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APOLIPOPROTEIN E: Far More Than a Lipid Transport Protein

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■ **Abstract** First recognized as a major determinant in lipoprotein metabolism and cardiovascular disease, apolipoprotein (apo) E has emerged as an important molecule in several biological processes not directly related to its lipid transport function, including Alzheimer's disease and cognitive function, immunoregulation, and possibly even infectious diseases. ApoE is a polymorphic protein arising from three alleles at a single gene locus. The three major isoforms, apoE4, apoE3, and apoE2, differ from one another only by single amino acid substitutions, yet these changes have profound functional consequences at both the cellular and molecular levels. ApoE3 seems to be the normal isoform in all known functions, while apoE4 and apoE2 can each be dysfunctional. Isoform (allele)-specific effects include the association of apoE2 with the genetic disorder type III hyperlipoproteinemia and with both increased and decreased risk for atherosclerosis and the association of apoE4 with increased risk for both atherosclerosis and Alzheimer's disease, impaired cognitive function, and reduced neurite outgrowth; isoform-specific differences in cellular signaling events may also exist. Functional differences in the apoE isoforms that affect (or did affect) survival before the reproductive years probably account, at least in part, for the allele frequencies of the present day.

INTRODUCTION

Over the past quarter century, studies of apolipoprotein (apo) E have continued to unlock the mysteries of this fascinating polymorphic protein [for review, see (59, 61)]. There are myriad isoform-specific roles for apoE acting at a cellular and molecular level.

APOLIPOPROTEIN E AND LIPOPROTEIN METABOLISM

ApoE Polymorphism

ApoE was discovered in the early 1970s as a protein component of triglyceride-rich lipoproteins [for review, see (59)]. It immediately became apparent that apoE

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was an important determinant in cholesterol metabolism, and apoE became the focus of intense scientific scrutiny. In the mid-1970s, Utermann and colleagues found that apoE was polymorphic, exhibiting multiple isoforms as detected by isoelectric focusing (98). They also found that particular isoforms were missing in the familial lipoprotein disorder type III hyperlipoproteinemia (HLP). Further work by Utermann and associates (100) and refinement of their hypotheses by Zannis et al (117), who used two-dimensional electrophoretic techniques to clarify the complex isoform patterns, led to an understanding of apoE polymorphism (115, 116).

Analysis of apoE isoforms from the plasma of multiple subjects revealed two kinds of apoE polymorphism, one genetically determined and one not. The genetically determined polymorphism is the result of three alleles at a single gene locus. The three apoE isoforms were designated apoE4, E3, and E2, with apoE4 being the most cationic and differing from apoE3 by one charge unit and from apoE2 by two charge units (Figure 1). Thus, six phenotypes were possible, and all were readily detectable in human subjects: three homozygous phenotypes (E4/4, E3/3, and E2/2) and three heterozygous phenotypes (E4/3, E3/2, and E4/2). The corresponding alleles for the three isoforms were termed $\varepsilon 4$, $\varepsilon 3$, and $\varepsilon 2$ (116).

The second, nongenetically determined polymorphism was found to result from variable posttranslational sialylation of apoE (115), which imparts an additional negative charge (Figure 1). These sialylated isoforms account for $\sim$10% to 20% of plasma apoE. The superimposition of this second polymorphism onto the first yields a complex isoform pattern that has to be interpreted carefully. For example, a monosialylated form of apoE4 has the same isoelectric point as nonsialylated apoE3.

The work of Gladstone investigators (80, 109) identified the molecular basis for both types of polymorphism. The genetically determined apoE4, apoE3, and apoE2 isoforms were found to differ in primary structure at two sites: residues 112 and 158 (Figure 1). ApoE3 has cysteine at residue 112 and arginine at residue 158, while apoE4 has arginine and apoE2 cysteine at both sites (the only cysteine residues in apoE). The arginine-cysteine interchanges completely accounted for the charge differences of the isoforms. The variable sialylation was due to the attachment of carbohydrate moieties to apoE at a single site: O-linked glycosylation of threonine 194 (109). The nonsialylated isoforms of apoE lacked not only sialic acid, but all neutral sugars as well.

Thus, apoE is a glycoprotein of 299 amino acids with a molecular mass of $\sim$34 kDa. The gene coding for the three isoforms of apoE resides on the long arm of chromosome 19 in humans. The gene has 3.6 kilobases with three introns and codes for a 317-residue precursor protein; an 18-amino acid prepeptide that signals secretion is cotranslationally removed. ApoE is synthesized and secreted by many tissues, primarily liver, brain, skin, and tissue macrophages throughout the body, sites where apoE plays critically important roles [for review, see (59, 61)].

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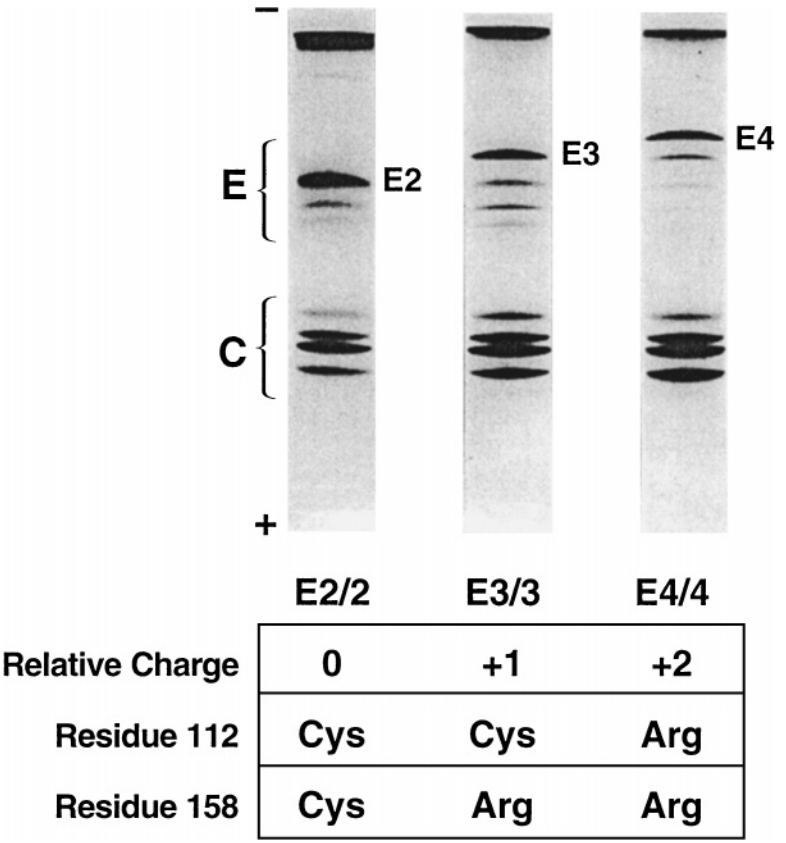

Figure 1 Isoelectric focusing of delipidated VLDL that shows the three homozygous apoE phenotypes: E2/2, E3/3, and E4/4. The apoE2, E3, and E4 isoforms differ sequentially by one charge unit as a result of the amino acid substitutions shown in the boxes. The minor isoforms beneath each major apoE isoform are the sialylated isoforms. The cathode (−) and anode (+) are indicated, as well as the positions of the apoC isoforms of VLDL. (Reproduced from RW Mahley & B Angelin 1984. *Adv. Intern. Med*. 29:385–411, and used by permission of WB Saunders.)

ApoE Structure/Function

The solution of the primary structure of apoE provided many insights into its function [for review see (59, 61, 105, 107)]. One particular region of the protein, residues 136–150, was especially enriched in basic amino acids. Before the amino acid sequence was solved, it was already known that lysines and arginines in apoE were essential for two of its functions, receptor binding and heparin binding. The 136–150 region was subsequently proven to be the site for both these functions. Further inspection of the apoE sequence revealed several regions in the

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carboxyl terminus that were predicted to form α-helices, many of which were amphipathic. Amphipathic α-helices are responsible for the lipid-binding capability of all apolipoproteins. Subsequently, it was also proven that this region constitutes the major lipoprotein (lipid)-binding site of apoE.

Secondary structure predictions suggested that apoE consists of two domains separated by a region of random structure. Physical and biochemical analyses demonstrated two independently folded domains separated by a "hinge" region (1, 110). The amino-terminal domain (as approximated by a thrombolytic fragment of apoE encompassing residues 1–191) also appeared to be a functional domain, containing both the receptor-binding and heparin (proteoglycan)-binding regions. Likewise, the carboxyl-terminal domain (as approximated by a second thrombolytic fragment, residues 225–229) appeared to be a distinct functional domain, containing the major lipid-binding site.

The tertiary structures of the amino-terminal domains of apoE4, E3, and E2 have been solved by x-ray crystallography, revealing more insights into the function of apoE (22, 24, 111, 112). The amino-terminal domain of apoE exists as a four-helix bundle (Figure 2) with the following boundaries: helix 1, residues 24–42; helix 2, residues 54–81; helix 3, residues 87–122; and helix 4, residues 130–164 (112); a short additional helix (residues 44–53) connects helices 1 and 2. The four helices are arranged in an antiparallel fashion. The receptor- and heparin-binding sites, which are essentially coincident, lie within helix 4. The structures of the three apoE isoforms vary in subtle, but important, ways. The substitution of cysteine for arginine at residue 158 in apoE2 (compared with apoE3) dramatically changes salt bridges within helix 4 (Figure 2*A*) and between helices 3 and 4 and also greatly lowers the positive ion potential of the receptor-binding region of residues 140–150 (22, 111). The substitution of arginine for cysteine at residue 112 in apoE4 (compared with apoE3) causes some noticeable shifts in side chain orientation of specific residues as well as a few salt bridge rearrangements (Figure 2*B*). The presence of arginine at residue 112 in apoE4 leads to a new salt bridge between glutamic acid 109 and residue 112 in helix 3, and more importantly (as will be discussed below), causes the side chain of arginine 61 to adopt a different, exposed position in helix 2 (24).

The receptor-binding function of apoE is remarkably isoform specific. In vitro assays revealed that apoE3 and apoE4 bind with similar affinity to the low density lipoprotein (LDL) receptors of cultured cells, whereas apoE2 has 2% or less of this binding capability (85, 106). Since apoE2 is associated with the genetic disorder type III HLP (see ApoE2 and Type III HLP, below), this reduced binding activity was thought to be causally related. The lower positive ion potential of residues 140–150 in apoE2 appears to be the major reason for the poor binding activity (i.e. by reducing the strength of the ionic interaction with the negatively charged ligand-binding regions of the LDL receptor). Earlier biochemical studies had suggested that arginine 158 is not directly involved in this ionic interaction and indicated that the cysteine substitution at residue 158 affects the receptor-binding region indirectly (49), a postulate supported by crystallographic data (22). Although in

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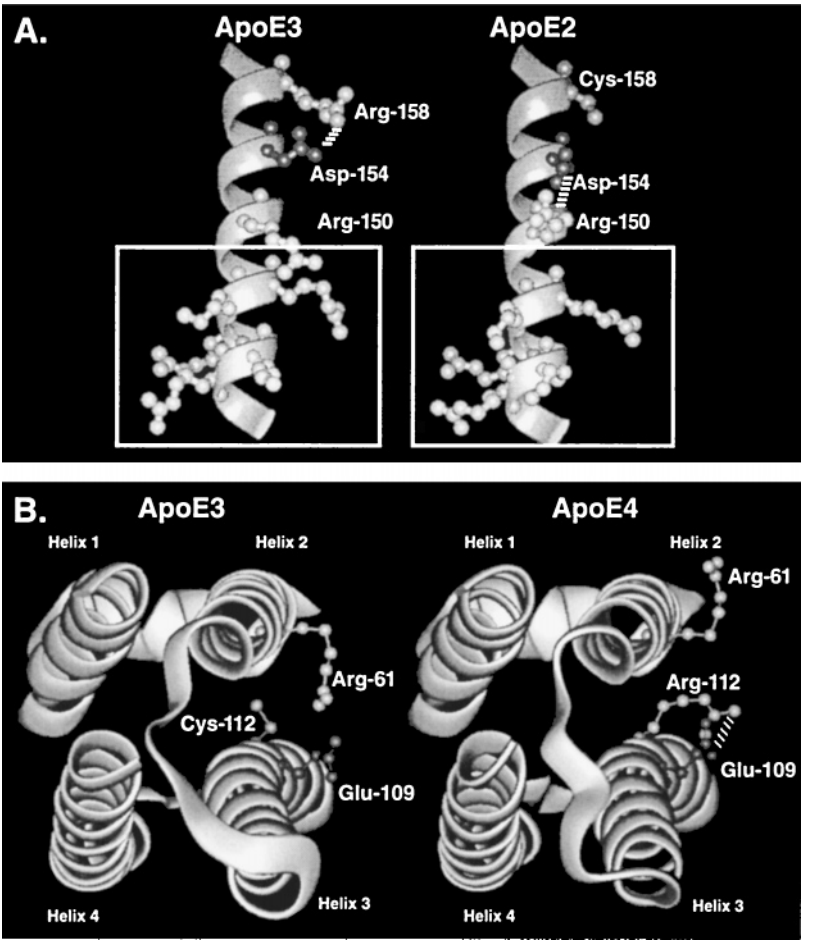

Figure 2 Three-dimensional structure of regions of apoE highlighting isoform differences. (*A*) The region of helix 4 where a critical salt bridge rearrangement in apoE2 reduces the positive ion potential of the LDL receptor–binding site (*boxed*). Aspartic acid 154 changes its ionic interaction to arginine 150 in apoE2 because of the cysteine 158 substitution, pulling the side chain of arginine 150 out of the positive ion cloud, reducing its potential, and causing a 100-fold reduction in apoE receptor binding. (Courtesy of Dr. Karl H. Weisgraber.) (*B*) The four-helix bundle of apoE showing the critical rearrangement of the arginine 61 side chain. The substitution of arginine 112 in apoE4 leads to an ionic interaction with glutamic acid 109 that excludes the arginine 61 side chain from its usual position, causing arginine 61 to be more exposed at the side of helix 2, and allowing it to become available for interaction with glutamic acid 255 in the carboxyl-terminal domain of apoE (not shown here). [Reproduced from *Curr. Opin. Lipidol*. (see Reference 61), and used by permission of Lippincott Williams and Wilkins.]

vitro analyses do not detect significant binding differences between apoE3 and apoE4, it is not clear whether this can be extrapolated to the in vivo situation (see Role of ApoE in Normal Lipoprotein Metabolism, below).

There do not seem to be any major differences among the isoforms in their ability to bind to heparin in vitro (apoE2 is somewhat less competent than apoE3 or apoE4). This suggests that although the heparin- and receptor-binding sites are roughly coincident in the primary structure, there are probably nuances in the nature of the interaction and/or the specific residues involved in receptor and heparin binding. The heparin-binding function of apoE is also important physiologically, since apoE interacts with heparan sulfate proteoglycans (HSPG) on cell surfaces in a second receptor-mediated process distinct from that involving the LDL receptor (see Role of ApoE in Normal Lipoprotein Metabolism, below) (for review, see 64).

The third major function of apoE, lipid binding, also shows isoform specificity. The lipoprotein-binding site in the carboxyl-terminal domain is centered on the amphipathic helices between residues 244 and 272 and is especially critical for binding to triglyceride-rich lipoproteins (24). ApoE3 and apoE2 preferentially bind to the smaller, more phospholipid-enriched high density lipoproteins (HDL), while apoE4 preferentially binds to the larger, triglyceride-rich very low density lipoproteins (VLDL). The difference in lipoprotein association appears to be determined by differences in the interaction of the amino-terminal and carboxyl-terminal domains among the isoforms. The side chain of glutamic acid 255 within the critical lipoprotein-binding region of the carboxyl-terminal domain forms a salt bridge with the exposed side chain of arginine 61 in apoE4, but not the other isoforms, because of the difference in orientation of arginine 61 in apoE3 and apoE2 (see discussion of x-ray structures above). This so-called domain interaction somehow directs the preference of apoE4 for binding to VLDL, as mutation of either arginine 61 or glutamic acid 255 changes the lipoprotein preference of apoE4 from VLDL to HDL (the "default" lipoprotein preference) (23). This unique domain interaction alters the conformation of apoE4 and is probably responsible for several apoE4-specific roles (see Effect of ApoE Isoforms on Normal Variation of Lipid Levels, Apolipoprotein E and Neurobiology, and Nontraditional Roles of Apolipoprotein E, below). The other isoforms do not show domain interaction.

Role of ApoE in Normal Lipoprotein Metabolism

A primary metabolic role for apoE is to transport and deliver lipids from one tissue or cell type to another (for review, see 59, 61, 64, 67). ApoE is a component of VLDL particles as they are secreted from the liver and is acquired by chylomicrons soon after their synthesis and secretion by the small intestine (one of the few tissues that does not synthesize apoE). Both of these lipoproteins become enriched in apoE as they circulate through the capillaries and are lipolyzed on the surface of endothelial cells by lipoprotein lipase, which hydrolyzes the triglycerides, releasing fatty acids for energy utilization by cells. In this way, apoE directs the metabolism of both endogenous triglycerides and cholesterol (VLDL) and dietary triglycerides

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and cholesterol (chylomicrons) by delivering them either to extrahepatic cells (via VLDL and their remnants) or to the liver (via chylomicron remnants), where the dietary fatty acids can be metabolized or resecreted as triglycerides with VLDL and where the cholesterol can be eliminated through the bile. In this regard apoE serves an "endocrine-like" function. It can also redistribute lipids within a tissue among the various cells and thus also serves a "paracrine-like" function for lipid transport and delivery.

ApoE accomplishes its lipid transport and delivery function mainly through two receptor pathways (Figure 3). The first is the highly regulatable LDL receptor pathway on both hepatic and extrahepatic cells. As a ligand for this receptor, apoE is much more potent than its LDL counterpart, apoB100, and thus is capable of regulating the levels of not only the lipoproteins on which it resides (VLDL and their remnants and chylomicron remnants) but also those on which it does not reside (LDL) [for review, see (63, 64)]. The second receptor pathway, more

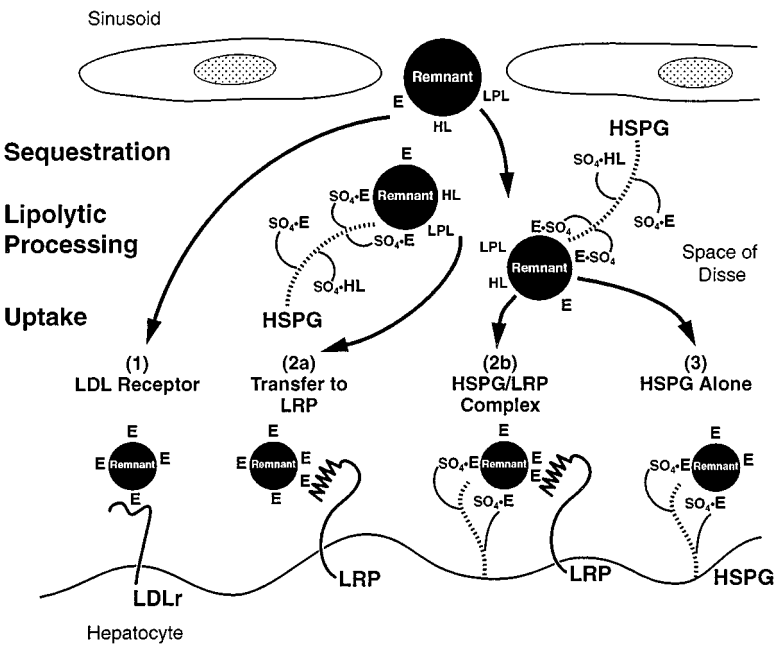

Figure 3 Receptor pathways involved in the clearance of lipoprotein remnants by the liver. Remnant particles are sequestered in the space of Disse, where they can undergo final lipolytic processing by lipoprotein lipase (LPL) or hepatic lipase (HL). Lipoprotein uptake can occur by any of three mechanisms: (*a*) the LDL receptor, (*b*) the HSPG/LRP pathway, and (*c*) HSPG alone. In HSPG/LRP uptake, lipoproteins could first bind to HSPG and then be transferred to LRP for uptake (*2a*) or bind to and be taken up by an HSPG/LRP complex (*2b*). [Reproduced from *J. Lipid Res.* (see Reference 64), and used with permission from Lipid Research, Inc.]

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recently discovered, is the HSPG/LDL receptor–related protein (LRP) pathway. The LRP, a very large protein of the LDL receptor gene family, binds a multitude of ligands, including apoE (36, 37). The HSPG/LRP pathway functions mainly in the liver for remnant lipoprotein metabolism [for review, see (12, 64)]. There, the lipoproteins are metabolized in a multistep process. They are first entrapped in the space of Disse, where the HSPG-rich surface binds the lipoproteins via apoE (sequestration step). Next, they can undergo final lipolytic processing by surface- or lipoprotein-bound lipases (processing step). Finally, they are either taken up by the LDL receptor, are transferred from the HSPG to the LRP for internalization, or are taken up as part of the HSPG/LRP complex (uptake step). In addition, lipoproteins may be taken up in a process that involves only HSPG (64).

ApoE also influences the metabolism of lipoproteins in ways unrelated to its role as a ligand for receptors. The accumulation of apoE on the surface of lipoproteins dramatically slows the rate of lipolysis of lipoprotein triglycerides by lipases (41, 42, 53, 82). Although all the apoE isoforms can inhibit triglyceride hydrolysis, there are isoform-specific differences depending on how much apoE accumulates on the lipoproteins (e.g. apoE4 preferentially enriches triglyceride-rich lipoproteins) (see ApoE2 and Type III HLP and ApoE4 and Increased Heart Disease Risk, below). ApoE can also affect plasma triglyceride and VLDL levels directly, as increased apoE synthesis and secretion by the liver or apoE accumulation in the plasma is associated with increased VLDL synthesis and secretion (40, 42, 102). Thus, apoE can regulate anabolic as well as catabolic metabolism of lipoproteins.

As a component of a subclass of HDL (HDL with apoE), apoE influences cholesterol efflux from cells as well as influx (45, 63). An important process termed reverse cholesterol transport rids cells of excess cholesterol so it can be transported to the liver for excretion. Small HDL particles are excellent acceptors for cellular cholesterol, and when they acquire apoE, the apoE acts as a ligand for delivery to the liver. The direct ligand role of apoE in HDL cholesterol metabolism appears to be more important in lower animals than humans; in humans, excess HDL cholesterol primarily is transferred to triglyceride-rich lipoproteins and LDL by the cholesteryl ester transfer protein for transport to the liver. Recently, it has been shown that the direct uptake of cholesteryl ester from HDL by various cells is facilitated by apoE-mediated binding of apoE-containing HDL to cell-surface HSPG (2). Subsequently, this apoE-HSPG interaction enhances the ability of the cell-surface protein SR-BI to mediate the selective delivery of HDL-cholesteryl esters to cells.

Effect of ApoE Isoforms on Normal Variation of Lipid Levels

Numerous population studies describing the role of apoE on the normal variation of plasma lipids have revealed important isoform-specific effects. These studies have established that apoE3 contributes little to the variation and thus is the “normal” benchmark. (It is also the most common of the apoE isoforms and the “normal”

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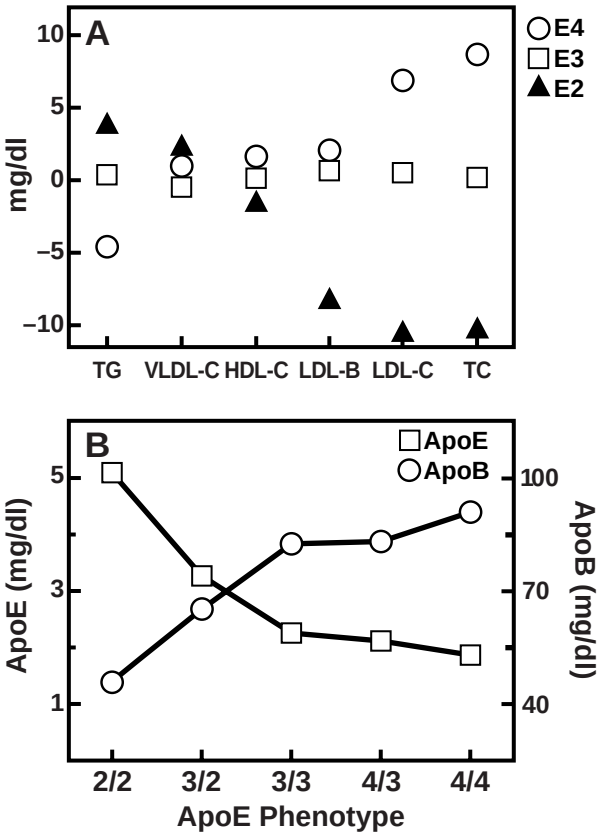

Figure 4 (*A*) Effect of apoE alleles on lipid parameters. TG, total plasma triglyceride; VLDL-C, VLDL cholesterol; HDL-C, HDL cholesterol; LDL-B, LDL apoB; LDL-C, LDL cholesterol; TC, total plasma cholesterol. [Adapted from *Am. J. Hum. Genet*. (see Reference 88), and used by permission of American Journal of Human Genetics.] (*B*) Effect of apoE phenotypes on plasma apoE (*squares*) and apoB (*circles*). (Reproduced from Reference 97, and used by permission of Elsevier Science Publishers.)

benchmark for apoE functions.) In contrast, apoE2 (or its allele, $\varepsilon 2$) and apoE4 (or its allele, $\varepsilon 4$) have definite impacts, some of them dramatic, on lipid and lipoprotein levels. Figure 4 summarizes the effects of apoE isoforms (alleles) on levels of apoE, apoB, plasma triglycerides, and total and LDL cholesterol (97). ApoE2 tends to be associated with increased levels of apoE and triglyceride and decreased levels of apoB and cholesterol. In contrast, apoE4 is associated with decreased apoE but increased apoB and cholesterol levels. The impact of apoE2 and apoE4 on lipid variation in the population is likely a direct consequence of their functional differences from apoE3 (see ApoE Structure/Function, above). It has been estimated

that apoE accounts for as much as 10% of the total variation in cholesterol levels in the population [for a more detailed discussion, see (19, 88)].

ApoE2 and Type III HLP

The lipoprotein disorder type III HLP (or dysbetalipoproteinemia) is one of the five familial HLPs defined by Fredrickson et al (29) in the 1960s [for review, see (62, 66)]. The biochemical and clinical features of the disorder are varied, and not all of the abnormalities are present in every patient. Plasma cholesterol and triglycerides are elevated to approximately equal levels, usually >300 mg/dl. A distinct feature of type III HLP is the presence of abnormal lipoproteins collectively termed β-VLDL, owing to their β-electrophoretic migration rather than the usual pre-β migration of typical VLDL. β-VLDL are cholesterol-enriched remnants of both intestinal and hepatic origin that are also enriched in apoE. Both HDL and LDL are somewhat reduced in type III patients, so the elevated lipids are almost entirely due to β-VLDL.

Type III HLP is an adult-onset disease that predisposes patients to the premature development of atherosclerosis (fourth or fifth decade), especially in the peripheral arteries. It is more prevalent in men than women, and almost all women with the disease are postmenopausal. Xanthomas are a prominent clinical feature, and one type, xanthoma striata palmaris, is unique to type III HLP. Found in over half of all type III patients, it is characterized by yellowish, macrophage-enriched lipid deposits in the palmar creases.

Virtually all patients with type III HLP are homozygous for apoE2 (i.e. the E2/2 phenotype). The primary causal molecular defect in type III HLP is homozygosity for apoE2, which is defective in binding to lipoprotein receptors. Therefore, type III HLP is essentially a recessive disorder, but there are exceptions when rare mutant apoEs are present (discussed below). However, not all apoE2 homozygotes have type III HLP. In fact, most E2/2 subjects (>90%) are normolipidemic or even hypolipidemic, owing to reductions in LDL or HDL or both. In these subjects, β-VLDL are detectable, but do not accumulate in the plasma. Overt type III HLP requires homozygosity for apoE2 plus secondary factors that precipitate the disorder (discussed below).

The major metabolic abnormality in type III HLP is delayed clearance of remnant lipoproteins, which is due to the receptor-binding defect of apoE2 [for review, see (62, 66)]. This delayed catabolism is usually insufficient to precipitate the disorder, however. Possible reasons for this include the presence of the second lipoprotein receptor system (see Figure 3) involving HSPG/LRP, with which apoE2 functions more efficiently than with the LDL receptor. Since apoE2's ability to bind to the LDL receptor is reduced but not zero, the level of the LDL receptor, which is highly regulatable, appears to be a factor modulating development of overt type III HLP.

Other metabolic abnormalities also are present. As mentioned in the section on the Role of ApoE in Normal Lipid Metabolism, above, the accumulation of

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apoE on the lipoprotein particles, as occurs with β-VLDL, leads to a reduction in lipoprotein lipase–mediated lipolysis of the lipoproteins. Since more extensively processed lipoproteins tend to be better ligands for removal by receptors, lipolytic inhibition by apoE can also lead to delays in remnant removal. Inhibition of lipolysis rather than LDL receptor regulation also seems to be responsible (at least in animal studies) for the low LDL (the end product of the lipolytic cascade), since the low LDL persists even in the absence of the LDL receptor (41).

In addition to catabolic defects, apoE also influences production of the hepatic lipoprotein component of β-VLDL. Overproduction of apoB-containing lipoproteins can precipitate type III HLP in apoE2-expressing transgenic animals (43) and presumably could do the same in humans. Furthermore, apoE accumulation in the plasma and increased levels in the liver can cause a severalfold increase in VLDL production (see Role of ApoE in Normal Lipoprotein Metabolism, above), thus contributing further to β-VLDL accumulation in the plasma of type III subjects.

The mode of inheritance of type III HLP is recessive, with secondary factors required in apoE2 homozygotes [for review, see (62, 66)]. But the mode of inheritance has been confounded by the discovery of as many as ten rare variants of apoE that are associated with type III HLP in which the subjects are heterozygous for these rare variants (66, 79). With one exception, the mutation in these rare variants is a substitution of a neutral or acidic amino acid for lysine or arginine in the cluster of basic amino acids in the receptor-binding/heparin (proteoglycan)-binding region of apoE (residues 136–150). Therefore, in these instances, the type III disorder is dominant, and furthermore, the need for secondary factors is not always apparent. In many instances, penetrance is very high, in some cases 100%.

The reason why the rare variants are associated with dominant expression of type III HLP lies in their specific properties (62, 66, 79). Since most of the rare apoE variants actually bind better to the LDL receptor than apoE2 does, other properties must account for the remnant accumulation. At least two properties have been identified that might account for the association with dominant expression of type III HLP. First, most of the rare variants bind poorly to the HSPG/LRP receptor system, with the severity of this defect correlating reasonably well with the degree of defective heparin-binding activity of the mutant apoE. Second, several of the rare variants of apoE associated with dominant type III HLP result from mutations in the apoE4 allele rather than the apoE3 allele. As a result, these mutants, like apoE4, preferentially associate with triglyceride-rich lipoproteins; thus, the β-VLDL from subjects heterozygous for these mutants show a marked increase in the ratio of defective to normal apoE, which could exacerbate β-VLDL accumulation.

The secondary factors that precipitate type III HLP in the minority of apoE2 homozygotes fall into three categories: genetic, hormonal, and environmental (62, 66). Multiple factors can be operable in any individual. Genetic influences involve gene interactions that can be either monogenic or polygenic. Among the gene interactions possible, those of other familial hyperlipidemias can precipitate the onset of type III HLP in E2/2 subjects: familial hypercholesterolemia

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(type IIa HLP), familial combined hyperlipidemia (type IIb HLP), and familial hypertriglyceridemia (types IV and V HLP).

Of the hormonal factors, estrogen is probably the most significant [for review, see (66)]. Almost all women with type III HLP are postmenopausal, and estrogen replacement therapy can completely eliminate the hyperlipidemia. Of several possible mechanisms, it is most likely that estrogen exerts this effect by increasing LDL receptor levels (at least in some animals) and lipolytic activity. Both of these activities would be expected to increase the rate of remnant removal, as demonstrated in apoE2 transgenic rabbits (44). Other negative hormonal influences include hypothyroidism and glucose intolerance, both of which occur frequently in type III HLP. In the case of hypothyroidism, there is probably a suppression of LDL receptor expression, which would be expected to retard remnant removal and therefore exacerbate the hyperlipidemic response.

The most important of the environmental factors are excess caloric intake (obesity), age, and gender. Dietary exacerbation of type III HLP likely is due to downregulation of LDL receptors or overproduction of lipoproteins or both.

ApoE4 and Increased Heart Disease Risk

Given its association with type III HLP, apoE2 might be expected to be the apoE isoform that places humans at the greatest risk for developing heart disease. However, except for the small percentage of E2/2 subjects who develop overt type III HLP, this does not seem to be the case. Instead, apoE2 may actually be the most beneficial apoE isoform (in the absence of type III HLP), while apoE4 carries the most risk. That this is true has been demonstrated in a number of studies. First, studies of normolipidemic populations have shown the association of apoE4 with increased total and LDL cholesterol and increased apoB (see Effect of ApoE Isoforms on Normal Variation of Lipid Levels, above). The increased heart disease risk of high cholesterol and high LDL is well established, so the elevated cholesterol level associated with apoE4, compared with apoE3, predicts increased risk. The same studies suggest reduced risk of apoE2. Second, clinical studies of patient populations have shown that apoE4 is overrepresented in both hyperlipidemic and heart disease populations (19, 70, 92, 99, 101).

While the above findings are well documented, what is less clear is the underlying mechanisms responsible for the phenomenon. One study of remnant metabolism showed that the presence of apoE4 leads to more rapid remnant catabolism (32); another showed that apoE4 carriers cleared dietary fat more rapidly than apoE3 carriers (104). The authors of both studies speculated that the metabolic consequence of their findings was to downregulate LDL receptors, thereby increasing plasma LDL in apoE4 carriers. They also speculated that the reason for the faster catabolism was the preferential association of apoE4 with triglyceride-rich lipoproteins. In contrast, one study found no phenotype differences in postprandial response (6), while another found that postprandial triglyceride clearance was delayed in apoE4 carriers (5). Although study protocol

differences might account for some of the discrepancy, it is more likely that circumstances not yet appreciated contribute to the variability.

No less confusing are studies of the association of apoE4 with hypertriglyceridemia. Population studies have reported that apoE4 is associated with lower triglycerides (88), others showed no consistent association (19), and still others have shown association with increased triglycerides (17). There also has been disagreement about the increased prevalence of hypertriglyceridemia in E4/4 subjects. The lack of accepted mechanistic explanations thus is understandable. LDL receptor downregulation is perhaps the most commonly accepted explanation for the increased cholesterol, but the triglyceride issue is very unsettled. One possible explanation revolves around the association of apoE with VLDL triglyceride production (see Role of ApoE in Normal Lipoprotein Metabolism, above). Regardless of the lack of clarity of the mechanism, what is clear is that the presence of apoE4 imposes increased risk for heart disease.

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ApoE and Atherosclerosis

Generally, it is considered that apoE protects against the development of atherosclerosis, but this benefit depends on the apoE isoform, the total plasma apoE level, and the cell type responsible for the synthesis and secretion of apoE. The protective effects of apoE in atherosclerosis are apparent from its roles in normal lipoprotein metabolism (see Role of ApoE in Normal Lipoprotein Metabolism, above), especially in targeting remnant lipoproteins for removal from circulation and its postulated role in reverse cholesterol transport. Likewise, studies of apoE-knockout mice dramatically demonstrate the role apoE plays in atherosclerosis protection. ApoE-null mice have extremely high lipid levels and massive amounts of cholesterol-rich β-VLDL and develop severe atherosclerotic lesions even on a normal chow diet (72, 81). These studies have also confirmed the atherogenic potential of β-VLDL first suggested from consideration of other fat-fed animals and from type III HLP in humans (62, 66).

In contrast, apoE can, under certain conditions, actually increase atherosclerosis risk. The role of apoE2 in type III HLP suggests such increased risk, a role confirmed in transgenic animals expressing either apoE2 (44) or other of the rare apoE variants associated with dominant expression of type III HLP (27, 103). In contrast, the lower LDL levels in apoE2 carriers without overt type III HLP are associated with less atherosclerosis risk. On the other hand, the association of apoE4 with increased cholesterol levels (see ApoE4 and Increased Heart Disease Risk, above) suggests that it increases the risk for atherosclerosis.

It is clear that the absence of apoE is associated with increased risk; however, having too much apoE may also be associated with increased risk. The role of high levels of apoE in inhibiting lipolysis or increasing VLDL production suggests a possible increased atherosclerosis risk because those triglyceride-rich lipoproteins could contribute to the formation of atherogenic remnant particles. It is quite likely that there is an optimal range of apoE plasma levels that is maximally beneficial,

and that levels above or below that range impose a risk rather than a benefit for atherosclerosis (61).

The role of macrophages in the development of arterial lesions is well appreciated (7). The beneficial nature of apoE production by macrophages has been shown in a number of animal studies. Mice expressing apoE only in macrophages were protected against atherosclerosis, even though plasma levels of apoE were exceedingly low and the animals were hypercholesterolemic (3). Also, mice expressing apoE normally except in macrophages were more susceptible to atherosclerosis (26). These and similar studies demonstrate that the site of synthesis of apoE can also be a crucial factor in susceptibility to atherosclerosis.

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APOLIPOPROTEIN E AND NEUROBIOLOGY

By the mid-1980s, clues indicating that apoE played an important role in neurological diseases had begun to surface: apoE was produced in abundance in the brain and served as the principal lipid transport vehicle in cerebrospinal fluid, was induced at high concentration in peripheral nerve injury and appeared to play a key role in repair by redistributing lipids to regenerating axons and to Schwann cells during remyelinization, and modulated neurite outgrowth in cultured rabbit dorsal root ganglion cells or Neuro-2a cells [for review, see (59, 60, 65, 107, 108)]. The stage was set for the discovery by Corder and associates that apoE is a major susceptibility gene associated with ~40%–65% of cases of sporadic and familial Alzheimer's disease and increases the occurrence and lowers the age of onset of the disease (13). Furthermore, the apoE4 allele (76, 90) is associated with poor clinical outcome in patients with acute head trauma and stroke, whereas apoE2 may be protective against neurodegenerative diseases.

A key to understanding the role of apoE in neurological diseases appears to reside in determining how apoE modulates neuronal repair, remodeling, or protection (Figure 5). Injurious agents can cause neuronal damage, requiring neuronal repair of synaptodendritic connections. We would suggest that apoE3 and apoE2 may be effective in mediating the repair process and in protecting neurons from excessive damage, whereas apoE4 may be relatively ineffective.

ApoE Modulation of Neurite Extension and Cytoskeletal Function

In in vitro studies, apoE3 and apoE2 associated with a lipoprotein transport vehicle capable of delivering the apoE to a specific cell-surface receptor pathway induce neurons to produce long neurites, whereas apoE4 is associated with an inhibition of neurite outgrowth (73, 74). The isoform-specific effects of apoE require that the apoE be associated with a lipoprotein transport vehicle (lipid-free apoE has no effect on neurite extension) directing it to the HSPG/LRP pathway. Inhibition of this pathway abolished the differential effects of apoE on neurite

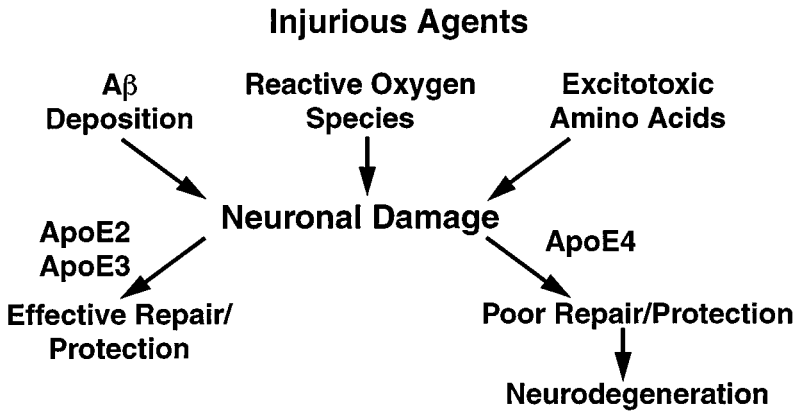

Figure 5 Isoform-specific effects of apoE on neuronal repair, remodeling, and protection. [Reproduced from *Curr. Opin. Lipidol.* (see Reference 61), and used by permission of Lippincott Williams and Wilkins.]

outgrowth (4, 39). These observations have been confirmed and extended by others (21, 95).

The isoform-specific effects of apoE on neurite outgrowth are associated with changes in the cytoskeleton, specifically microtubule assembly (74). ApoE3 stimulates the polymerization of β-tubulin and stabilizes the formation of microtubules in cultured neurons, whereas apoE4 apparently destabilizes microtubule assembly. Strittmatter et al (93) hypothesized that apoE3, which preferentially interacts with the microtubule-associated protein tau, protects tau from hyperphosphorylation. Hyperphosphorylation prevents tau from interacting with microtubules and results in intracellular deposits of tau (i.e. neurofibrillary tangles characteristic of Alzheimer's disease). While it is clear that the apoE isoforms have differential effects on the cytoskeleton, the mechanism is unclear. ApoE might modulate the cytoskeleton by interacting differentially with cytoskeletal components, a process that would require internalized apoE to enter the cytosol. Alternatively, the effects of apoE on the cytoskeleton may occur through a signaling pathway or another pathway not requiring a physical interaction between apoE and tau or other microtubule-associated proteins. Signaling mechanisms involving members of the LDL receptor gene family, all of which bind apoE, have recently been described (18, 38, 96).

There is a differential intracellular handling of the apoE isoforms that could be associated with the isoform-specific effects on the cytoskeleton and neurite outgrowth. ApoE3 (and apoE2) accumulate within cells, including neurons, to a greater extent (two- to fourfold) than apoE4 (52). It appears that apoE3 is sequestered within the cell body and extends along the neurites of cultured neurons, while apoE4 is retained by the cells to a significantly lesser extent (74). The intracellular localization of internalized apoE remains a critical question. Although

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Han et al (33) reported apoE in the cytosol and on the cytoplasmic surface of intracellular organelles, this would require that apoE escape the endosomal-lysosomal pathway and enter the cytosol. At the present time, there is no known mechanism whereby this occurs. Alternatively, apoE (E3>E4) may enter the late endosomes and be sorted into a unique membrane compartment, thus escaping lysosomal hydrolysis. Within this compartment, apoE may modulate intracellular processes, with apoE3 preferentially stimulating and apoE4 inhibiting neurite outgrowth.

ApoE, Amyloid β Peptide, and Amyloid Plaques

It has been hypothesized that the apoE isoforms differentially affect amyloid plaque formation, amyloid β (Aβ) peptide metabolism, or both. Lipid-free apoE4 forms a stable complex with the Aβ peptide in vitro, whereas apoE3 does not (94). Specifically, the lipid-binding region of apoE (residues 244–272) mediates its interaction with Aβ. However, lipidated apoE displays the opposite effect. Lipidated apoE3 binds to the Aβ peptide with a 20-fold higher affinity than lipidated apoE4 (56). The increased binding of apoE3 could enhance the clearance of the Aβ peptide, preventing the accumulation of the neurotoxic Aβ species (55, 57).

ApoE is associated with the extracellular amyloid plaques in the brains of Alzheimer's disease patients, where it might serve as a nidus facilitating plaque formation. ApoE4 has been shown to result in the formation of insoluble, high-molecular-weight complexes with the Aβ peptide, leading to the formation of dense amyloid monofibrils in vitro (84). On the other hand, apoE3 is less reactive and does not result in such extensive complexes. These morphologic studies have been confirmed and extended by quantifying fibril counts or by thioflavin fluorescence (58, 113).

One mechanism whereby the Aβ peptides may be neurotoxic (114) could be by causing neurodegeneration secondary to oxidative stress. ApoE3 has a greater capacity to protect cells against H_2O_2-induced oxidative damage than apoE4 (71). Despite several different mechanisms whereby apoE isoform–specific effects may impact Aβ metabolism and amyloid plaque formation, a definitive role awaits further studies.

ApoE Isoform–Specific Effects on CNS Structure and Function in ApoE Transgenic Mice

Human apoE3 expressed in the brains of apoE-null transgenic mice protects the mice against the age-related neurodegeneration seen in apoE-null mice and against kainic acid–induced excitotoxic neurodegeneration (9). Transgenic mice expressing similar levels of human apoE4 display less immunostaining of synaptophysin, a marker for presynaptic terminals, and less microtubule-associated protein 2, a marker for dendrites. In addition, transgenic mice expressing human apoE3 in the CNS have smaller cerebral infarcts after ischemia than mice expressing apoE4 (87).

The differential apoE isoform–specific effects on the morphological integrity of the CNS have been extended to spatial learning and memory in transgenic mice.

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In the water maze, apoE3-expressing female mice on the apoE-null background performed the task of locating a hidden underwater platform similarly to wild-type control mice (apoE-null mice are impaired) (78). However, apoE4-expressing female mice did not perform the task well. This deficit was gender-specific: the female mice were more susceptible than males to the detrimental effects of apoE4 on spatial learning and memory. Likewise, apoE4 female mice on the apoE-null background displayed fewer and shorter rearing events in an open field test than wild-type controls (78). These results suggest that there are apoE isoform–specific effects affecting learning, memory, and possibly curiosity and exploratory activity. Data are beginning to establish that apoE4 expression may negatively affect cognitive function in humans before any signs or symptoms of Alzheimer's disease appear and at a relatively early age (11, 48, 91).

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Insights into Isoform-Specific Effects Derived from ApoE Structure/Function Studies

As discussed in the section on ApoE Structure/Function, the single amino acid change at residue 112 in apoE4 appears to alter the overall conformation of the molecule. Specifically, in the amino terminus of apoE4, arginine 61 is oriented in such a way that it can interact with glutamic acid 255 in the carboxyl terminus. It has been hypothesized that this unique domain interaction, distinguishing apoE4 from apoE3 and apoE2, is responsible for the isoform-specific effects of apoE in the nervous system (61, 107). A formal test of this hypothesis may be possible if a small molecule can be identified that prevents the interaction between the amino-terminal and carboxyl-terminal domains. This would convert apoE4 to an "apoE3-like" molecule and might prevent the detrimental effects of the apoE4 on CNS structure and function.

APOLIPOPROTEIN E POLYMORPHISM AND GENETICS

ApoE Allele Frequencies

Population studies that showed the impact of apoE isoforms on lipid parameters in normal populations (see Effect of ApoE Isoforms on Normal Variation of Lipid Levels, above) also yielded valuable information on apoE allele frequencies in various geographic and ethnic populations around the world [for review, see (31, 66)]. In all populations, the $\varepsilon 3$ allele is the most frequent, with a range of 50%–90% (Figure 6). The range of $\varepsilon 4$ allele frequency is about 5%–35% and of $\varepsilon 2$ about 1%–15%. With few exceptions, $\varepsilon 4$ allele frequency exceeds that of $\varepsilon 2$. In North American populations derived from Europe, the frequencies of apoE alleles fall in the mid-range, with $\varepsilon 3 \sim 77\%$, $\varepsilon 4 \sim 15\%$, and $\varepsilon 2 \sim 8\%$. As a result, the frequencies of the six apoE phenotypes are (approximately): 55% E3/3, 25% E4/3, and 15% E3/2, with the other phenotypes E4/4, E2/2, and E4/2 making up the remainder at about 1%–2% each (Figure 6).

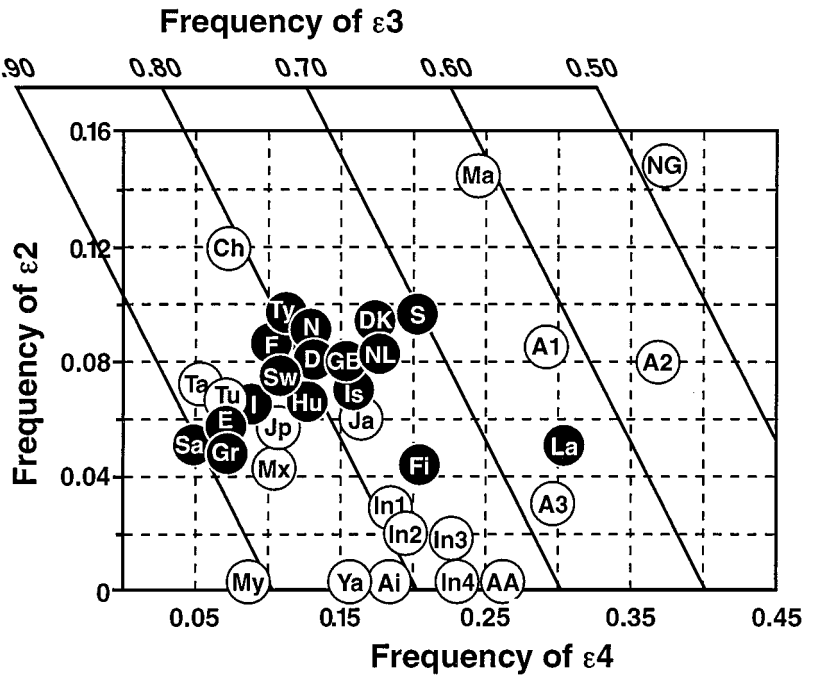

Figure 6 ApoE allele frequencies in various populations. The $\varepsilon 4$ allele frequency is shown on the *x*-axis, $\varepsilon 2$ on the *y*-axis, and $\varepsilon 3$ on the *z*-axis. European populations are shown as shaded symbols and are (from the left): Sa, Sardinian; Gr, Greek (in Cyprus); E, Spanish; I, Italian; F, French; Ty, Tyrolean; Sw, Swiss; N, Norwegian; D, German; Hu, Hungarian; GB, British (England, Scotland, Ireland); Is, Icelandic; DK, Danish; NL, Dutch; S, Swedish; Fi, Finish; and La, Lapps (Saami in Finland). Other populations are Ta, Chinese in Taiwan; Ch, Chinese in Beijing and Singapore; Tu, Turks; My, Mayans of Mexico; Jp, Japanese; Mx, Mexicans; Ja, Javanese; Ya, Yanomamo from Brazil; Ai, Amerindians (nine tribes); In1, Inuit from Nuuk; In2, Inuit from Alaska; In3, Inuit from Nanortalik; In4, Inuit from Ammassalik; Ma, Orang Asli from Malaysia; AA, Australian aboriginals; A1, Africans from Sudan; A2, Khoi San from South Africa; A3, Africans from Nigeria; and NG, Huli and Pawaia of Papua New Guinea. The data for I, F, N, D, GB, S, Fi, Ch, Jp, and A3 were pooled from two to nine individual studies. (Reproduced from *Hum. Genet*. (see Reference 31), and used by permission of *Human Genetics*.]

The geographic and ethnic differences in the apoE allele frequencies raise fascinating questions. For example, what phenomena explain the rather wide variations in frequencies among these populations? (The issue of the very existence of multiple alleles is considered in the section on The ApoE Allele Genetic Dilemma, below.) The genetic drift phenomena involved are likely to include both founder effects and bottleneck effects such as catastrophic events. The populations with the most extreme frequencies of $\varepsilon 2$ and $\varepsilon 4$ generally appear to be those most isolated,

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geographically or culturally or both. Geographically isolated populations are likely to be more susceptible to bottleneck effects such as disease or natural disaster phenomena. In this regard, it is noteworthy that the frequency of $\varepsilon 4$ is lowest around the historical centers of agriculture, which were the first places where population sizes exceeded the thresholds needed to maintain viral diseases (LU Gerdes, personal communication). In addition, both geographically and culturally isolated populations could display a profound impact from founder effects.

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The ApoE Allele Genetic Dilemma

At least three major questions arise as a result of the existence of the three apoE alleles: Which one came first? How did the other two attain such high frequency? Why have they all survived? It has been suggested that, even though $\varepsilon 3$ is the most common allele in humans, $\varepsilon 4$ may be the ancestral allele (34, 59). The arguments for either $\varepsilon 3$ or $\varepsilon 4$ being the parent allele are relatively straightforward. The case for $\varepsilon 3$ primarily rests on two facts. First, it is the most frequent allele. Second, the $\varepsilon 4$ and $\varepsilon 2$ alleles can each arise from $\varepsilon 3$ by a single nucleotide change in the gene, $\varepsilon 4$ by a T→C transition in codon 112 and $\varepsilon 2$ by a C→T transition in codon 158. If this were the origin of apoE alleles, it might be expected that both $\varepsilon 4$ and $\varepsilon 2$ confer some selective advantage that has allowed them to persist in the human population.

The case for $\varepsilon 4$ being the ancestral allele centers on the apoE gene in animals. Almost all animal apoE genes code for arginine at the position corresponding to residue 112 in human apoE (i.e. they are apoE4-like) (105). Especially significant is that all the great apes, the closest living ancestors of humans, have this $\varepsilon 4$-like allele and do not display multiple isotypes (34). The other apoE alleles would then have arisen by sequential one-base mutations, $\varepsilon 3$ as a result of a C→T transition in codon 112, and then $\varepsilon 2$ arising from $\varepsilon 3$ by a C→T transition in codon 158. Given the allele frequencies, it could be surmised that $\varepsilon 4$ was (is) at a selective disadvantage compared with $\varepsilon 3$ (and probably compared with $\varepsilon 2$ as well). A further argument for this sequential progression of alleles is that the two polymorphic sites, at codons 112 and 158, are both CpG "hot spot" mutation sites. Both arginine 112 and 158 codons are CGC. The methylation of C in CpG sequences and their ready deamination to T suggests that the C→T transition should be favored over the reverse T→C transition, which would be necessary to obtain $\varepsilon 4$ from $\varepsilon 3$. Still, these reverse transitions do occur, and one might also ask why other "hot spot" mutations in apoE have not occurred. (Fully two thirds of apoE arginine codons are CGC, yet these sites are not polymorphic.)

The other issue that needs to be considered is the functional properties of the corresponding apoE isoforms. Even though most animals have E4-like apoE, with arginine at the position equivalent to residue 112, all nonhuman forms of apoE that have been studied do not display the one known property that distinguishes human apoE4 from human apoE3: preferential binding to triglyceride-rich lipoproteins; instead, animal apoE prefers HDL, like human apoE3 (see ApoE

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Structure/Function, above) [for review see (105)]. This is because animal apoE does not have arginine 61, the residue that is crucial in promoting domain interaction and in driving the lipoprotein preference (see ApoE Structure/Function, above). Instead, all animals whose apoE structure is known, including the apes, have threonine at this position. Therefore, the point at which the mutation of threonine 61 to arginine appeared is of critical importance regarding the functional consequences for the protein.

Although it is not possible to say for certain whether $\varepsilon 4$ or $\varepsilon 3$ came first (no one believes $\varepsilon 2$ is the ancestral allele), it is possible to surmise which alleles might have a selective advantage in each instance. Regardless of whether $\varepsilon 4$ or $\varepsilon 3$ came first, $\varepsilon 2$ must have conferred some selective advantage to achieve its relatively high frequency. This is supported by the data on its roles in both lipoprotein metabolism (see Apolipoprotein E and Lipoprotein Metabolism, above) and neurobiology (see Apolipoprotein E and Neurobiology, above). If $\varepsilon 4$ were the parent allele, the $\varepsilon 2$ allele would almost certainly have conferred advantages over $\varepsilon 4$. Likewise, if $\varepsilon 4$ came first, $\varepsilon 3$ must also have been beneficial compared with $\varepsilon 4$. However, the reverse should be true if $\varepsilon 4$ arose from $\varepsilon 3$ and achieved such a high frequency. Everything known about $\varepsilon 4$ so far suggests that it is a deleterious allele (see Apolipoprotein E and Lipoprotein Metabolism and Apolipoprotein E and Neurobiology, above), an argument that would favor $\varepsilon 4$ as the parent allele, but there is a problem with this concept. If $\varepsilon 4$ is a deleterious allele, why does it exist at such a high frequency in certain populations and persist in all populations? This issue is further complicated by the fact that the known deleterious consequences of carrying apoE4 all occur well after the reproductive years. Both the propensity for developing heart disease and the propensity for developing Alzheimer's disease are primarily later-life events. This dilemma is discussed in the next section.

ApoE Alleles and the Reproductive Schedule

Genetic drift could account for the present-day apoE allele frequencies around the world; however, if $\varepsilon 4$ is the parent apoE allele, it is difficult to imagine how $\varepsilon 3$ achieved its dominant status without having a large selective advantage over $\varepsilon 4$. But since the known deleterious effects of $\varepsilon 4$ occur in the postreproductive years, we are left to consider how the selective advantage of $\varepsilon 3$ could have its impact during the reproductive years (or before) and therefore affect gene transmission.

One straightforward way that apoE alleles could satisfy this requirement is to have a direct impact on reproductive efficiency. Such a study has been performed and the result supports a selective advantage for $\varepsilon 3$ (30). In a study of 40-year-old men of known apoE genotype, men carrying only the $\varepsilon 3$ allele had more children than those carrying either the $\varepsilon 4$ or $\varepsilon 2$ allele. If that were the only operable factor, the $\varepsilon 4$ and $\varepsilon 2$ alleles should disappear eventually, so the situation is likely to be more complicated than just reproductive efficiency.

A second possibility is related to the deleterious cognitive effects of the $\varepsilon 4$ allele (see Apolipoprotein E and Neurobiology, above). If transmission of learning from

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older members of families to younger ones is important for survival of the young (as it was in more primitive hunter-gatherer times), there could be a disadvantage in "families" whose later-life cognitive function was impaired. This concept was offered in an essay by Finch & Sapolsky (28) and termed "grandmothering" by them. Another way in which cognitive impairment from $\varepsilon 4$ carriers could affect survival in the reproductive years is if either cognitive or other brain function impairment were a lifelong phenomenon and not just restricted to later years. We now know that the $\varepsilon 4$ allele affects cognitive function at a relatively young age (11, 91) and that the $\varepsilon 4$ allele can impair recovery from head trauma and stroke at any age (76, 90). Thus, there may be some effect in the young that could reduce survivability and therefore allele frequency distribution. The reverse of this hypothesis was also offered by Finch & Sapolsky (28). They theorized that $\varepsilon 4$ could have survived because it might be advantageous early in life but disadvantageous later in life. Such an argument might also help to explain the high frequency attained by the $\varepsilon 4$ allele if $\varepsilon 3$ were the ancestral allele.

Another distinct possibility contributing to the evolution of apoE alleles is the role of infectious disease(s) that selectively increased one allele at the expense of the others. The $\varepsilon 3$ allele might have provided protection against epidemics of infectious disease that selectively reduced $\varepsilon 4$ allele frequency. Conversely, the $\varepsilon 4$ allele might have provided protection against infectious diseases common to the African subcontinent and certain other isolated populations, such as in Papua New Guinea, where the frequency of apoE4 is the highest. Evidence is beginning to accumulate that apoE plays a role in immune system modulation and in the susceptibility to infectious disease (see Nontraditional Roles of Apolipoprotein E, below).

NONTRADITIONAL ROLES OF APOLIPOPROTEIN E

ApoE and Immunoregulation

Lipoproteins, including apoE-containing lipoproteins and LDL, have the ability to either inhibit or stimulate antigen- and mitogen-induced T lymphocyte activation and proliferation [for review, see (59)]. Cuthbert & Lipsky (15) have shown that low concentrations of these lipoproteins binding to LDL receptors can enhance the responsiveness of lymphocytes to mitogens, resulting in lymphocyte proliferation. On the other hand, these lipoproteins, when binding to different receptors or cell-surface binding sites (referred to as the immunosuppressive receptor), can have the opposite effect and inhibit lymphocyte activity.

The ability of lipoproteins to suppress the mitogen-induced proliferative response of lymphocytes was first recognized in 1976, when it was shown that a subpopulation of "LDL" could inhibit lymphocyte proliferation (14). Later, it was appreciated that only apoB- and apoE-containing lipoproteins could perform this function, and that apoE-containing lipoproteins were three to four times more effective in the inhibition (35, 47). It has also been demonstrated that lipid-free

apoE can still inhibit lymphocyte proliferation and that apoE2, which is defective in LDL receptor binding, retains the inhibitory activity. Further evidence suggesting that the inhibitory activity does not proceed through the LDL receptor comes from the observation that LDL receptor–negative lymphocytes are still susceptible to proliferation suppression by apoE (15, 16).

Multiple activation events are suppressed by apoE, including early and late events (35). ApoE also interferes with signals from multiple mitogens, including interleukin 2 and transferrin, probably the best-studied responses (35, 54). It has been suggested that apoE inhibits both CD4 and CD8 lymphocyte proliferation, at least in part, by reducing the production of biologically active interleukin 2 (activity reduced by 50%–60%). The reduced bioactivity inhibits the interleukin 2–dependent lymphocyte activation by interfering with signaling involved in cell cycle progression. The mechanism whereby apoE alters interleukin 2 activity without altering mRNA levels or the mass of secreted protein is unknown (54).

The cell-surface receptor or binding site that functions as the apoE-responsive immunosuppressive receptor has not been definitively identified. The immunosuppressive receptor is inefficient in internalizing apoE-containing lipoproteins and is present even on FH lymphocytes, which are LDL receptor deficient; therefore, the LDL receptor is not involved. Although the LRP has not been excluded as the immunosuppressive receptor, the fact that apoB-containing LDL can inhibit lymphocyte proliferation argues against it, since apoB is not a ligand for the LRP. (However, it would probably be necessary to repeat some of the earlier studies with LDL to make certain there was no apoE in the LDL preparation.)

Two more likely candidates for the immunosuppressive receptor are HSPG and the transferrin receptor. Much of the evidence is consistent with the notion that HSPG are involved in the binding, and both apoE and apoB bind to heparin and heparin-like macromolecules. It is known that heparin will rescue the cellular response to mitogens, and the rescue correlates with the removal of apoE from the cell surface (46). The transferrin receptor also has been proposed as the immunoregulatory receptor for apoE. Transferrin, which is required for the proliferative response, can inhibit the apoE effect (35). The transferrin receptor, like the LRP, can function in combination with HSPG to act more effectively; therefore, the transferrin receptor and HSPG could function in concert to bind apoE and initiate the suppression of proliferation.

The sequence within apoE involved in binding to the immunosuppressive receptor is likely in the same region as its LDL receptor– and heparin-binding sites (i.e. in the vicinity of residues 140–150). ApoE peptides encompassing residues 141–155 and multimers of this region mimic the native apoE by similarly inhibiting mitogen-stimulated lymphocyte proliferation (10, 25).

Although it is clear that apoE can modulate key elements of the immune response, either positively or negatively, these observations are primarily obtained in in vitro systems. These results need to be reevaluated in light of the most recent knowledge concerning the functional classification of T-cell subpopulations and signature cytokines that modulate lymphocyte activation and proliferation.

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ApoE and Susceptibility to Infectious Disease

As mentioned in ApoE Alleles and the Reproductive Schedule, above, apoE isoform frequency may be determined by differential isoform effects on susceptibility to disease and on survival. Although there is virtually no evidence of apoE isoform–specific effects on infectious diseases, there is evidence that apoE could play a role in reducing susceptibility to three categories of infectious agents: viruses, bacteria, and protozoan parasites.

At least three viruses bind to the HSPG on cell surfaces as an initial means of gaining entry: herpes simplex virus (HSV), human immunodeficiency virus (HIV), and Dengue virus. Each binds to HSPG via a specific envelope glycoprotein. Although there is no direct evidence that apoE can inhibit this step, it seems possible this could occur through competition for HSPG. There is some support for this possibility. First, apoA-I, another important apolipoprotein with repeating amphipathic helices, interacts with the HIV gp41 protein (which also contains amphipathic helices) and prevents HIV-induced syncytium formation and entry of the virus into the host cells (69, 77). Second, the Tat protein, a transactivator of HIV gene expression, can be released from HIV-infected cells and stimulates the growth of HIV-related Kaposi's sarcoma cells. This process can be inhibited by apoE, which blocks proliferation and chemotaxis of Kaposi's sarcoma cells in vitro (8). It is probably not coincidental that Tat, like apoE, has an arginine-rich domain capable of interacting with HSPG. Also, apoE can inhibit tumor formation caused by inoculation of HIV-related Kaposi's sarcoma cells into nude mice (8). Third, the presence of HSV-1 increases the risk for Alzheimer's disease and the $\varepsilon 4$ allele is overrepresented in Alzheimer's patients with HSV but not in those lacking HSV (51), suggesting a differential apoE isoform–specific effect involving viruses that is connected directly to the risk of developing Alzheimer's disease. The combination of direct and circumstantial evidence suggests that apoE may have profound effects in susceptibility to viral infections.

The evidence that apoE plays a role in resistance to bacterial infections is much more direct. ApoE-null mice appear to have an impaired immune response and display a susceptibility to bacterial infection. The enhanced susceptibility of apoE-null mice, compared with control mice, has been shown in studies with *Listeria monocytogenes* (83) and *Klebsiella pneumoniae* (20). On the other hand, LDL receptor–null mice, which also have a marked hypercholesterolemia, are protected against lethal gram-negative bacterial infection (75). Thus, it appears that the absence of apoE itself, and probably not the hyperlipidemia per se, results in the inability of the mice to be protected from these bacterial infections.

Finally, there is some evidence that apoE plays a role in protection against malaria. The malaria circumsporozoite protein utilizes the HSPG/LRP pathway to invade hepatocytes, requiring both cell-surface molecules for maximal entry (86). Inhibitors or competitors of one component of the HSPG/LRP pathway (e.g. the receptor-associated protein or lactoferrin for LRP or heparinase for HSPG) can partially inhibit host cell invasion. Furthermore, remnant lipoproteins, which use

apoE as the ligand and interact with the HSPG/LRP pathway, also can inhibit host cell invasion of the circumsporozoite protein (89).

Thus, it is probable that apoE plays a role in modulating infectious disease by as many as three classes of infectious agents. The mechanism may or may not be related to the immunoregulatory roles for apoE. There is some reason to believe that apoE isoform–specific effects could be operable, but this has not been proven, and the differential role of apoE4 in HSV/Alzheimer's disease has been demonstrated only indirectly. It remains as speculation that such apoE involvement in susceptibility to infectious disease translates into the determination of apoE allele frequency by differential survivability/susceptibility.

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Other Possible Roles for ApoE

A number of other roles suggested for apoE have as a common theme effects on cell signaling mechanisms. The ability of apoE to suppress mitogen-induced lymphocyte proliferation (see ApoE and Immunoregulation, above) probably involves intracellular signaling (35). ApoE also appears to inhibit mitogen-induced smooth muscle cell proliferation by suppressing signal transduction (50). Earlier, it was found that smooth muscle cells express apoE in the quiescent state but not during active proliferation (68). Now it is apparent that apoE prevents transit to the G1 phase of the cell cycle in smooth muscle cells by suppressing signal transduction (MAP kinase activity). It has been suggested that diverse cytostatic functions of apoE could be due to the variety of receptors apoE can utilize. It is increasingly apparent that members of the LDL receptor family are involved in many signal transduction pathways, initiated through signals in the cytoplasmic tails of these receptors (18, 38, 96).

Another phenomenon that may further link apoE to intracellular regulatory events is the observation that apoE accumulates intracellularly in an isoform-specific fashion. ApoE4 accumulates intracellularly to a lesser extent than apoE3, suggesting different intracellular pathways for the specific isoforms (52). This observation suggests myriad possibilities for how specific isoforms could affect intracellular pathways. One intriguing possibility, still in its germinal stages of investigation, is the role of apoE in apoptosis, a programmed process for cell death that may involve multiple signaling pathways.

Thus, it is clear that apoE plays a part in many processes beyond its traditional role in cholesterol and lipoprotein metabolism. Studies continue to unravel the varied biological impact of this intriguing protein.

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