhancement of viral attachment and fusion. The structural differences between apoE4 and apoE3 may shed light on the mechanism by which apoE4 modulates infectivity and fusion.

Ampiphatic helices of apolipoproteins interact with similar helices of the HIV gp41 protein and inhibit viral fusion (52). In apoE, these helices encompass the lipid binding region. Since domain interaction modulates lipid binding, isoform-specific differences in HIV infectivity and fusion may be explained by the structure of apoE4. Alternatively, both apoE and HIV bind to cell-surface HSPG, whose interaction with viral particles may be rate limiting in HIV infection. Another potential explanation is the differential effects of apoE on cholesterol content or other lipids in infected cells or the HIV envelope.

Do infectious diseases drive apoE evolution?

The source of differences in the apoE allele frequencies among population groups is unknown. ApoE3 is the most common, but apoE4 may be the ancestral allele (1, 3, 53, 54). Many animals, including all the great apes, have an apoE4-like allele (Arg-112) and do not display multiple isoforms (2). It is unlikely that the detrimental effects of apoE4 in cardiovascular or neurological disease provided the evolutionary pressure, as these effects are postreproductive. Any genetic drift from apoE4 to apoE3 to apoE2 most likely results from the selective pressures of infectious diseases.

For example, apoE4 is more frequent in Africa, perhaps because it afforded protection against specific endemic infectious diseases (e.g., parasitic diseases). However, 50,000 years ago, when populations migrated from Africa to Europe and beyond, new infectious diseases (e.g., viral infections) may have arisen in historical centers of agriculture, where population density might maintain viral diseases, resulting in selection against carriers of apoE4. Thus, apoE3 might have provided a selective advantage over apoE4. A cataclysmic event in human history driving the evolution of apoE4 to apoE3 to apoE2 could have been an infectious disease, such as the Great Plague, which killed 30–50% of Europeans in the 14th century, or smallpox. Much remains to be learned about the evolution of this fascinating protein and the selective pressures that account for the population differences in the frequency of the apoE alleles (3, 55–57).

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