LIPIDS AND CARDIOVASCULAR DISEASE

Apolipoprotein E in lipoprotein metabolism, health and cardiovascular disease

A. David Marais

Chemical Pathology Division, Pathology Department, University of Cape Town Health Science Faculty and National Health Laboratory Service, Cape Town, South Africa

Summary

Apolipoprotein E (apoE), a 34 kDa circulating glycoprotein of 299 amino acids, predominantly synthesised in the liver, associates with triglyceride-rich lipoproteins to mediate the clearance of their remnants after enzymatic lipolysis in the circulation. Its synthesis in macrophages initiates the formation of high density-like lipoproteins to effect reverse cholesterol transport to the liver. In the nervous system apoE forms similar lipoproteins which perform the function of distributing lipids amongst cells. ApoE accounts for much of the variation in plasma lipoproteins by three common variants (isoforms) that influence low-density lipoprotein concentration and the risk of atherosclerosis. ApoE2 generally is most favourable and apoE4 least favourable for cardiovascular and neurological health. The apoE variants relate to different amino acids at positions 112 and 158: cysteine in both for apoE2, arginine at both sites for apoE4, and respectively cysteine and arginine for apoE3 that is viewed as the wild type. Paradoxically, under metabolic stress, homozygosity for apoE2 may result in dysbetalipoproteinaemia in adults owing to impaired binding of remnant lipoproteins to the LDL receptor and related proteins as well as heparan sulphate proteoglycans. This highly atherogenic condition is also seen with other mutations in apoE, but with autosomal dominant inheritance. Mutations in apoE may also cause lipoprotein glomerulopathy. In the central nervous system apoE binds amyloid β-protein and tau protein and fragments may incur cellular damage. ApoE4 is a strong risk factor for the development of Alzheimer's disease. ApoE has several other physiological effects that may influence health and disease, including supply of docosahexaenoic acid for the brain and modulating immune and inflammatory responses. Genotyping of apoE may have application in disorders of lipoprotein metabolism as well as glomerulopathy and may be relevant to personalised medicine in understanding cardiovascular risk, and the outcome of nutritional and therapeutic interventions. Quantitation of apoE will probably not be clinically useful. ApoE is also of interest as it may generate peptides with biological function and could be employed in nanoparticles that may allow crossing of the blood-brain barrier. Therapeutic options may emerge from these newer insights.

INTRODUCTION

Scope of the review

Apolipoprotein E (apoE) was a curious 'arginine-rich protein'[1] discovered in triglyceride-rich lipoproteins but now is one of the ten most studied genes encoded in the genome.[2] Insights into the structural and functional aspects of apoE not only clarify lipoprotein metabolism and cardiovascular health and disease, but are relevant to neurological function and potentially for therapeutic applications. This review aims to integrate developments around apoE over the recent few years following previous reviews on apoE,[3] dysbetalipoproteinaemia[4] and Alzheimer's disease.[5] Although the emphasis of this article will be on role of apoE in lipid metabolism in the circulation and nervous system, the pathophysiology, genetics and basic and applied science aspects are included.

A brief history of apoE

Severe hyperlipidaemia causing xanthoma tuberosum[6] was linked to apoE through its association with type III hyperlipidaemia according to the Fredrickson classification.[7] Ultracentrifugation characterised the cholesterol-rich, very low-density lipoprotein (VLDL) and different migration as β-lipoproteins, explaining the term of dysbetalipoproteinaemia. Isoelectric focusing found varying distributions of isoforms amongst populations.[8] The binding of apoE2, a common variant, to low density lipoprotein (LDL) receptors is defective, but is restored with cysteamine.[7] Mutations of apoE were discovered to cause dominantly inherited dysbetalipoproteinaemia.[9] Another common variant, apoE4, was linked to Alzheimer's disease[10] while the apoE2 isoform is associated with longevity.[11]

APOLIPOPROTEIN E

Evolution of apoE

Apolipoprotein B (apoB) and the related microsomal triglyceride transfer protein enabled the assembly of lipoproteins for lipid transport[12] in multicellular organisms. ApoE developed as part of a set of similar interchangeable apoproteins, enabling improved metabolic control of lipoproteins.[13] ApoE confers efficient clearance of remnants of triglyceride-rich lipoproteins, but can itself create lipoproteins to shuttle lipids between cells, especially in the nervous system.

The sole fish expresses two forms of apoE[14] prominently in the gut and brain. Human apoE differs from apoE in other mammals, including primates, probably by mutations that occurred at genetic 'hot spots'.[15] Common variants occur at two amino acids, creating three isoforms. Isoelectric focusing identified isoforms labelled apoE2, apoE3 and apoE4 according to their charge. These isoforms derive from the corresponding genes referred to as ε2, ε3, and ε4. Additional mutations in apoE may change the isoelectric point by altering the net charge on the protein.

The corresponding gene for apoE is ε4 in animals, but amino acid 61 of the mature protein is threonine in animals and arginine in humans. The consequent modest hyperlipidaemia attributable to this mutation altered the properties of apoE. The consequent LDL hypercholesterolaemia may have conferred a survival advantage by enhancing resistance to infection, albeit with a risk of atherosclerosis and neurological impairment in older people. ApoE3 or apoE2 may have been selected by long-term survival due to better 'grandmothering' in groups of humans over the past 10,000 years.[15]

Variations in the *APOE* gene

The *APOE* gene is located on chromosome 19q13.2 and comprises 4 exons. The variants that give rise to the apoE isoforms are rs429358 for codon 112 and rs7412 for codon 158. There are thus six permutations of the apoE or ε variants. In the United States of America[7] the approximate prevalences for E2/2, E2/3, E2/4, E3/3, E3/4 and E4/4 are 1, 22, 2, 58, 14 and 3%, respectively; data suggest that apoE4 is more prevalent in the black population.

The common variants of apoE explain about 4% of the variance in plasma cholesterol.[16] Another variant in apoE (rs35136575) affects LDL cholesterol concentrations, explaining 1% of the variance in Caucasians, 3% in African Americans and 2% in Mexican-Americans.[17]

Functions of apoE

ApoE is most important for lipid and lipoprotein metabolism, promoting the clearance of remnants of triglyceride-rich (apoB-containing) lipoproteins from the circulation into the liver because it is the ligand for the LDL receptor family of proteins and heparan sulphate proteoglycans (HSPG). In the brain, apoE itself assimilates and transfers lipids. ApoE influences adipogenesis from triglyceride-rich lipoproteins.[18] In mice, apoE adapts bile acid metabolism by upregulating sterol 6-β hydroxylase which converts chenodeoxycholic acid to the more hydrophilic muricholic acid and decreases fat absorption.[19]

Vascular function is affected in various ways by apoE: from maintaining blood-brain barrier integrity[20] to inflammatory responses.[21] ApoE also influences platelet aggregation.[22] Inflammatory effects include transformation of macrophages to M2 phenotypes[23] and proliferation of lymphocytes and T helper cells.[20] ApoE influences micro-RNA levels, especially miRNA-146a which is enriched in the brain. Lowered concentrations of miRNA-146a are found in the plasma and brain of apoE4 carriers.[24] Modulation of NF-κB activity by apoE influences pericyte and blood-brain barrier function.[25] ApoE influences paraoxonase expression at which apoE4 is less effective than the other isomers.[26]

Intracellular organelle homeostasis may be influenced by apoE. Early neuronal dysfunction in apoE4 carriers is associated with mitochondrial dysfunction.[27] ApoE4 may also increase endoplasmic reticulum stress by mimicking misfolding of protein.[28] Biologically active peptides from apoE may also have pathophysiological roles.[29] Short cationic peptides (e.g., amino acids 133–167), may have immunomodulatory effects and could disrupt bacterial biofilms.

Production of apoE

ApoE is expressed in many cells,[30] but especially in the liver, and widely in the central nervous system: in astrocytes, glial cells, vascular cells and in the choroid plexus. Neurons produce a splice variant of RNA that is minimally translated.[31] ApoE is expressed in macrophages and adipocytes.[30] The binding of apoE to heparin and HSPG in extracellular matrices or on cell membranes may influence cellular responses to signals and could explain why apoE is expressed in the spleen, lung, kidney, ovary, peripheral nerves and muscle. The biosynthesis of apoE is depicted in Fig. 1.

The mRNA for apoE is translated to a protein of 317 amino acids with subsequent cleavage of its signal peptide of 18 amino acids. For comparability with previous publications, this document retains the use of the numbering applicable to the mature protein. *O*-glycosylation is at threonine 194 in the liver,[32] but less extensive glycosylation is present in the circulation than in hepatocytes and more glycosylation is documented in the nervous system. Maturation through the Golgi apparatus and secretion of apoE are slowed down when the endoplasmic reticulum membrane is enriched with cholesterol.[33] In the Golgi apparatus of hepatocytes, apoE associates with VLDL, enhancing its development and secretion. Interestingly, apoE is also incorporated into the hepatitis C virion at the Golgi apparatus and promotes its infectivity, especially with apoE4.[34] The final conformation of apoE may differ according to the cell type and isoforms.[35]

Although self-association into a lipid-free tetramer may occur,[36] the bulk of apoE is associated with lipoproteins. The normal plasma concentration of apoE[37] of approximately 1 μmol/L, compares with that of apoB and indicates that not all of the apoB-containing lipoproteins carry apoE.

Some newly synthesised apoE may be degraded, but secretion is increased by the availability of lipid as seen with the uptake of LDL in HepG2 cells.[38] Secreted apoE can be re-uptaken into hepatocytes and re-secreted, but less so in the case of apoE4 with consequent accumulation of cholesterol in the cell.[39] ApoE can be internalised in the absence of LDL receptors because it has broad ligand activity, and is recycled after endocytosis.[40] Macrophages partition endogenously produced apoE from exogenous apoE[41] with different effects on lipid metabolism. Degradation of endogenous apoE is less when macrophages are incubated with HDL,[42] presumably from a salvage mechanism similar to that provided by LDL.

A cysteine protease that can be inhibited by N-acetyl-leucyl norleucinal,[43] degrades apoE. ApoE also undergoes chymotrypsin-like proteolytic cleavage extracellularly to smaller peptides ranging between 17 and 25 kDa in the nervous system,[44] with variations in different parts of the brain. Neurons degrade apoE4 differently. About 30 amino acids are cleaved from the carboxy-terminus to leave a 29 kDa
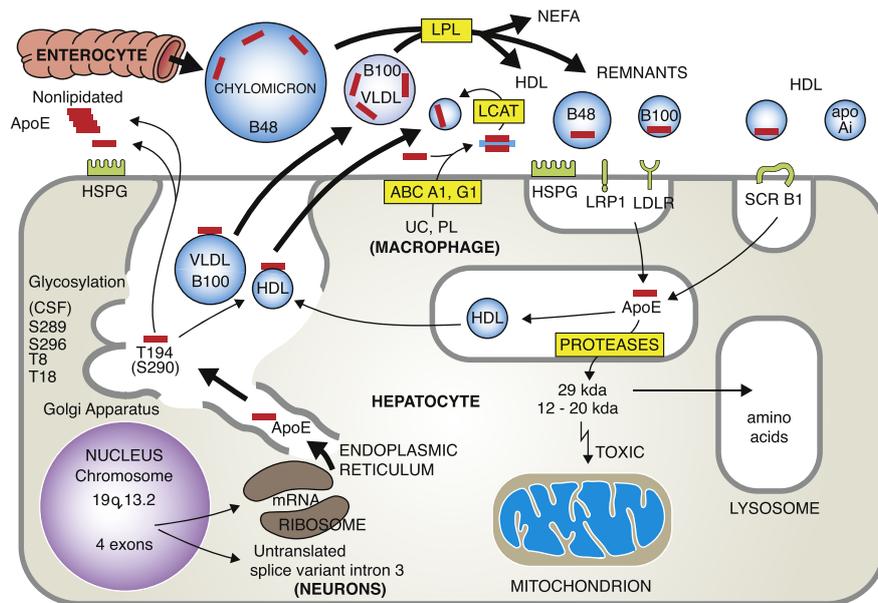
Fig. 1 Apolipoprotein E biosynthesis. The gene on chromosome 19 contains 4 exons but may be spliced to a minimally translated variant in neurons. Glycosylation at the Golgi apparatus is chiefly at threonine 194 but also at serine 290 while in the CSF other glycosylated forms are found. ApoE promotes the synthesis of VLDL and its secretion can be promoted with acquisition of lipoproteins by the cell. Some apoE binds extracellular heparan sulphate proteoglycans while other apoE may self-associate into tetramers. ApoE on triglyceride-rich lipoproteins remain on remnants and promote their uptake by binding heparan proteoglycan, LDL receptor related protein 1 and LDL receptor. ApoE can accept cholesterol and phospholipid at the cell surface through ABC transporters A1 and G1. Such discoidal particles gain cholesterol ester by the action of LCAT. High density lipoproteins with apoE can bind scavenger receptors to deliver apoE into the cell from where it can be secreted or be degraded, with some differences from degradation of apoE4 that may yield toxic peptides to the mitochondria and lysosomes. ABCA1, G1, adenosine binding cassette transporters A1 and G1; ApoAi, apolipoprotein Ai; ApoE, apolipoprotein E; B48, apolipoprotein B$_{48}$; B100, apolipoprotein B$_{100}$; CSF, cerebrospinal fluid; HDL, high density lipoprotein; HSPG, heparan sulphate proteoglycan; kda, kilodalton; LCAT, lecithin:cholesterol acyltransferase; LDLR, low density lipoprotein receptor; LRP1, LDL receptor related protein 1; LPL, lipoprotein lipase; NEFA, non-esterified fatty acids; PL, phospholipid; SCRB1, scavenger receptor B1; S, serine; T, threonine; UC, unesterified cholesterol; VLDL, very low-density lipoprotein.

fragment followed by secondary fragments of 12–20 kDa. These smaller peptides have multiple effects on other cellular proteins, cytoskeleton and even mitochondria. The carboxy-terminal peptide from apoE4 (amino acids 272–299) causes neurological damage in transgenic mice.[45]

The *in vivo* turnover of apoE isoforms was studied by stable isotopes.[46] ApoE concentration was highest with apoE2 and lowest with apoE4 isoforms. Clearance of apoE2 is lower relative to apoE3 and production rate of apoE4 is lower than that of apoE3 and apoE2. There was preferential association of apoE4 with VLDL. The fractional catabolic rate for total apoE was 0.49 pools/day and within lipoproteins was 2.95 pools/day. The production rates for apoE on HDL and VLDL were 0.47 mg/kg/day and 2.6 mg/kg/day.

Interaction of apoE with receptors

ApoE is a ligand for the constitutively expressed HSPG and LDL receptor related protein in the liver, as well as the LDL receptor. The activity of the LDL receptor is regulated by the cholesterol content of the cell and ensures a supply of cholesterol when up-regulated by internalising LDL and remnant lipoproteins. The high affinity of apoE for the LDL receptor could be due to several copies being present on a lipoprotein or a distinct epitope may be exposed on association of the apoprotein with lipoproteins.[47] After lipolysis of triglyceride-rich lipoproteins in the circulation, the remnants penetrate the fenestrated vessels for uptake by the liver. The large clearance capacity of HSPG will prevent remnant accumulation even when there is no LDL receptor activity.

The structure of apoE is indicated in Fig. 2. The N-terminus provides the ligand activity of apoE[48] while the C-terminus interacts with the phospholipids, β amyloid and tau protein. At the N-terminus, four helices bundle together and the interposed hinge domain locks in position the C-terminus, which is folded back over the LDL receptor-binding region. This region only becomes exposed when the C-terminus moves away on embedding in phospholipids. This ensures that apoE is an active ligand only on the lipoprotein. The ligand region comprising amino acids 136–150 is rich in positively charged arginine and lysine residues and binds HSPG and the LDL receptor. The LDL receptor binding activity is robust as it persists in a chimaeric protein of the N-terminal domain of apoE with the C-terminus of apoAi.[49]

In the absence of arginine at position 158, apoE2 allows the aspartate at position 154 to form a salt bridge with arginine at position 150. This disrupts LDL receptor binding significantly, but binding to HSPG is disrupted less, explaining why only a proportion of apoE2 homozygotes develop dysbetalipoproteinaemia. Mutations within the LDL receptor binding domain of apoE disrupt both LDL receptor and HSPG binding and thus strongly predispose to remnant accumulation evidenced by dominant inheritance, albeit still with delayed onset until metabolic changes unmask the problem.

The arginine at position 61 in human apoE forms a salt bridge with glutamate 255 to keep the C-terminus in apposition; but not in apoE4[15] so that greater phospholipid binding exposes the LDL receptor-binding region and clearance of lipoproteins containing apoE4 is enhanced.

Fig. 2 ApoE structure. The protein of 317 amino acids loses the signal peptide of 18 amino acids to become the mature protein containing the N-terminus, hinge and C-terminus domains. The latter contains regions that associate with phospholipid, tau protein as well mediating as self-association. The LDL receptor and heparan sulphate proteoglycan binding domains are as indicated, as are the amino acids that determine the isoforms. Amino acid 61 is arginine in humans only. Amino acid 194 is O-glycosylated. The glutamates at 254 and 255 interact with arginine 61. The aspartic acid at 154 forms a salt bridge with arginine at position 158 in apoE3 and apoE4 but with arginine 150 in apoE2. Amino acids: C, cysteine; D, aspartic acid; E, glutamic acid; R, arginine. HSPG, heparan sulphate proteoglycan; LDLR, low-density lipoprotein receptor; τ, tau protein.

Chemical modification of apoE may harm its function. Acrolein, a reactive aldehyde in smoke, impairs the formation of small lipoprotein-like particles by apoE as well as the binding to its receptors.[50] Glycation of apoE also disrupts HSPG interaction.[51]

LIPOPROTEIN METABOLISM AND APOE

Distribution of apoE on lipoproteins

The lipoprotein pathways and the roles of apoE in lipoprotein metabolism are illustrated in Fig. 3. In the fasting state apoE is found on VLDL and HDL. LDL, like IDL, carries practically no apoE. Post-prandially, chylomicrons undergo lipolysis and their remnants, like those of VLDL, are cleared rapidly. ApoE and apoCiii often coexist on apoB-containing lipoprotein, on almost half VLDL.[52] ApoCiii hinders clearance mediated by apoE.[53] Post-prandially, apoB-containing lipoproteins containing apoE can double in concentration by 4.7 hours, decrease in size to that of LDL by 6 hours and disappear by 10 hours.[54]

ApoE initiates the biogenesis of HDL by accepting cholesterol and phospholipid from the ABCA1 transporter,[55] but apoE4 is less effective than apoE3. The efficacy at generating such particles with apoE4 is improved by

Fig. 3 Apolipoprotein E and lipoprotein metabolism. The lipoprotein metabolism in the circulation concerns distribution of lipid from the gut and liver and recycling to the liver with little transfer of apoB-containing lipoproteins to the nervous system, though small lipoproteins containing apoE can bind receptors at the blood-brain barrier to exchange by transcytosis, supplying especially essential n-fatty acids to the brain and distributing these as well as other lipids within the nervous system. ApoE is synthesised by most cells in the nervous system and interacts with amyloid β and tau protein, apoE4 being linked with Alzheimer's disease and cerebral amyloid angiopathy. Macrophages can assimilate lipid and promote reverse cholesterol transport with apoE. Atherosclerosis relates to increased exposure to apoB containing lipoproteins, especially LDL or remnants (dysbetalipoproteinaemia). Aggregation of lipoprotein can cause glomerulopathy. Aβ, amyloid β protein; ABCA1, G1, adenosine binding cassette transporters A1 and G1; apoE, apolipoprotein E; Av, apolipoprotein Av; B48, apolipoprotein B48, B100, apolipoprotein B100; Cii, apolipoprotein Cii; CM, chylomicron; CMR, chylomicron remnant; FC, free cholesterol; HDL, high density lipoprotein; HSPG, heparan sulphate proteoglycan; LCAT, lecithin:cholesterol acyltransferase; LDLR, low density lipoprotein receptor; LPL, lipoprotein lipase; LRP1, LDL receptor related protein 1; NEFA, non-esterified fatty acids; PL, phospholipid; SCR, scavenger receptor; S, serine; T, threonine; τ, tau protein; UC, unesterifed cholesterol; VLDL, very low-density lipoprotein; VLDLRe, VLDL receptor at blood-brain barrier; VLDLR, VLDL remnant in circulation compartment.

enhancing lipid efflux through ABCA1 and ABCG1 with the peptide CS 6253, generating similar lipoproteins to those of apoE3 and improving cognitive function.[56]

Newly formed discoidal particles, containing two molecules of apoE, gain cholesterol ester under the action of lecithin:cholesterol acyltransferase (LCAT), to almost the size of LDL. Recombinant apoE can create particles with varying composition of unesterified cholesterol, cholesterol ester and phospholipid. Large spherical apoE-containing particles are more prone to remodelling by phospholipid transfer protein than apoAi-containing particles. Even after modification by phospholipid transfer protein,[57] prominent in cerebrospinal fluid (CSF), apoE tends to occupy larger HDL particles.

Impact of isoforms on lipoproteins

ApoE isoforms influence post-prandial lipoprotein metabolism as demonstrated by oral fat challenges in which retinyl palmitate marks chylomicrons.[58] Clearance is rapid with apoE3 and E4, but delayed with apoE2.

ApoE is found on a range of VLDL particles, but in lower concentration in subjects with apoE4, consistent with enhanced clearance.[59] Proportionally, there is more apoE4 on VLDL than on HDL in which apoE3 and apoE2 predominate.

The chief effects of apoE isoforms on fasting plasma lipids are on LDL and apoB concentrations.[60] On average, apoE2 lowered LDL cholesterol by 0.24 mmol/L in men. In women the lowering was 0.35 mmol/L; pre- and post-menopausally by 0.21 mmol/L and 0.53 mmol/L, respectively. ApoE4 raised LDL cholesterol concentrations in men and women by 0.07 and 0.14 mmol/L, respectively.

Although LDL does not contain apoE, the isoforms influence its concentration and size along with many other factors including sex, age and triglyceride concentration. Smaller LDL is associated with higher cardiovascular risk and lower HDL concentration. Increased or prolonged post-prandial triglyceride concentration could allow cholesterol ester transfer protein and hepatic lipase to remodel LDL to smaller particles in apoE2 carriers. Various effects of apoE isoforms have been reported in studies that differ in methods and participants. ApoE2 carriers had smaller LDL with normal HDL size, unlike apoE4 carriers who had a preponderance of smaller HDL.[61] In middle-aged Croatians,[62] LDL particle size was smaller in men with an apoE2 allele, but not in women. Middle-aged healthy Arabian carriers of apoE2 had no small LDL compared with controls without apoE2; however, (older) subjects with apoE2 and coronary heart disease had smaller LDL.[63]

In a diet study,[64] apoE4 carriers had larger LDL. Individuals carrying apoE4 increased their particle size when changing from a lipid-rich to a carbohydrate-rich diet, presumably because increased VLDL production attributable to oleic acid resulted in hypertriglyceridaemia that remodelled the more slowly turning over LDL particles. In this study, apoE2 was associated with smaller particles. Smaller LDL size was found independently of triglyceride concentration in apoE4 carriers,[65] with an interaction of this isoform and a variant in the hepatic lipase gene. In familial combined hyperlipidaemia, small LDL correlated with higher concentrations of large VLDL which is relatively poor in apoE.

ApoE is present on a subset of lipoprotein(a) [Lp(a)].[66] Lipoparticles containing apoB and apo(a) compared with those additionally containing apoE, are found in the smaller size range (closer to LDL) compared with the latter (predominantly in VLDL). In normolipidaemic individuals, the apoE-containing Lp(a) account for about 16% of the Lp(a),[67] and possibly more in hypertriglyceridaemia. ApoE isoforms did not influence the apoE-containing Lp(a), although the the final concentration of Lp(a) is affected.

ApoE4 homozygosity rendered Lp(a) concentration 65% higher than apoE2 homozygosity.[68] Furthermore, higher Lp(a) concentrations prevailed in subjects with LDL concentration compatible with familial hypercholesterolaemia (inferring low LDL receptor activity). Interestingly, apoE2 homozygosity had less impact on Lp(a) concentration than expected, but it aggravated the elevation of Lp(a) concentration in those suspected of having impaired LDL clearance. Nevertheless, the apoE2 status did not modify the relationship with coronary artery or aortic valve disease.[69]

APOE IN HEALTH AND DISEASE

Animal models

Although mammals share similar genes for lipoprotein metabolism, the plasma lipoprotein profiles differ markedly between man and other mammals. Humans display higher concentrations of LDL and longer lifetimes of exposure to lipoproteins. Most animals, including rodents, are resistant to atherosclerosis, but animal models have been devised to study dyslipidaemia and atherosclerosis.[70] Popular models either knock out apoE, insert a dysfunctional protein such as apoE Leiden,[71] or human apoE isoforms are expressed. High fat diets in such models can result in hypercholesterolaemia exceeding 20 mmol/L without very severe hypertriglyceridaemia and apoE concentrations can reach values of 1 g/L.

Atherosclerosis involves both lipid accumulation and an inflammatory process that features macrophages. ApoE decreases lipid accumulation in circulating monocytes and the expression of inflammatory molecules on monocytes and vascular endothelium.[72] Abrupt cessation of apoE production promptly leads to hyperlidaemia and changes in T cells and antibodies. A conditionally apoE deficient mouse revealed that rapidly induced hyperlipidaemia effected changes in T lymphocyte helper cell 1, T regulatory subsets and antibody production.[73]

ApoE-knockout mice have lower brain cholesterol content and impaired cognitive function.[74] The apoE4 transgenic mouse model has some neurological deficit and lower brain docosahexaenoic acid (DHA) content. Dietary supplementation of DHA improved both the brain content and neurological function.[75]

Brain injury in human apoE4 carriers has a worse prognosis for recovery.[76] In mice, the role of apoE can be evaluated in brain trauma,[77] subarachnoid haemorrhage[78] and auto-immune injury[79] at primary and secondary injury, for changes in cellular behaviour and autophagy that deals with increased protein and organellar degradation. ApoE contributes to the maintenance of the blood-brain barrier[80] and to the transport of lipid across it. The peptide COG1410 comprising amino acids 138–148 of apoE (but for two altered amino acids), penetrates the blood-brain barrier and prevents the potential harm of apoE4 in secondary injury.[78]

Cardiovascular risk

Many risk factors for vascular disease influence apoE concentration. Routine genotyping of apoE or measuring its concentration may improve risk assessment, but requires further study.

ApoE isoforms influence cardiovascular risk from birth, including responses to infant feeding.[81] The hypolipidaemic effect of apoE2 is evident in childhood[82] with lower LDL and higher HDL concentrations. The apoE4 isoform is associated with increased carotid intima-media thickness,[83] along with higher LDL cholesterol, Lp(a) and apoB concentrations. In diabetes mellitus, apoE4 is also an indicator of cardiovascular risk.[84]

The metabolic syndrome characterised by hyper-triglyceridaemia, low HDL cholesterol, abdominal obesity and insulin resistance is associated with increased apoE concentration.[85] Premature coronary artery disease is associated with higher triglyceride concentration, more apoE-rich lipoproteins[86] and lower HDL cholesterol concentration, smaller LDL particles and higher Lp(a) concentration, irrespective of the apoE isoforms.

The impact of the common variants on health and cardiovascular risk through mild alterations in the lipid profile is clear in epidemiological studies, but more severe and pathological sequelae also occur. Table 1 summarises various effects of apoE on health and disease. The onset of dysbetalipoproteinaemia occurs in middle adulthood in men and post-menopausally in women. Earlier onset is possible with more severe metabolic stress. Penetrance is low in apoE2

homozygosity, but high in the dominantly inherited forms. Lipoprotein glomerulopathy is generally associated with the dysbetalipoproteinaemia phenotype and is due to aggregated lipoprotein and thrombus. The pathological changes with apoE Toyonaka resembles membranous nephropathy. Hypertriglyceridaemia may predominate in dysbetalipoproteinaemia, but some mutations were also reported in association with hypertriglyceridaemia. Other mutations involve the natural variants that lower or raise LDL concentration relative to the wild type, and one mutation confers LDL hypercholesterolaemia in a dominantly inherited fashion that may be diagnosed as familial hypercholesterolaemia. Some mutations predispose to cognitive impairment while others lower the risk of Alzheimer's disease.

Dysbetalipoproteinaemia

In dysbetalipoproteinaemia, remnant lipoproteins accumulate as a result of impaired clearance that occurs mostly in adult men or post-menopausal women when overproduction of lipoproteins or further decreased clearance stresses metabolism. Dysbetalipoproteinaemia is highly atherogenic, but may also result in accumulation of triglyceride-rich lipoproteins that precipitate pancreatitis. Both cutaneous and tendinous xanthomata may occur. In clinical practice, elevations of both triglyceride and cholesterol concentrations are not rare, but dysbetalipoproteinaemia is seldom proved. While apoE2 homozygosity is supportive of this diagnosis, dominantly inherited dysbetalipoproteinaemias could be overlooked.[87]

Table 1 Apolipoprotein E mutations affecting health and disease relative to wild type apoE3, according to the numbering of the mature protein (18 aa less than translated)

Dysbetalipoproteinaemia	Glomerulopathy	Hypertriglyceridaemia	Other associations
-13T*	R25C (ApoE2 Kyoto)	E13K; R145C (ApoE5 Philadelphia)	Normolipidaemia
E13K; R145C (ApoE5 Philadelphia)	R114C (ApoE Tsukuba)	C112R; R142C	C112, R158 (ApoE3 Wild type)
W20*	L141_K143del (ApoE Tokyo)	G127A	R274H
G31FS*29 c.237-2A>G	R142_L144del (ApoE Maebashi)	Dup135-142 (apoE5 Ess)	S296R
K95/E96insG*50/51 (ApoE Groningen)	R145P (ApoE2 Sendai)	R180C (ApoE1 Baden)	Lower LDL
E121_G127dup (ApoE Leiden)	R147P (ApoE2 Chicago)	R228C (ApoE2 Dunedin)	C112, R158C (ApoE2)
G127D (ApoE1)	R150C (ApoE Modena)	V236D (ApoE2)	Higher LDL
K128Q	R150G (ApoE Okayama)	E244K; E245K (ApoE Suita)	R112, R158 (ApoE 4)
R136C (ApoE Heidelberg)	R150P (ApoE2 Guang-Zhou)	R251G (ApoE3)	Familial hypercholesterolaemia
R136S (ApoE Christchurch)	D151dupD (ApoE Kanto)	L252E (ApoE1)	L149Del
R136fs*96	A152D (ApoE Las Vegas)	E E244K; E245K (ApoE7 Suita)	Neurological dysfunction
R142C	L155P (ApoE Chengdu)	212K (ApoE5)	R112, R158 (ApoE 4)
R142L	Q156_G173del (ApoE1)		L28P (ApoE Pittsburgh, Freiburg)
R142S (ApoE Nagoya)	R158P (ApoE Kurashiki)		E244K; E245K (ApoE7 Suita)
R145C (ApoE2)	S197C (ApoE Toyonaka)		Neuroprotective
R145H (ApoE Kochi)			C112, R158C (ApoE2)
K146D (ApoE1 Harrisburg)			V218E (ApoE2)
K146N; R147W (ApoE1 Hammersmith)			
K146Q			
L149del			
K164E			
Q187E (ApoE2 Toranomon)			
R209_T212delfs*17			
W210* (ApoE Washington)			
V236D (ApoE2)			
E244K; E245K (ApoE7 Suita)			

The onset of dysbetalipoproteinaemia occurs in adulthood in men and post-menopausally in women. Earlier onset is possible with more severe metabolic stress. Penetrance is low in apoE2 homozygosity while it is high in the dominantly inherited forms. Glomerulopathy is generally associated with the dysbetalipoproteinaemia phenotype. Lipoprotein glomerulopathy is due to aggregated lipoprotein and thrombus but Apo E Toyonaka resembles membranous nephropathy. Hypertriglyceridaemia may predominate in dysbetalipoproteinaemia. Other mutations involve the natural variants that lower or raise LDL concentration relative to the wild type, and one mutation that confers LDL hypercholesterolaemia in a dominantly inherited fashion. Some mutations predispose to cognitive impairment while others lower the risk.

Most persons with apoE2 homozygosity display hypo-cholesterolaemia and distinct LDL particles until conversion to dysbetalipoproteinamia. During the transition, LDL particles may become smaller due to remodelling as a result of hypertiglyceridaemia and LDL may become undetectable when remnant lipoproteins accumulate. Initially IDL and small VLDL may accumulate, but ultimately VLDL and chylomicrons may accumulate.

HDL concentration and insulin resistance were evaluated as predictors of dysbetalipoproteinamia which was present in almost half of 52 apoE2 homozygotes.[88] As expected, apoE and apoB concentrations were higher in dysbetalipoproteinaemia, the HDL cholesterol and apoAi concentrations were lower, and the insulin concentration as well as the homeostatic model assessment (HOMA) index was higher. Interestingly, the C-reactive protein concentrations did not differ.

When there is only mild hypertriglyceridaemia, dysbeta-lipoproteinaemia can be mistaken for familial hyper-cholesterolaemia, as defined by total cholesterol concentrations of >7.5 mmol/L, tendon xanthomata and premature atherosclerosis. Mutations conferring dominantly inherited dysbetalipoproteinaemia are listed in Table 1. One mutation in apoE, the deletion of leucine 149, results in accumulation of LDL very clearly illustrated on size exclusion chromatography.[89] This mutation confers a high affinity for the LDL receptor[90] and prevents dissociation in the endosomal compartment so that the LDL receptor binding apoE on remnants is targeted for degradation in the lysosome.

Lipoprotein glomerulopathy

Lipoprotein glomerulopathy is diagnosed histologically by lipid material staining with Sudan black and the demonstration of apoE in the glomeruli. The presentation can be with proteinuria or renal failure. The exact pathogenesis is uncertain,[91] but aggregation of lipoproteins and thrombosis at the glomerulus recur after renal transplantation. Many of the mutations of apoE conferring lipoprotein glomerulopathy also disturb lipoprotein metabolism, but homozygosity for apoE2 does not seem to be strongly associated with lipoprotein glomerulopathy. However, apoE2 is associated with diabetic nephropathy, whereas apoE4 may even be protective.[92] The mutations conferring glomerulopathy have mostly been in the N-terminus of apoE, with significant structural changes from substitutions with proline or changes at cysteine. Though the conformation of apoE is affected, HSPG binding and even LDL receptor binding have been preserved. Table 1 lists apoE mutations associated with glomerulopathy. Lipoprotein-modifying treatment appears to lessen the amount of aggregation in this disorder. Structural correctors may be of benefit by changing the tendency of apoE to aggregate. A case may be made for gene editing for this severe disorder when such treatment is perfected.

ApoE in the nervous system

Apart from contributing to atherosclerotic cardiovascular disease, apoE can affect neurological function and repair more directly and changes in the CSF could be informative. Recovery from a range of neurological stresses or injuries has been investigated in relation to apoE concentration and isoforms. ApoE, at about 20% of the plasma concentration, is the major apoprotein in CSF while apoB is practically absent.[93] The concentration of apoE in CSF decreases by 70% acutely after severe brain injury.[94]

The link between Alzheimer's disease and apoE4 is firmly established. Careful cognitive testing shows that apoE2 ameliorates the risk compared with apoE3.[95] The intensity of delirium, a risk factor for Alzheimer's disease, is inversely correlated with the concentration of apoE in the CSF.[96] The pathogenesis of Alzheimer's disease relates to impaired clearance of amyloid beta and tau proteins.[97] ApoE4, with its higher affinity for these proteins, its lower concentration in the CSF and possibly increased cellular uptake, and its toxic fragments, seems central in the process.

Cerebral amyloid angiopathy, responsible for a haemorrhagic stroke presentation with a rapid progression from extensive deposition of amyloid protein, also has an increased prevalence of apoE4.[98]

The risk of Alzheimer's disease is influenced by more genetic variants than those routinely assessed. A rare variant, apoE7, in which lysines replace glutamate at positions 244 (rs140808909) and 245 (rs190853081) causes dyslipidaemia and impaired memory, with a prevalence of 1% that was confined to patients with memory problems.[99] Modelling the impact of the mutation reveals similarities to apoE4.[100] The variant V218E was associated with a much lower risk of Alzheimer's disease.[101]

ApoE isoforms can also influence other pathological processes in the nervous system. Wilson's disease, in which malfunction of the P-type ATPase (ATP7) results in progressive hepatic cirrhosis and neurological disease, displays much variation in clinical manifestations. ApoE4, the isoform with the least binding of copper and associated with impaired neuronal repair, is over-represented, while apoE3 homozygotes have better cognitive status.[102]

INVESTIGATION OF APOE AND DYSLIPIDAEMIA

In the modern laboratory investigations,[103] dysbetalipoproteinaemia may not be recognised when using the calculated LDL cholesterol concentration, but may be considered if there is a large difference of calculated LDL cholesterol concentration from that of directly measured LDL cholesterol. Ultracentrifugation of lipoproteins or isoelectric focusing of apoproteins is rarely available in diagnostic laboratories. While electrophoresis is less costly, this is also not available at many clinical laboratories.

Electrophoresis of proteins and lipoproteins

Agarose gel electrophoresis displays remnant lipoproteins as a broad-β band with high specificity, but low sensitivity in dysbetalipoproteinaemia.[104] When the broad-β band is present, investigation for causes other than apoE2 homozygosity should be considered. The low yield of apoE2 homozygosity in subjects displaying a broad β-band[105] raises the possibility that other mutations of apoE may explain dysbetalipoproteinaemia, as reported from the Netherlands and South Africa.[9]

Acrylamide gel electrophoresis was found to be diagnostic of dysbetalipoproteinaemia by Blom et al.[104] and contributes to the work-up of dysbetalipoproteinaemia[106] though it is available in very few laboratories. Size exclusion

chromatography can similarly discern remnant lipoproteins from LDL.[89]

Genetic investigation

Genetic testing has become practicable and affordable. Genotyping of the three common variants may soon become standard practice, but there may be ethical considerations regarding the risk of dementia. Although it is generally believed that apoE2 protects against and apoE4 enhances vascular disease, the impact may not be useful in clinical practice for apoE2[107] while the impact of apoE4 on health is more at an older age.[108]

The commonly used polymerase chain reaction[109] for genotyping apoE amplifies the portion of the apoE gene that discriminates the three isoforms by a restriction enzyme digest and includes many of the other mutations that confer dysbetalipoproteinaemia. If remnant lipoproteins are confirmed by ultracentrifugation, electrophoresis or chromatography, additional genetic testing should be undertaken to identify the cause of dysbetalipoproteinaemia.

ApoE concentration

The value of apoE concentrations in clinical practice is unclear.[110] The concentration is strongly influenced by the isoform and the concentration of triglyceride-rich lipoproteins, and varies with age and sex.[37] The concentration of apoE in the CSF also varies according to the isoform.[111]

The highest apoE concentrations are found in apoE2 homozygotes[37] and the lowest apoE concentration in apoE4 homozygotes. The plasma concentration may not be as revealing of risk as association of apoE with specific lipoproteins. A subset of women with higher cardiovascular risk had more apoE in HDL.[112]

There is little information on the range of apoE concentrations in dysbetalipoproteinaemia. While high levels of apoE may indicate dysbetalipoproteinaemia, this disorder may also be due to the absence of apoE.[113]

Plasma lipid-modifying medication generally lowers apoE concentration. A 17% reduction of apoE concentration was reported with lovastatin.[114] Hypertriglyceridaemic subjects receiving 20 mg or 80 mg of atorvastatin had mean reductions of apoE concentration by 38% and 41%, respectively, but with much variation in the responses.[115] Atorvastatin also lowered apoE concentration in HDL.

The risk of neurological dysfunction may relate to low apoE concentration in general and especially with an ABCA1 transporter defect.[116] Point-of-care testing for apoE concentration is available by a nano-body based assay for CSF analysis.[117] The small portion of the total plasma apoE that resides on HDL can be measured by polyethylene glycol precipitation, whereafter HDL undergoes analysis by cation exchange.[118] The larger species are richer in apoE and correlate with higher triglyceride concentration but not with coronary calcium scores.

THERAPEUTIC APPLICATIONS

ApoE may offer novel treatment for atherosclerosis and neurological dysfunction as well as in various new applications.[119]

Increasing apoE expression in activated macrophages may be neuroprotective and can be achieved with retinoic acid.[120]

Probucol, an anti-oxidant with mild plasma lipid-modifying activity, also increases apoE expression and favourably affects clinical manifestations of Alzheimer's disease.[121] Glucocorticoids increase the expression of apoE in macrophages.[122]

ApoE4 maturation may be favourably modified by high affinity small molecules or 'structural correctors'.[123]

Changing apoE isoforms

The mutations in apoE are compatible with health for several decades and the attributable dyslipidaemia can be treated effectively by lifestyle, statins and fibrates. Nevertheless, if gene editing translates into safe clinical practice, patients with dysbetalipoproteinaemia or lipoprotein glomerulopathy could benefit from this treatment. The prevention of Alzheimer's disease by gene editing will be a challenge unless the blood-brain barrier can be crossed. Unless the pathogenesis of dysbetalipoproteinaemia is fully understood and predictable, conversion to apoE2 status may result in a highly atherogenic condition.

Dysbetalipoproteinaemia may, theoretically, respond to long-term administration of (recombinant) apoE3. Plasma infusion from apoE3 donors has not been reported in acute severe dyslipidaemia causing pancreatitis.

Aminothiols such as cysteamine could find application in modifying apoE,[124] to convert apoE2 to a more functional form. In homozygous apoE3 subjects with cystinosis, treatment with cysteamine resulted in some apoE4 formation.

Specific applications of apoE

ApoE-like proteins or peptides may modify the lipoprotein profile, atherosclerosis, inflammation or could influence cellular metabolism and, ultimately, neurological function. These strategies were reviewed by White et al.[125] and Anantharamaiah et al.[126]

The apoE mimetic peptide EpK improves cholesterol efflux from cells and showed benefit in an animal model of atherosclerosis.[127] Peptide Ac-hE18-NH 2 enhances clearance of lipoproteins by HSPG, containing a large portion of apoE extending from the LDL receptor binding domain to the carboxy-terminus.[128] Another peptide, hEp could be expressed in the liver, and lowered lipoprotein concentration and protected against atherosclerosis.[129] Incidentally, this peptide also interfered with hepatitis C virus binding.

ApoE-mimetic peptides could protect the blood-brain barrier from disruption after trauma,[130] conserving the metabolic activity in the nervous system. The blood-brain barrier could also be protected in subarachnoid haemorrhage.[131] ApoE mimetic peptides may also find application in mitochondrial disease.[132]

Coating solid lipid nanoparticles with apoE at approximately 120–160 nm size and thus similar to remnant lipoproteins, enables transport transcellularly by clathrin-mediated endocytosis across the blood-brain barrier[133] and could deliver drugs to the brain. Interestingly, covalently linked apoE peptides on solid lipid nanoparticles had good delivery to the brain after administration to the lungs.[134]

ApoE in personalised medicine

In personalised or precision medicine, management is individualised based on the knowledge of genetic variants and

how they affect risk, response to intervention or complications. Knowledge of apoE status may add value in clinical practice in the settings of dyslipidaemia, cardiovascular risk and neurodegenerative conditions even though apoE is mostly not the primary causal factor.[135]

The plasma cholesterol-raising effect of fatty diets was used to good effect in subjects with apoE4 to select healthier foods.[136] Men carrying apoE2 alleles, typically having mildly increased triglyceride concentrations, had greater reductions of plasma cholesterol and triglyceride when embarking on a low fat diet.[137] Men homozygous for apoE2 had reductions in LDL and triglyceride while those with apoE4 alleles experienced only LDL reductions. ApoE2 carriers had better improvements in the lipid profile with exercise. Plasma lipid profiles did not change with exercise, but VLDL particles decreased in size, while small LDL particles improved only in apoE3/3 subjects.[138]

The biosynthesis of DHA in the brain may not meet demands. Subjects carrying apoE4 alleles, despite having similar concentrations of plasma DHA, have decreased uptake of DHA into the brain[139] and would benefit from greater dietary supplementation. Alcohol consumption may need to be curtailed in the presence of apoE4.[140] ApoE4 enhances the LDL-raising effects of n-3 fatty acids in the setting of atherogenic lipoprotein levels.[141]

ApoE genotype may influence responses to medication.[142] Atorvastatin raised HDL cholesterol best in carriers of apoE2. Fibrates, being effective in lowering the triglycerides as well as apoB and apoE, had a powerful effect in carriers of apoE2 and least effect in carriers of apoE4. High doses of n-3 polyunsaturated fatty acids lower triglycerides, and 4.8 g/day for EPA and 4.9 g/day for DHA decreased apoE by about 15%.[143] Extended release niacin lowered apoE concentration by 25% in subjects with the metabolic syndrome.[144]

ApoE may influence the spread and growth of neoplasms. Liposarcoma cells express and secrete apoE.[145] ApoE expression correlated with the invasion of oral squamous carcinoma.[146] Macrophages found within gastric carcinoma, secrete exosomes containing apoE and promote spread.[147] ApoE is an independent prognostic factor for breast cancer with concentrations of >43 mg/L being unfavourable.[148]

CONCLUSION

ApoE is a molecule with common variants imparting significant implications for health and disease over the lifespan of an individual. The apoE2 variant is usually favourable for a low risk of cardiovascular disease as well as neurodegenerative disease but occasionally precipitates dysbetalipoproteinaemia, a highly atherogenic condition. Rarer mutations in apoE are also associated with dysbetalipoproteinaemia, often with dominant inheritance. Lipoprotein glomerulopathy is a rarer disease due to mutations in apoE, often with dyslipidaemia. The common variant, apoE4, is associated with less cognitive ability with ageing and Alzheimer's disease as well as modest hypercholesterolaemia and more atherosclerosis.

Owing to the original discovery of apoE in lipoproteins, much research has been done to elucidate the role of apoE in the production and clearance of apolipoprotein B-containing lipoproteins. More recently, its important role at the cellular level has been elucidated in the provision of lipoproteins in the HDL class to assist with cholesterol efflux from cells, transfer of lipids between cells and supporting cell growth

and repair processes. Studies have shown interactions of apoE with HSPG that could modulate many other processes. Peptides from apoE may have additional effects on the cell, and differ in the various isoforms. ApoE mediates much lipid transport into and inside the nervous system to ensure normal function but also interacts with amyloidogenic proteins in the nervous system with cumulative harm from especially apoE4.

In medical practice, the recognition of the risk of vascular disease with measurements of lipoproteins and lipid-modifying treatment is successful without having to determine the genotype of apoE. But when dysbetalipoproteinaemia is considered, genetic work-up is useful to confirm this severe disorder and trace potentially affected family members. The concentration of apoE in the blood or CSF is currently of research interest. Genotyping of apoE is preferred for assessing the risk of dementia with due consideration for the implications of such knowledge. Knowledge of apoE isoforms could assist clinical decisions in precision medicine spanning lifestyle interventions, cardiovascular disease, neurodegenerative, inflammatory and even neoplastic disease, but much research will be required to apply this information to best advantage in the context of the patient.

Research is indicating great complexity in the metabolism of apoE in various cell lines. It is possible that, with much more research, apoE mimetic peptides can influence atherosclerosis and neuronal function favourably.

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