ApoE From A to Z -

Atherosclerosis

to

Alzheimer's

Helen H. Hobbs

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INTRODUCTION

Apolipoprotein E (apoE) is a 34 kilodalton glycoprotein that plays an important role in the transport of lipids between tissues and among cells. ApoE is produced by many different cell types and circulates in plasma as a major constituent of lipoproteins. Individuals with high plasma levels of apoE-containing lipoproteins have an increased incidence of both peripheral and coronary atherosclerosis. Recently, apoE has been implicated in another disease process unrelated to lipoproteins; a common variant of apoE has been linked to Alzheimer's disease. The establishment of a connection between apoE and this degenerative neurological disorder has taken both the lipidologist and the neurologist into new and unfamiliar fields.

ApoE SYNTHESIS AND STRUCTURE

In normal subjects, the plasma concentration of apoE ranges from 2.5 to 6 mg/dl (1). Approximately 90% of the apoE circulating in plasma is produced in the liver (2). ApoE mRNA is translated on membrane-bound ribosomes and the N-terminal 18 amino acids are removed upon insertion of the protein into the lumen of the endoplasmic reticulum (ER). In the ER, a single O-linked sugar is attached to the 299 amino acid polypeptide. A variable numbers of sialic acid residues (2, 4, or 6) are added to the sugar chain in the Golgi complex (3, 4). ApoE is then secreted from the cell into the plasma where it rapidly associates with triglyceride(TG)-rich lipoproteins. As apoE circulates in plasma it becomes partially or completely desialylated (4).

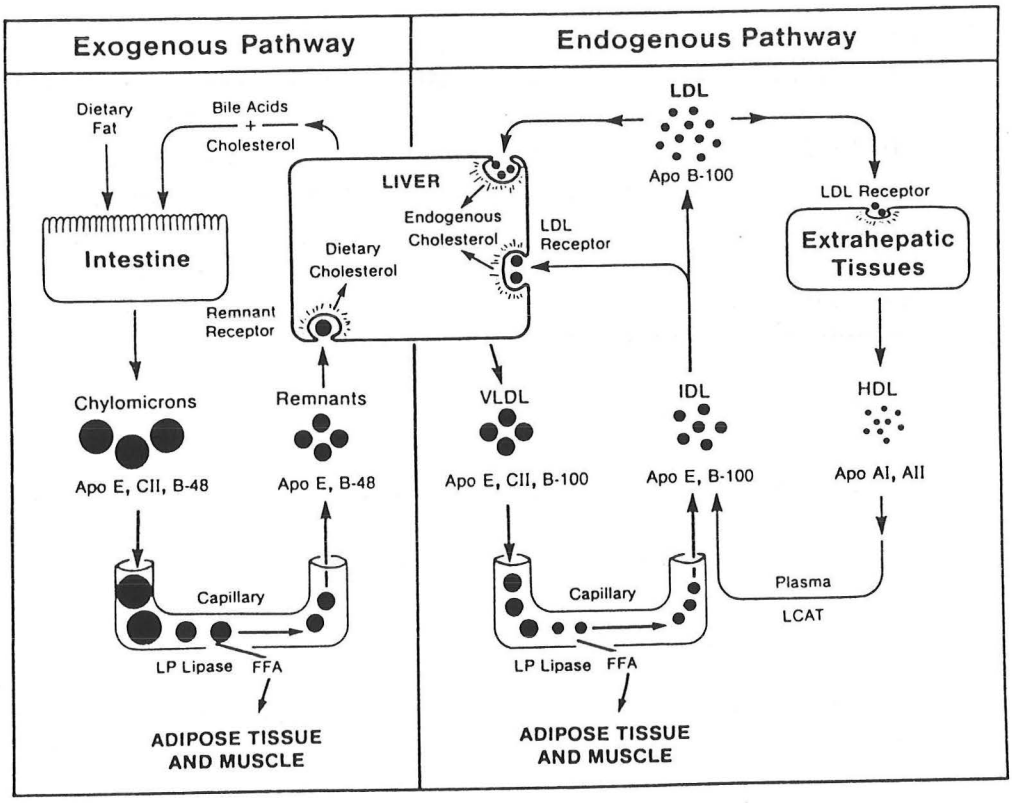
Subjection of apoE to thrombin digestion results in the formation of two major fragments, each of which has a different functional role. A 22 kDa fragment from the N-terminal region contains the sequences which mediate the binding of apoE to cell surface lipoprotein receptors and to heparin (5). A C-terminal 10 kDa fragment includes the sequences required for lipoprotein association (6).

ApoE AND THE METABOLISM OF TG-RICH LIPOPROTEINS

TG-rich lipoproteins enter the plasma by two routes - the endogenous and the exogenous pathway [Figure 1]. In hepatocytes, TG's, cholesterol esters, phospholipids and free cholesterol are coupled with a single copy of apoB100 and secreted as very low density lipoprotein (VLDL) particles. In the plasma, the VLDL acquires multiple copies of apoE as well as apolipoproteins of the C series. In a parallel pathway, exogenously derived lipids are absorbed from the diet by intestinal epithelial cells. Within the enterocytes the dietary lipids are coupled to apoB48, apoAI, and apoAIV, and packaged into chylomicrons particles. These very large, TG-rich, buoyant particles are secreted into the lymphatic system rather than the portal circulation so as to bypass the liver. Once in the plasma, chylomicrons acquire multiple copies of apoE from HDL. ApoE,

unlike apoB, readily exchange between lipoprotein particles.

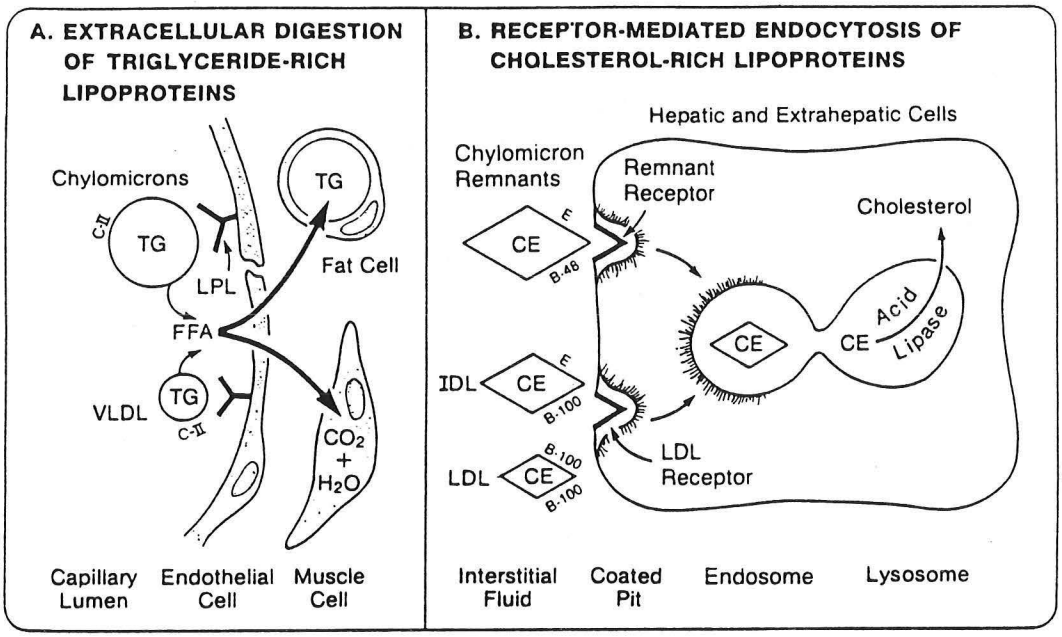
Figure 1



The predominant lipid component of VLDL and chylomicron particles is TG; in VLDL particles, the TG to cholesterol ratio is 5:1 and in chylomicrons it is 20:1. Both classes of TG-rich particles contain apolipoproteins of the C series (apoCI, apoCII, and apoCIII). The apoC's play two important roles in the metabolism of TG-rich particles. First, they inhibit the uptake of TG-rich particles by lipoprotein receptors in the liver (7). In general, there is an inverse relationship between the number of apoC's and apoE's on lipoprotein particles. Particles containing many apoC's and few apoE's have a lower affinity for hepatic lipoproteins receptors (8). Thus, addition of apoC's to the TG-rich particles ensures that the TG's are delivered to peripheral tissues prior to lipoprotein removal by the liver. Second, apoC's are required for the hydrolysis of TG-rich particles by lipoprotein lipase. Lipoprotein lipase is an enzyme that is synthesize by adipocytes and myocytes and secreted into the extracellular space. It migrates through the capillary wall and attaches to proteoglycans which decorate the endothelial surfaces. As VLDL and chylomicrons circulate through capillaries, the apoCII tethers the particle to lipoprotein lipase so that the TG's in the core of the lipoprotein can be hydrolyzed [Figure 2A]. Free fatty acids are released and diffuse into the adjacent tissues where they are either re-esterified and stored as triglyceride, or burned as fuel by ß-oxidation.

The importance of apoCII in the lipolysis reaction is exemplified by the fact that individuals who have apoCII deficiency have very high plasma levels of chylomicrons.

Figure 2



In the presence of apoCII and lipoprotein lipase, both chylomicrons and VLDL decrease in size and become much more cholesterol ester- and apoE-rich. The newly formed VLDL and chylomicron remnants have a cholesterol ester to triglyceride ratio that approaches one. These remnant particles have a density of <1.006 g/ml and yet migrate with β lipoproteins on agarose gel electrophoresis and, thus, together are referred to as β-VLDL.

Coincident with the lipolysis and remodeling of the TG-rich lipoproteins, the apo C's are transferred to HDL and apoE's are added to the particle. In addition, the conformation of the apoE's on the lipoprotein surface is altered so as to expose epitopes that are capable of binding lipoprotein receptors (9). Almost as soon as chylomicron remnants are formed, they are cleared from the circulation via apoE-mediated uptake by two hepatic cell surface receptors-the LDL receptor and the closely related LDL receptor-related protein (LRP) (also called the α2-macroglobulin receptor) [Figure 2B]. The relative roles of each of these receptors in the clearance of remnant particles by the liver is still debated, but both are clearly capable of binding and internalizing β-VLDL (10). The VLDL remnants that are formed in the peripheral tissues have two possible fates. Approximately half of the VLDL remnants are removed from the plasma by the liver via the LDL receptor (11). ApoE-containing lipoproteins actually bind to the LDL receptor with a higher affinity then does the receptor's namesake, LDL (12). The remaining VLDL remnant particles are converted to LDL and this process is thought to involve hepatic lipase.

Animal experiments suggest that the amount of apoE in plasma can be rate-limiting in the removal of lipoproteins by hepatic receptors. Intravenous infusion of apoE into cholesterol-fed rabbits is associated with a dramatic fall in plasma lipoprotein levels (13). The importance of apoE in the clearance of apoB-containing lipoproteins is also dramatically illustrated by the overexpression of rat apoE in transgenic mice (14). These mice have markedly reduced plasma lipoprotein levels and the rate of clearance of ^{125}I-VLDL is three times faster than non-transgenic controls. Of more significance, the administration of a high cholesterol diet, is not associated with any rise in plasma lipoprotein levels in the apoE transgenic mice [Figure 3]. Thus, high levels of apoE expression can overcome the effect of an increased entry of lipoproteins into the plasma compartment.

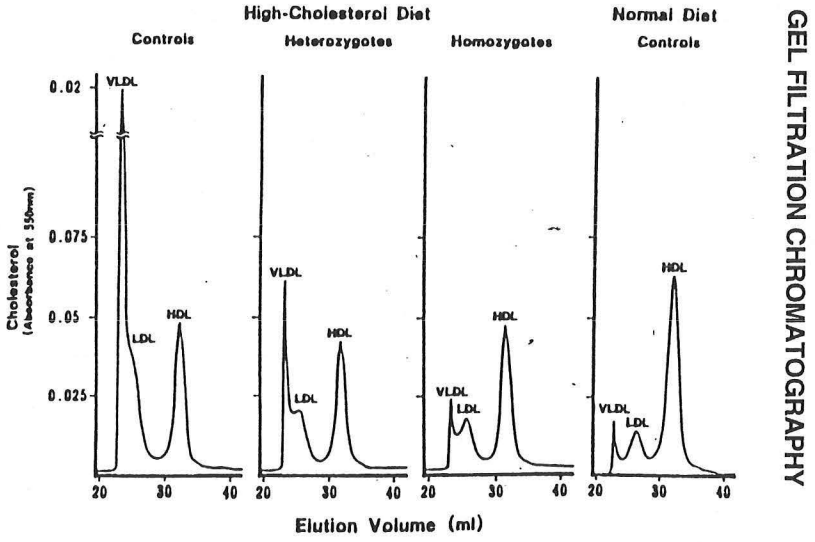
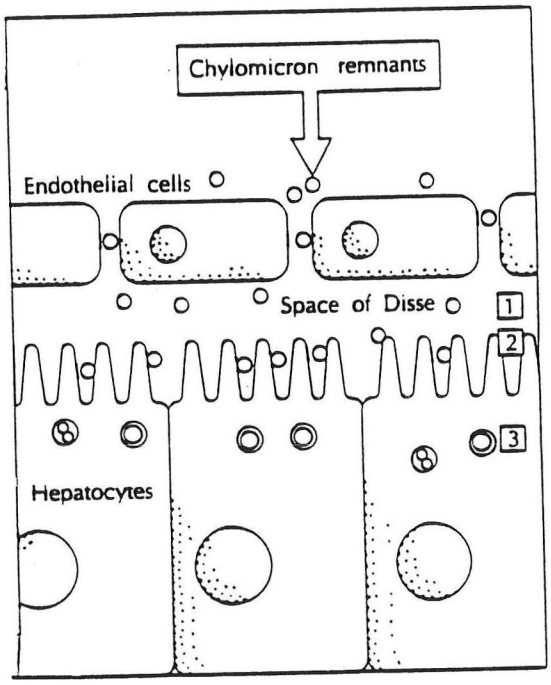


Figure 3. Profile of lipoprotein cholesterol levels in cholesterol fed apoE transgenic and control mice (14).

Both the amount and the conformation/orientation of apoE on the surface of lipoprotein particles influence their rate of clearance by hepatic cell surface receptors (8-10). To demonstrate binding of β-VLDL to LRP on cultured cells requires the prior addition of ApoE to the β-VLDL particles (10). There is now evidence to suggest that this requirement for "apoE doping" may simulate the *in vivo* situation. Immunocytochemical studies have demonstrated that ApoE coats the absorption surfaces of the hepatic microvilli, probably by linkage to proteoglycans (15, 16). Thus, when remnant particles enter the space of Disse, they are literally bathed in apoE. In this way the remnants can be trapped, apoE added to the particle and the remnant delivered to adjacent lipoprotein receptors for endocytosis [Figure 4] (3).

Figure 4



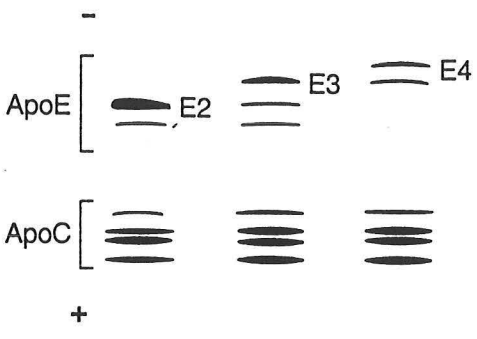
ApoE AND REVERSE CHOLESTEROL TRANSPORT

Unlike most other apolipoproteins, apoE is made and secreted by a number of different cell types outside of the liver including macrophages, astrocytes, Leydig cells, adrenal cells, proximal tubular cells, epithelial cells of Bowman's capsule and keratinocytes (18-23). The exact role that apoE plays in many of these tissues has not been elucidated. ApoE has been implicated in playing an important role in the reverse cholesterol transport of cholesterol from peripheral tissues to the liver. Macrophages phagocytize dead and senescent cells and accumulate large amounts of cholesterol esters in their cytoplasm. When mouse peritoneal macrophages are loaded with cholesterol *in vitro*, they dramatically increase their synthesis and secretion of apoE (24). In these lipid-laden cells, apoE has been estimated to comprise 2% of the total protein synthesized. ApoE is secreted into the extracellular space independently of cholesterol and rapidly associates with HDL particles (25). The cholesterol-rich, apoE-rich HDL circulates in plasma and is removed by hepatic LDL receptors. In this way, excess cholesterol is transported from peripheral tissues back to liver.

ApoE IS POLYMORPHIC IN SEQUENCE

The apoE glycoprotein is polymorphic in two respects. Plasma apoE contains a variable number (1-3) of sialic acid residues attached to the single O-linked sugar chain (4,26). The amino acid sequence of the polypeptide chain also varies. There are three common isoforms in the general population which are referred to as apoE2, apoE3, and apoE4, depending on their relative mobility on two dimensional isoelectric focusing gel

[Figure 5]. These differences in gel migration are due to sequence variations at two amino acid positions--112 and 158. ApoE2 and apoE4 have two cysteines or two arginines in these positions, respectively. ApoE3, the most frequent isoform in the population, has a cysteine at residue 112 and arginine at residue 158.



	E2/2	E3/3	E4/4
Relative Charge	0	+1	+2
Residue 112	Cys	Cys	Arg
Residue 158	Cys	Arg	Arg

Figure 5. Isoelectric focusing of VLDL from three individuals homozygous for different apoE phenotypes. The minor bands are due to other sialylated forms (1).

These polymorphic amino acids are the results of single base pair changes in the sequence of the *APOE* gene, which resides on chromosome 19q13.1 in close linkage with *APOCI* and *APOCII*. The frequencies of each *APOE* allele (ε2, ε3 or ε4) in the European and North American populations are ~ 0.10 (ε2), 0.75 (ε3) and 0.15 (ε4) (27) with the exception of the Finnish and the African-American population where the frequency of ε4 is significantly higher ~ 0.22 (28). The frequencies of the six possible genotypes differ significantly between different populations [Table 1] (28).

Table 1. The average frequency of the apoε alleles in four populations (28).

	∈2	∈3	∈4
Caucasians	0.08	0.77	0.15
Japanese	0.035	0.85	0.112
Chinese	0.08	0.82	0.06
Amerindians	0.00	0.82	0.184

ApoE POLYMORPHISM AND LIPOPROTEIN METABOLISM

The different apoE isoforms vary in their relative distribution among plasma lipoproteins. ApoE3 and apoE2 are partitioned approximately equally between apoB-containing lipoproteins and HDL. Both apoE2 and apoE3 have a free reactive sulfhydryl group that can form mixed disulfide linkages with apoAII. The apo(E-AII) complexes tend to be associated with HDL particles, which may contribute to the higher concentration of apoE2 and apoE3, relative to apoE4, in the HDL fraction (29). ApoE4 associates preferentially with VLDL/IDL due in part to the lack of formation of these heterodimers. Also, it has been demonstrated that the presence of a positively charged residue at amino acid 112 (Cys to Arg) contributes to the predilection of apoE4 for more TG-rich particles (30).

The polymorphism in apoE not only influences the localization of apoE to different lipoprotein classes, but also has important functional sequelae. ApoE2 has a markedly decreased affinity for the LDL receptor when compared to either apoE3 or apoE4 (31). This is shown in Figure 6. In these experiments apoE has been purified from individuals homozygous for the three different isoforms and the apoE has been complexed with phospholipids (DMPC). The apoE/phospholipid vesicles have been incubated with cultured fibroblasts in the presence of radiolabeled LDL to compare the ability of the different apoE isoforms to compete with LDL for LDL receptor binding. ApoE2 has only 2% of the binding affinity of either apoE3 or E4 (31). It has also been shown that apoE2 binds to the putative chylomicron remnant receptor, LRP, with reduced affinity, when compared to apoE3 or apoE4 (8). If the apoE2 is treated with cysteamine, which converts the cysteine residue at amino acid 158 to a lysine analogue, it can now bind with near normal affinity. Thus, the LDL receptor binding defect associated with apoE2 is due to the substitution of a neutral amino acid for a basic amino at amino acid 158 (i.e. Arg to Cys).

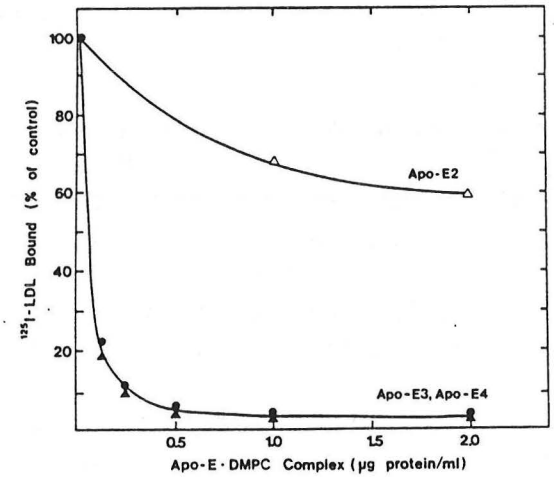


Figure 6. Comparison of the receptor binding activities of apoE/phospholipid complexes to human fibroblasts in the presence of radiolabeled human LDL.

What are the consequences of the apoE polymorphism in apoE on lipoprotein metabolism? Normolipemic individuals who are homozygous for apoE2 clear both chylomicron and VLDL particles from their plasma at a reduced rate (33,34). As a result, ε2 homozygotes have detectable levels of β-VLDL in their plasma even after an overnight fast. ApoE2 homozygotes have been classified as having dysbetalipoproteinemia (34).

The relationship between the apoE phenotypes and mean plasma concentrations of lipoproteins, apoE and apoB in a large German population are shown in Figures 7 (35). Individuals who are homozygous for apoE4/E4 have significantly higher plasma levels of total cholesterol and LDL-cholesterol(C) and lower plasma levels of ApoE when compared to apoE3/E3 individuals (35). Individuals homozygous for apoE2 have significantly lower plasma levels of cholesterol and LDL-C, and significantly higher plasma levels of apoE. These same trends have been seen in every population studied to date (36).

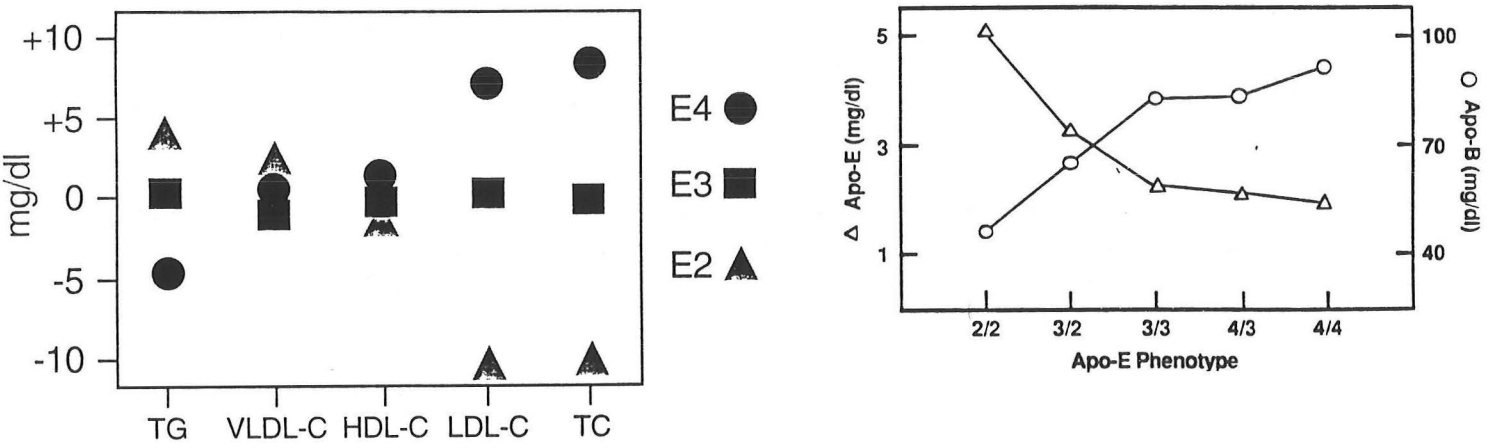


Figure 7. The effect of different apoE isoforms on the plasma concentrations of lipoproteins (left) and apoE and apoB (right) (1).

It seems paradoxical that individuals who are homozygous for apoE2 (the isoform associated with a decrease in LDL receptor binding capacity) have lower plasma levels of LDL-C. There are at least two possible explanations for this seeming paradox. The decrease in the rate of delivery of both intestinally and endogenously-derived cholesterol to the liver in ε2 homozygotes may result in an increase in hepatic LDL receptor activity, and thus a decrease in plasma LDL-C levels. Also, for unknown reasons, there is impaired conversion of VLDL to LDL in ε2 homozygotes (37, 38).

Conversely, why do individuals with ε4 have higher plasma LDL-C levels despite the fact that apoE4 binds to the LDL receptor an affinity equal to apoE3? Despite these *in vitro* data, *in vivo* metabolic studies have shown that apoE4-containing lipoproteins are removed more rapidly from the circulation than apoE3-containing particles (39). The

preferential association of apoE4 with TG-rich particles may contribute to their rapid catabolism. The observed increase in plasma LDL-C concentration in individuals with apoE4 may be a consequence of down-regulation of the hepatic LDL receptor in response to an increased rate of delivery of cholesterol ester-rich lipoproteins to the liver.

ApoE POLYMORPHISM AND CORONARY ARTERY DISEASE (CAD)

Some but not all studies, have found a slight increase in the frequency of the ε4 allele and a decreased frequency of the ε2 allele in individuals with coronary artery disease (28, 40-42). Differences in apoE isoform frequencies may contribute to the observed differences in the incidence of ischemic heart disease between population. The Finnish population has a 50% higher frequency of apoE4 than the general European population and also has one of the highest incidence of CAD in the world, whereas the ε4 allele is of low frequency in the Chinese and Japanese populations where there are low rates of CAD (28). It has been recently suggested that the association of apoE4 with coronary atherosclerosis is independent of the plasma lipid level (43). As part of the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study, the thoracic and abdominal aortas and right coronary arteries of young male accident victims (age 15-34) were examined; the amount of atherosclerotic involvement was related to the apoE genotypes of the individuals. The ε3/ε4 individuals tended to have more atherosclerotic involvement than the ε3/ε3 individuals and this difference was independent of plasma LDL-C concentrations. However, the differences between the two groups were small and accounted for less than 6% of the variation in lesion amount. It has not yet been convincingly demonstrated that the apoE polymorphism has an effect on the incidence of CAD. If it does have an effect, it is small and most likely attributable to associated differences in plasma lipid levels.

ApoE POLYMORPHISM AND LONGEVITY

A French group reported that in centenarians there was an increase in the frequency of the ε3 allele and a decrease in the frequency of the ε4 allele, when compared to controls (age 20-70)[Table 2] (44). It is not known what is responsible for the under-representation of the ε4 allele in this elderly population. Did more of the individuals with the E4 allele die at an earlier age from cardiovascular disease due to the higher plasma concentrations of cholesterol? In support of this interpretation is the fact that there was a statistically significant decrease in the frequency of the ε2 allele in the ancient age group. Alternatively, it has been suggested that the lower frequency of ε4 in the centenarians may be due to a sampling artifact. As will be discussed later, more individuals with ε4 have Alzheimer's disease, and since many of them are institutionalized, they are not as likely to be included in samples of healthy elderly individuals (45).

Table 2. Frequency distribution of apoε alleles in 325 centenarians and 160 controls

ApoE Alleles	Centenarians Numbers (Frequency)	Controls Numbers (Frequency)	Other Control Population (Paris) Numbers (Frequency)
ε2	83 (0.128)	22 (0.068)	39 (0.079)
ε3	533 (0.820)	264 (0.820)	399 (0.801)
ε4	34 (0.052)	36 (0.112)	60 (0.120)

ApoE4 AND TYPE V HYPERLIPIDEMIA

In some studies the ε4 allele has also been strongly associated with Type V hyperlipoproteinemia (46). This hyperlipidemia is characterized by elevations in both VLDL and chylomicrons (but not remnant particles) in the plasma. The disorder is multifactorial in etiology and tends to have an autosomal dominant pattern of inheritance in families. Not all individuals with Type V have an apoE4 isoform, but the frequency of apoE4 isoform was found to be significantly higher in 30 severely affected Type V patients when compared to 37 controls (33% vs 3% (ε4/ε4), 40% vs 21.6% (ε4/ε3 and ε4/ε2). This result has been questioned by another group who did not find a single apoE4 isoform in 20 patients with Type V (47). The reason for the association between apoε4 and Type V hyperlipidemia has not been elucidated. It is possible that this association has nothing to do with apoE4, but is caused by a gene closely linked to the *APOE* locus, possibly *APOCI* or *APOCII*.

TYPE III HYPERLIPOPROTEINIA (HLP)

This disorder, which is alternatively referred to as familial dysbetalipoproteinemia (FDB), was originally described by Gofman in 1952 when he noted the coincident occurrence of "xanthoma tuberosom" and elevations in the plasma concentration of VLDL (48). It was also described by Fredrickson and classified as Type III hyperlipoproteinemia (HLP) based on the analysis of the VLDL particles by agarose gel electrophoresis (49); the VLDL (i.e. fasting lipoproteins with d<1.006 g/ml) migrates as a β lipoprotein rather then as a pre-β particle so a broad band which extends from the pre-β position through to the β band is seen [Figure 8]. Not surprisingly, the disorder has also been referred to as broad band beta disease. In 1973 Havel and Kane showed that individuals with Type III HLP had elevated plasma apoE levels (50). Two years later Utermann made the important observation that individuals with Type III hyperlipidemia all lacked the "normal" form of apoE (i.e. apoE3 and apoE4) on isoelectric focusing analysis of VLDL-associated proteins (51).

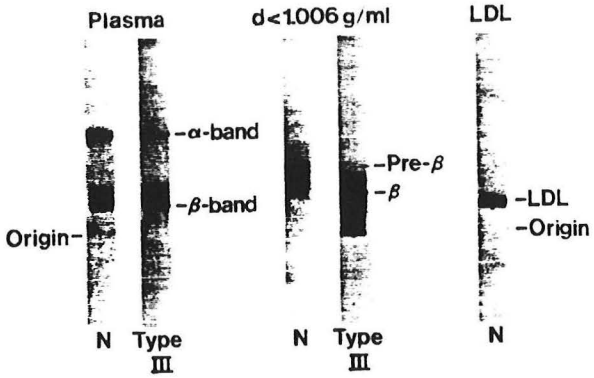


Figure 8. Agarose gel electrophoretic pattern of plasma (left) and <1.006 g/ml lipoproteins from a normal subject (N) and a subject with Type III HLP (1).

There are three classes of molecular defects in the *APOE* gene which are associated with Type III HLP:

1) Over 95% of affected individuals have two ε2 alleles. Homozygosity for ε2 is necessary, but not sufficient, to cause the disorder; only 1% of ε2 homozygotes develop Type III HLP.

2) Less than 5% of individuals with Type III HLP have an autosomal dominant (AD) variant and are heterozygous for a mutation in apoE.

3) Even more rarely, the disease is caused by the absence of apoE in the plasma (ApoE deficiency).

The molecular basic for these defects will be discussed after the clinical features of Type III HLP are reviewed.

CLINICAL FEATURES OF TYPE III HYPERLIPIDEMIA

Type III HLP rarely develops prior to age 20 and usually has its onset during middle age. Affected individuals tend to be obese, diabetic, have proteinuria, or consume alcoholic beverages. There is an increased incidence of the disorder in men, and in post-menopausal women. In the post-menopausal woman, the disease responds dramatically to estrogen replacement therapy [Figure 9]. The disorder developments in association with hypothyroidism and the administration of exogenous thyroid hormone ameliorates the disease.

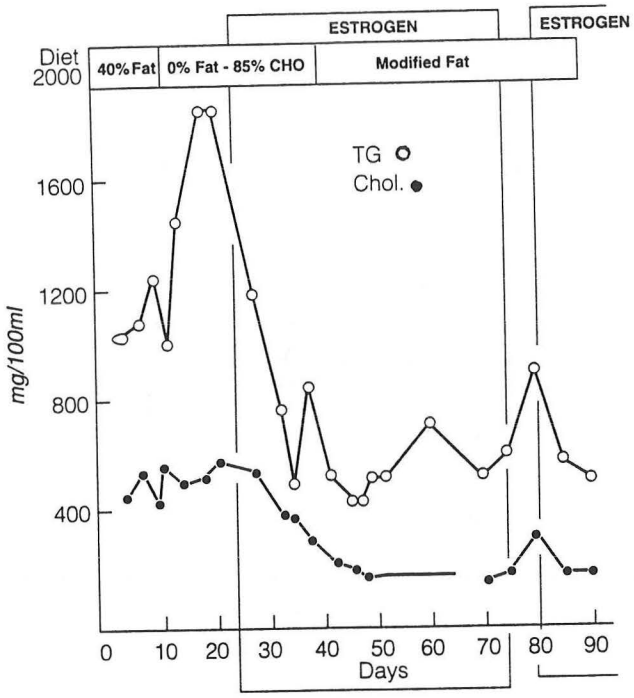


Figure 9. Plasma cholesterol and TG levels in a post-menopausal woman with Type III HLP after initiation on estrogen replacement therapy.

Approximately 50% of first degree relatives of individuals with Type III HLP have some form of hyperlipidemia (52). There is a particularly high frequency of familial combined hyperlipidemia (FCHL) in the families of Type III patients (53). Childhood onset of the disorder has been reported in individuals who are ε2/ε2 and also heterozygous for an LDL receptor mutation (54). In some FH heterozygotes, the disease is expressed in individuals with only a single copy of the ε2 allele (55).

Occasionally, FDB is associated with the development of a monoclonal gammopathy or immunological disorder (1). Individuals with homozygous hepatic lipase deficiency can have a lipoprotein pattern which is similar, but not identical, to that of Type III HLP (56). With these two exceptions, all of the factors which are associated with the development of FDB have one of two major effects on lipoprotein metabolism. Either they cause an increase in the formation of VLDL particles by the liver (obesity, increased caloric intake, diabetes mellitus, FCHL, alcohol consumption), or they result in a decrease in LDL receptor activity (FH, hypothyroidism, estrogen deficiency, high fat diet).

The typical plasma lipoprotein concentrations of untreated individuals with Type III HLP is a plasma cholesterol level over 350 mg/dl and a TG level that either equals or exceeds the level of cholesterol. Most of the increase in the plasma cholesterol level is in the VLDL/IDL fraction. Therefore, the plasma VLDL-C levels are very high, whereas both the LDL-C and HDL-C levels are low.

The clinical sequelae of having high plasma levels of remnant particles in the blood are summarized in Table 3. About 55% of affected individuals develop a

pathognomonic skin lesion - xanthomata striata palmaris (or palmar xanthomas). These lesions consist of lipid deposits in the digital and palmar creases of the hands so that the creases have an iridescent orange-yellow hue. Tuberous and tuberoeruptive xanthomas are found in greater than 50% of affected individuals and most commonly involve the buttocks, elbows, knees and hands. These lesions consist of firm, protuberant nodules that appear in patches and often coalesce. Pathological evaluation of these lesions reveals lipid-laden macrophages.

Table 3. Clinical findings in 185 Type III hyperlipidemic individuals (1).

Clinical Finding	Percent of Patients
Xanthomas	
Striata palmaris	55
Tendon	13
Tuberous and tuberoeruptive	64
Xanthelasma	7
Corneal arcus	11
Coronary heart disease	28
Peripheral vascular disease	21
Cerebrovascular disease	4
Gout	4
Diabetes mellitus (clinical)	4
Hypothyroidism	4

A relatively small percentage (2-20%) of affected individuals have periorbital lipid accumulations resulting in the formation of xanthelasma. Approximately 13% of affected individuals have tendon xanthomas which are indistinguishable from those seen in individuals with FH. No study has systematically examined this subset of patients to determine if these individuals have both FH and Type III HLP.

A more serious consequence of Type III HLP is the accumulation of lipids in the walls of coronary arteries. Like, FH, Type III HLP is associated with the development of coronary atherosclerosis but unlike FH, it is also associated with the development of peripheral atherosclerotic disease, especially involving lower extremities (1). About one-third of all FDB patients have either coronary or peripheral vascular disease. In males, the onset is usually in the early 40's and in females it is delayed by 10 years. Although the frequency of vascular disease in these patients is high, they comprise less than 1% of all myocardial infarction victims (57).

In vitro studies have demonstrated that apoE2-containing β-VLDL particles are avidly taken up by monocyte-macrophages. Early pathological reports suggested that the atherosclerotic lesions of Type III patients were distinctive in that they tended to be less complex and have a larger number of foam cells, but subsequent studies revealed

that the mature atherosclerotic lesions in Type III HLP patients are indistinguishable from those of other etiologies (58).

DIAGNOSIS OF TYPE III HLP

The diagnosis of Type III HLP is straightforward if the typical skin lesions are present. In the absence of the cutaneous lesions, a more detailed analysis of the blood lipoprotein levels is required. The diagnosis should be suspected in individuals with plasma cholesterol level over 300 mg/dl and an equal or higher plasma level of triglyceride. FDB can not be diagnosed using the standard methodologies employed to measure lipoprotein levels at Parkland Hospital (the so-called lipid panel). For a lipid panel, the total cholesterol and triglycerides are measured prior to precipitation of the apoB-containing lipoproteins (i.e. VLDL, IDL & LDL). The cholesterol is then measured in the supernatant and this value represents the HDL-C level. The LDL-C is then computed using the following formula: LDL-C= TC-[TG/5 + HDL-C]. This formula gives quite an accurate estimate of the LDL-C except in the following two circumstances: 1) if the triglyceride levels are greater than 350 mg/dl and 2) if there are remnant particles in the plasma sample. The formula is based on the assumption that almost all the triglyceride in a fasting plasma sample is in the VLDL fraction. In the presence of remnants, the VLDL-C can not be estimated by dividing the plasma TG level by 5.

To determine if remnant particles are present in plasma, a β-quantification analysis is required. The plasma is subjected to ultracentrifugation at a density (d=1.006 g/ml) such that the chylomicrons, VLDL, and remnants float and the IDL, LDL, and HDL sink. The cholesterol concentration in the upper phase is measured and compared to the total plasma triglyceride level. In the presence of remnants, the ratio is higher than normal (i.e > 0.2). In most individuals with Type III, the ratio is > 0.3. Since most of the plasma cholesterol is in β-VLDL, Type III subjects have low plasma LDL-C and HDL-C levels.

An alternative method which can be used to make a diagnosis of Type III HLP requires fractionating VLDL or whole plasma by both size and charge using agarose gel electrophoresis. This method is less sensitive but is simple, cheap, and specific.

At the present time, there is no clinical indication for apoE phenotyping or genotyping, except as a research tool. The apoE isoforms can be analyzed using either isoelectric focusing, or immunoblotting with anti-apoE isoform specific antibodies (59). There are a number of easy methodologies which can be used to analyze the *APOE* gene directly. All methods employ the polymerase chain reaction (PCR) to amplify the sequences surrounding codons 112 and 158 from total genomic DNA. The sequences of these fragments can then be analyzed using allele-specific oligonucleotide hybridization (60), restrictions analysis (61), or the single-stranded conformation polymorphism technique (62) to determine the apoE genotype.

TREATMENT OF TYPE III HLP

A complete dietary history should be obtained and the family should be screened for dyslipoproteinemias. It is imperative that patients with Type III HLP be screened and treated for any of the above mentioned secondary causes of the disorder: estrogen to the post-menopausal woman, weight reduction diet to the overweight patient, and L-thyroxine to the hypothyroid patient.

Dietary modification should be the first line of therapy and often has a dramatic lipid lowering effect. The saturated fat, cholesterol and total caloric intake should be reduced. It is important that a high carbohydrate diet is not instituted since this tends to increase the hepatic production of VLDL and thus worsen the hyperlipidemia (63). By decreasing the intake of dietary fats, fewer chylomicron particles are generated delivered to the liver. This results in an up-regulation of hepatic LDL receptor activity which increases the clearance of VLDL remnants.

An example of the effect of dietary modification and weight reduction on plasma lipid levels of an individual with Type III HLP is shown in Table 4. Coincident with losing weight, there was a dramatic decrease in the plasma lipid levels and this was shown to be associated with an increase in LDL receptor binding affinity of his β-VLDL. Presumably, these temporal differences in receptor affinity of his remnants are due to conformational changes in the apoE protein associated with differences in the lipid composition of the particles.

Table 4. Serial weights and plasma lipid levels in an individual with Type III HLP who was started on a low fat, weight reduction diet.

Date	Weight (lb.)	Chol (mg/dl)	TG (mg/dl)	VLDL-C (mg/dl)	ApoE (mg/dl)
1981	270	725	670	465	–
4/26/82	–	248	246	113	25.75
7/08/82	265	215	193	85	–
10/13/82	240	108	72	10	13.4
2/16/83	257	316	325	112	–

In patients in which dietary therapy is not entirely effective, nicotinic acid and fibric acid derivatives should be initiated. HMG-CoA reductase inhibitors can also be

effective agents in treating this disorder. Nicotinic acid reduces VLDL production and increases their clearance. Administration of large doses of niacin are associated with a ~ 40% drop in VLDL-C and a significant (~ 20%) increase in HDL-C. The fibric acid derivatives are more convenient to take and tend to be better tolerated. The administration of clofibrate, gemfibrozil, or fenofibrate are all associated with a ~ 30% fall in plasma cholesterol and TG and an increase in the level of both the plasma LDL-C and HDL-C [Table 5] (63-65). Treatment with these agents often result in dramatic regression, and resolution, of the tuberoeruptive xanthomas. There is an associated symptomatic decrease in the frequency of angina and/or claudication (65).

Table 5. Mean plasma lipoprotein levels before and after initiation of clofibrate to 13 individuals with Type III HLP (65).

	Before Treatment	After Treatment
Total Chol	498 ± 144	245 ± 35
Total TG	685 ± 424	181 ± 52
VLDL-Chol	244 ± 160	70 ± 30
LDL-Chol	117 ± 29	126 ± 58
HDL-Chol	34 ± 13	50 ± 17
HDL-TG	24 ± 11	15 ± 7
VLDL-chol/Total TG	0.38 ± 0.09	0.37 ± 0.08

Since the goal of treatment is to reduce the plasma concentration of atherogenic β-VLDL particles, serial β-quantification must be performed to monitor the VLDL-C level. The plasma VLDL-C to TG ratio should not be expected to normalize due to the underlying dysbetalipoproteinemia.

MOLECULAR DEFECTS IN CLASSICAL AND AUTOSOMAL DOMINANT (AD) TYPE III HLP

The vast majority of Type III patients are homozygous for ε2 and have an identifiable risk factor responsible for the development of Type III. Less than 5% of individuals with Type III HLP have an AD form of the disease. In these individuals, heterozygosity for the mutant apoE allele is sufficient for expression of the disease. A major difference between these two forms of the disease is the degree of penetrance. As stated previously, only 1% of apoE2 homozygotes develop Type III HLP whereas the AD form is 100% penetrant. The chemical composition of the remnant particles that accumulate in the recessive and dominant forms of Type III HLP are indistinguishable,

but in the AD variant, the concentrations of the mutant apoE to normal apoE in the β-VLDL fraction are as high as 7:1 (66). This is in contrast to apoE2 heterozygote who do not have a disproportionately higher level of apoE2 in the remnant fraction.

Clues as to why some apoE defects are dominant and some are recessive can be gleaned by an analysis of the molecular defects in the apoE molecule. A 22 kDa thrombin fragment which contains the first 191 amino acids of apoE was crystallized (67). The domain consists of four antiparallel α helices. Helix 4 contains the LDL receptor binding region. The sequences necessary for LDL receptor binding are between amino acid residues 140 to 160 (68) [Figure 10]. Tandem arrays of a peptide including amino acids 141-155 can bind the LDL receptor with high affinity (69). Between amino acids 140-150, there is a cluster of six basic residues (i.e. lysine, arginine or histidine) and the crystallographic analysis revealed that all the positively charged side chains are exposed (67). These basic residues are thought to mediate the binding of apoE to both the LDL receptor and LRP by interacting with highly conserved acidic residues found in multiple copies within the ligand-binding domains of both receptors.

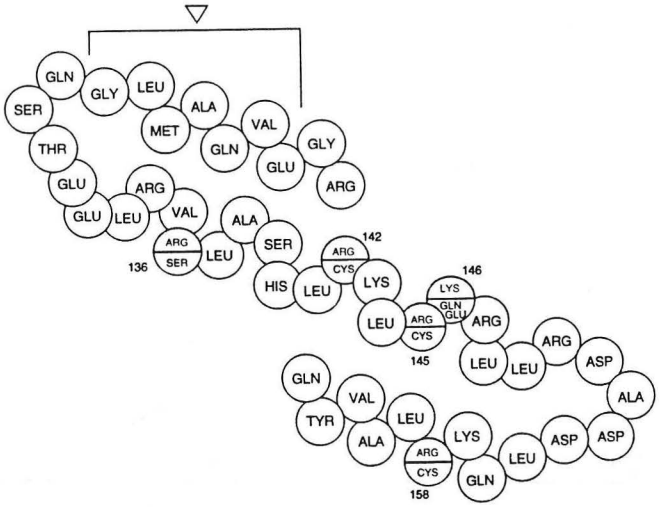


Figure 10. Amino acid sequence of LDL receptor binding domain of apoE. The site of naturally-occurring mutations which result in single amino acid substitutions are given. ApoE-Leiden has a seven amino acid duplication which is demarcated by a bracket.

Most of the naturally-occurring mutations the cause the autosomal dominant form of Type III HLP are located in the LDL binding domain and result in a substitution of a neutral for a basic amino acid [Figure 17]. Some of the mutations, such as apoE $_{\text{Leiden}}$ (which is due to a duplication of amino acids 121-127), are located outside of the binding domain and only indirectly disrupt the folding of this region (70).

Surprisingly, even though most of these AD apoE mutations are located within the ligand binding domain, they do not impair LDL receptor binding as much as apoE2. The results of binding competition studies using cultured fibroblasts are shown in Figure 18. Increasing concentrations of unlabeled apoE/phospholipid complexes from ε3/ε3 (O), ε2/ε2 (A.G. and D.R.; Figure 11), and AD Type III subjects were incubated in the presence of radiolabeled LDL. Some of the mutants apoE's were able to compete as well as apoE3 for receptor binding (M.McC and E.S.), whereas others (J.T.) were only slightly less competitive (71). In another series of experiments, pre-βVLDL (which consists of chylomicron remnants and nascent VLDL particles) and β-VLDL were isolated from individuals with both the AD and the recessive form of Type III HLP (72). The particles were tested *in vitro* for ability to bind LDL receptors on cultured cells. The pre-β-VLDL from both groups were defective in LDL receptor binding. As expected, the β-VLDL from ε2/ε2 individuals were also defective in receptor binding. The unexpected finding was that the β-VLDL from the AD Type III HLP patients bound to receptors with an increased affinity (72). This observation differs the results of the only *in vivo* turnover study that has been performed in subjects with AD Type III HLP. In that study, remnants were cleared at a reduced rate (73).

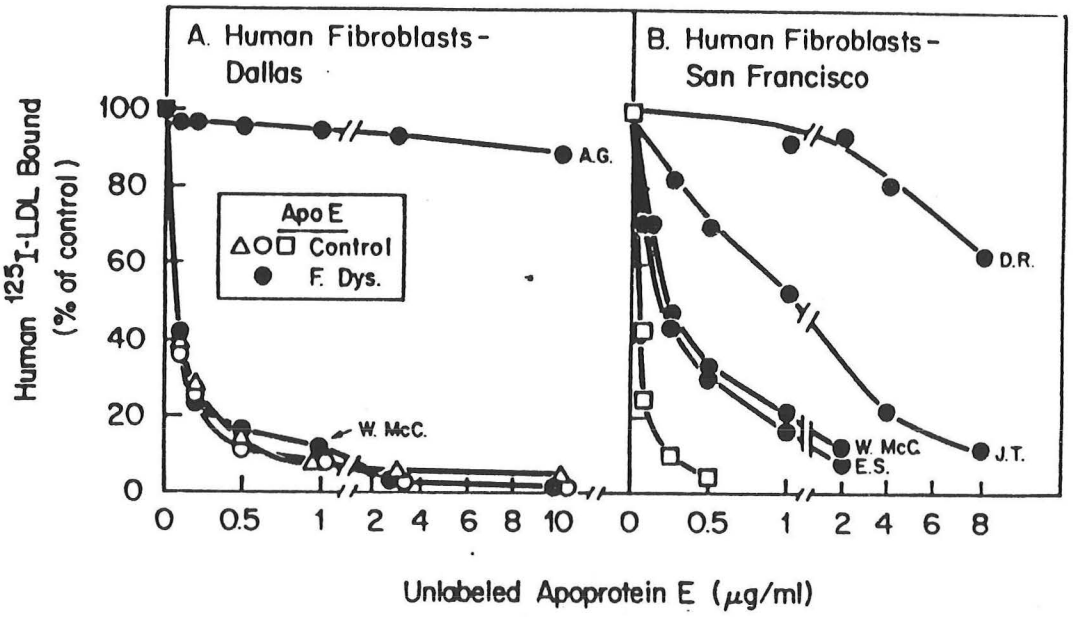


Figure 11. Ability if apoE/phospholipid complexes to compete with LDL for binding to human fibroblasts (71).

Thus, the mechanisms responsible for the development of Type III in the autosomal recessive and dominant forms of Type III HLP must differ. In both forms of hyperlipidemia, there is an increase in the production of β-VLDL due to a decrease in the clearance of pre-βVLDL particles. In the autosomal recessive form, the development

of Type III HLP occurs when there are additional physiological perturbations which exacerbate the defect in hepatic lipoprotein receptor binding. The reason for the lower penetrance of Type III HLP in ε2/ε2 homozygotes appear to reflect a greater structural flexibility and sensitivity of this variant to the physicochemical milieu.

The reason why the other forms of Type III are autosomal dominant is likely due to a combination of factors. First, many of the mutations responsible for AD Type III have an arginine at position 112, so a higher proportion of the mutant apoE is associated with the VLDL fraction. Within the remnant fraction, the abnormal apoE proteins outnumber the normal apoE's by as much as 7 to 1. But this does not explain completely why the remnant particles are cleared from the circulation at a much slower rate. A possible clue comes from recent studies which report that the AD apoE's are defective in heparin binding (74). The same region in apoE which mediates LDL receptor binding also contains a heparin binding site (5). When apoE-Leiden was expressed in cultured hepatoma cells, it failed to associate with the cell surface or to augment the uptake of β-VLDL (74). As discussed previously, the efficient uptake of large remnant particles by the liver may require binding to cell-surface heparan sulfate proteoglycans and supplementation with apoE (16). These studies would support *in vitro* evidence that heparin binding plays an important role in the removal of the larger remnant particles from the plasma [Figure 12]

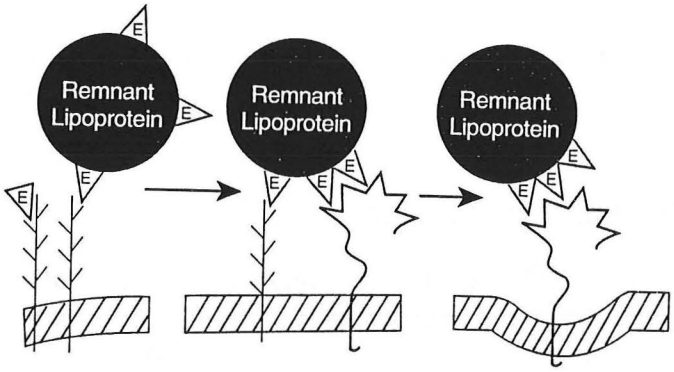


Figure 12. Proposed model suggesting the role of heparan sulfate proteoglycans and LRP in remnant binding and internaliztion.

ApoE DEFICIENCY IN MICE AND MEN

Three pedigrees have been described in which the probands have early onset Type III HPL and little to no detectable plasma apoE (75-77). The parents of the probands, who are obligate heterozygotes for the mutant apoE allele, have normal lipid levels. Thus, apoE deficiency is an autosomal recessive disorder.

All the apoE deficient individuals have markedly elevated plasma levels of cholesterol and β-VLDL, as would be expected. However, unlike the other forms of Type III, these individuals have a normal or only modest elevation in plasma TG so the ratio of VLDL-C to plasma triglyceride levels is much higher (~ 0.90) then in either classical or AD Type III. The affected individuals have massive tuberoeruptive xanthomas (in some cases including involvement of the pinna of the ear) and accelerated atherosclerosis (75). Interestingly, in one patient who was treated with a low fat diet and clofibrate, the xanthomas completely resolved despite there being only a modest reduction in the plasma cholesterol level (376 mg/dl to 319 mg/dl) and an increase in the triglyceride levels (75).

Two groups have successfully inactivated the apoE gene in mice (78,79). Both groups of mice have a phenotype which resembles that of apoE-deficient humans. The plasma levels of cholesterol are dramatically increased (494 mg/dl vs. 60 mg/dl in controls) but there is no significant increase in plasma concentrations of TG. The normal levels of triglyceride in both the apoE deficient humans and mice suggest that the presence of apoE must in some way inhibit the lipolysis of lipoprotein particles. When the mice were placed on a Western-type diet, the plasma cholesterol level increased to 1821 mg/dl in the apoE deficient mice versus 132 mg/dl in the control mice. All of the increase in plasma cholesterol was in the VLDL/IDL fraction. Of most importance, after 3-4 months on a regular chow diet, the mice developed large advanced atherosclerotic lesions (replete with fibrous plaques) in the proximal aorta, pulmonary and coronary arteries (78-80).

The development of this mouse model has provided a dramatic reminder of the importance of apoE in lipid homeostasis and provides an important system in which to study atherosclerosis.

EXTRAHEPATIC SYNTHESIS OF ApoE

As alluded to previously, apoE is produced by many different cell types in the body. The exact role of apoE in these other tissues has not been fully elucidated but apoE may participate in the distribution and transport of lipids within tissues. In the adrenal gland, apoE expression is regulated by ACTH and may influence steroidogenesis (21). In the ovary, apoE-rich HDL has been shown to inhibit ovarian androgen synthesis (22). ApoE has also been implicated in playing a role in immune regulation (81). For some tissues, the sequences responsible for tissue-specific expression of apoE have been pinpointed. The sequences which control hepatic-specific expression of apoE are not located in the 5′ flanking sequences but rather 18 kb 3′ of the *APOE* gene (82).

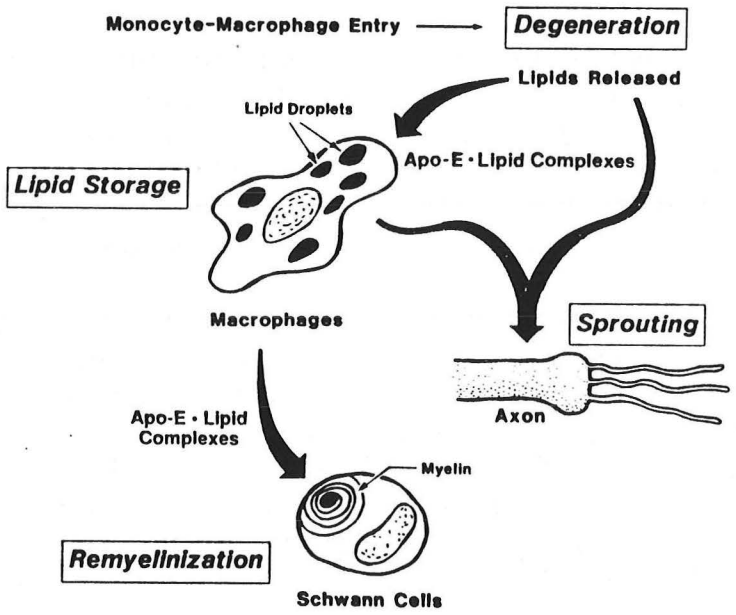
The highest concentration of apoE mRNA in the body is in the liver and the second most abundant tissue is the brain (18). ApoE, as well as apoAI, is found in the cerebrospinal fluid (CSF) where the two apolipoproteins circulate on different populations of small spherical particles (83). Unlike apoE, the ApoAI is not produced

in the brain but is derived from plasma. In brain tissue, astrocytes are the major cell type which express and secrete apoE (84). Neurons, microglia, oligodendroglia, ependymal and choroidal cells do not normally produce apoE. The apoE is seen within the astrocytic processes which interact with the basement membrane that coats the brain and blood vessels. Astrocytes play a supportive role to other tissues in the nervous system and they maintain the proper extracellular milieu by taking up ions, small metabolites and neurotransmitters. They also contribute to the extracellular matrix.

In the peripheral nervous system, apoE is found in some glial cells, satellite cells and enteric glial cells as well as non-myelinating Schwann cells (84).

ApoE has been implicated in playing an important role in the regeneration of injured nerves [Figure 13, from ref 106] (85-90). The level of apoE increases over a 100-200 fold range within a week after injury to the rat sciatic nerve, and does not return to baseline until the nerve has completely regenerated. It has been estimated that apoE comprises ~3% of all extracellular proteins in the vicinity of the injured nerve (85). The apoE is made and secreted by macrophages which are recruited to the lesion to scavenge the cholesterol and other lipids released by the degenerating nervous tissue. It has been proposed that the apoE-lipid complexes are then delivered to the regenerating nerve and Schwann cell. Both the axon and the Schwann cell upregulate their LDL receptors after nerve injury. Thus, it is likely that apoE participates in the uptake and storage of lipids, and then in their delivery to regenerating neural tissues for reutilization. ApoE may also play a role in modulating neuronal growth (90).

Figure 13



Crush injury to the rat optic nerve results in a dramatic increase in the secretion of apoE by surrounding glial cells. However, in the central nervous system, unlike the peripheral nervous system, the concentrations of apoE do not increase and the axons never regenerate (85, 86).

ApoE is not the only apolipoprotein that accumulates at the site of nerve injury. ApoD increases in concentration by 500 fold, whereas apoAI and AIV both increase by 15-25 fold (89). ApoD circulates in human plasma as part of HDL and is a member of the α2-microglobin family, which includes other protein that transport small hydrophobic molecules like retinol or odorants. The apoD is produced locally by both astrocytes and to a lesser extent by oligodendrocytes.

These studies suggest that apoE plays a very important role in the health maintenance of nervous tissue and in its regeneration. However, a potent argument against this scenario is that both humans and mice that lack apoE have no neurological deficits and no evidence of any impairment in the ability to regenerate nerves (91) (Nor do they have any endocrine or immune deficiencies). Presumably, other apolipoproteins, such as apoAI, and perhaps apoD, can substitute for apoE in its absence.

ApoE AND ALZHEIMER'S DISEASE

A number of observations by different investigators, including neurobiologists, geneticists, and biochemists, has contributed to the discovery of a link between apoE and Alzheimer's disease.

Alzheimer's disease is the most common cause of dementia. At least 1 out of every 20 individuals over the age of 65 suffers from this disorder. The disease is characterized by an early loss of recent memory with difficulties in reasoning, judgement and insight. Over a 5-12 year period, the affected individual has increasing difficulty in performing simple tasks. The pathological hallmarks of Alzheimer's disease are 1) senile plaques which consist of amyloid deposits encircled by dystrophic neurons 2) amyloid deposition in the leptomeningeal vessels ("Congophilic angiopathy") 3) intracellular neurofibrillary tangles and 4) the loss of neurons and synapses.

The backbone of the Alzheimer senile plaque is comprised of a ~43 amino acid peptide, the βA4 peptide, which is derived from a transmembrane protein called amyloid precursor protein (APP). APP is found in high concentrations at neuronal synapses. The protein contains an internalization signal but its physiological ligand remains to be found. The relationship of βA4 to the full length protein sequence is shown in [Figure 14] (92). APP has several isoforms due to alternative splicing and also undergoes a series of processing events which are mediated by both lysosomal and cell surface enzymes. Normally, APP is cleaved by a cell surface enzyme, secretase, which clips within the ßA4 peptide region which releases a 10 kDa fragment. This is a peculiar processing event since the site of cleavage is within the membrane segment. The

mechanism by which the βA4 peptide is generated from APP remains to be elucidated the peptide but is not specific for Alzheimer's disease. The peptide is also produced in the normal brain, but is present in very low concentrations.

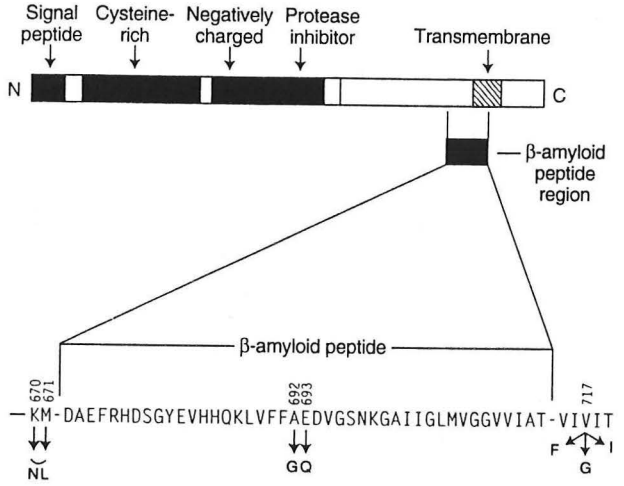


Figure 14. Schematic diagram of APP and THE LOCATION OF THE βA4 peptide. The amino acid substitutions which have been identified in families with early onset Alzheimer's disease are given (92).

The disease can be classified into five major categories based on its mode of inheritance, typical age of onset, and known molecular defects:

DOWN'S SYNDROME- Individuals with Down's Syndrome invariably develop Alzheimer's disease in their 50's to 60's. The APP gene is on chromosome 21, so these individuals have 3 copies of the gene and consequently synthesizes βA4 at an increased rate.

EARLY-ONSET (<60 YEARS), AUTOSOMAL DOMINANT. A minority of these patients have mutations in the gene encoding APP. A total of 6 mutations in the APP gene have been molecularly characterized and found to cosegregate with Alzheimer's disease in families (for review, ref. 92). Interestingly, most of these mutations are located outside the βA4 peptide region. It has been proposed that the mutations may interfere with the proper processing of AP, and thus promote the release of the βA4 peptide. One of two mutations located within the βA4 peptide region (Glu_{693} to Gln) does not cause typical Alzheimer's disease, but rather the Dutch form of hereditary cerebral hemorrhage with amyloidosis (93).

In another group of families with an early-onset, autosomal dominant phenotype,

the disease is linked to chromosome 14 (94). The identification of the mutant gene responsible for this form of Alzheimer's disease is still pending.

EARLY-ONSET, SPORADIC- The molecular defects responsible for this form of the disease are not known. Presumably some of these individuals have somatic or germline mutations.

LATE-ONSET, SPORADIC- The *APOE* gene has been implicated in this form of the disease, but is only one of probably many factors, both genetic and environmental, that contribute to this disease.

LATE-ONSET, AUTOSOMAL DOMINANT - In 1991, the disease was shown to be "loosely" linked to 19q13.1-q13.3, a genomic region which includes the *APOE* locus, in a subset of the families with late-onset disease (95).

Independent of this linkage data, there are pathological studies which point to apoE as a possible candidate gene. Immunoreactive apoE has been noted to be present in the blood vessels, neurons and neurofibrillary tangles in Alzheimer's patients (96). But this finding was not specific for Alzheimer's disease; ApoE was also associated with the prion amyloid deposits of Creutzfeld-Jacobs disease. An observation that sparked more interest was made by Wisniewski et al. who showed that even the pre-amyloid βA4 fibrils found in the brains of Down's Syndrome patients stained with apoE (97). He proposed that apoE may act as a "pathological molecular chaperone" and contribute to the actual formation of the β-pleated amyloid fibrils (97). Though the term chaperone is a misnomer (since this desidnation has been given to a specific class of proteins that bind to nascent intracellular proteins), the idea that apoE, or perhaps one of the other amyloid-associated proteins, might play a role in amyloid formation, focused a number of laboratories on the the identification of the company amyloid keeps.

In the effort to identify other possible potential βA4-binding proteins, Strittmatter and his colleagues found that apoE, a contaminant of their protein preparation, bound to βA4 with high affinity (98). Then they immobilized βA4 on a filter and incubated it with human CSF. ApoE bound the peptide with high affinity and could not be dissociated using either detergents or denaturing agents. Then they tested to see if there was any differences in the binding characteristics of apoE3 and apoE4. Both isoforms bound tightly to soluble βA4 peptides, but apoE4 was noted to bind more rapidly than apoE2 (5 min vs. 2 h) (99). By using thrombin fragments of apoE, the binding was localized to the C-terminal region of apoE, (amino acids 244-272), the same region which mediates the binding of apoE to lipoproteins.

Thus, there was pathological, biochemical, and linkage data which suggested a possible role of apoE, and particularly apoE4, in the pathogenesis of Alzheimer's disease. Next, a series of association studies were performed which served to strength the linkage between apoE4 and Alzheimer's disease.

First, it was reported that there was a significantly higher frequency of ε4 in 30 unrelated families with late-onset Alzheimer's disease than in 91 age-matched unrelated controls (0.5 vs 0.16) [Table 6] (98). There was also a significantly higher frequency of ε4 (0.4 vs 0.16) in a sample of patients with the sporadic form of late-onset Alzheimer disease (100). Taken together, it was estimated that ~80% of the late-onset familial and 64% of the late-onset sporadic cases had at least one ε4 allele, in contrast to 31% of the controls.

Table 6. The frequency of APOε alleles in 83 patients from 30 families with late onset familial Alzheimer's disease (FAD). n= number of chromosomes.

		Controls	
Allele	FAD		
ε2	0.04	0.10	0.08
ε3	0.44	0.73	0.78
ε4	0.52	0.16	0.14
	(*n* = 166)	(*n* = 182)	(*n* = 2000)

Importantly, the association of the ε4 allele with Alzheimer's disease was shown to be specific. There was not a higher frequency of ε4 in patients with amyloidosis formed by proteins other than βA4 (101). Nor was there an enrichment for ε4 in the early-onset types of Alzheimer's disease, Down's Syndrome, or in the subset of individuals with defined mutations in APP (102).

There is also pathological data which links the ε4 allele to Alzheimer's disease. Pathological quantification of the amount of βA4 and apoE staining in the cerebral cortex of late-onset Alzheimer's patients revealed significantly more amyloid deposition and apoE staining in the brains of the ε4/ε4 Alzheimer patients (103). However, there was no significant difference in the number of neurofibrillary tangles between the two groups. (This is important because of recent unpublished claims that apoE3 may protect against the formation of neurofibrillary tangles).

The ε4 allele appears to have a gene dosage effect on the expression of Alzheimer's disease. Analysis of 42 late-onset families with known ε4 alleles, revealed that only 20% of the ε2/ε3 or ε3/ε3 individuals in the families developed Alzheimer's, whereas 47% of the ε2/ε4 or ε2/ε4, and 91% of E4/E4 developed the disease (102) [Figure 15]. Thus, in these families, the inheritance of one ε4 allele conferred a 2.84-fold

increased risk and two ε4 alleles an 8-fold increase in the likelihood of developing Alzheimer's disease.

APOE Genotype	% Affected
2/2	–
2/3	18.8
3/3	20.8
2/4	20.0
3/4	47.8
4/4	91.3
Total	40.6

Corder et al., Science, 261:922

Figure 15. Frequency of APOε genotypes in members of 42 late-onset families with Alzheimer's disease (102).

In this set of families, the ε4/ε4 homozygotes tended to have a more severe disease than the ε4 heterozygotes which was reflected in an earlier age of onset of symptoms. In the ε4/ε4 homozygotes, the mean age of onset was 68.4 years and in the ε4 heterozygotes the onset was 75.5 years. These were both significantly earlier then the age of onset in the non-ε4/ε4 family members (84.4 years) [Figure 16].

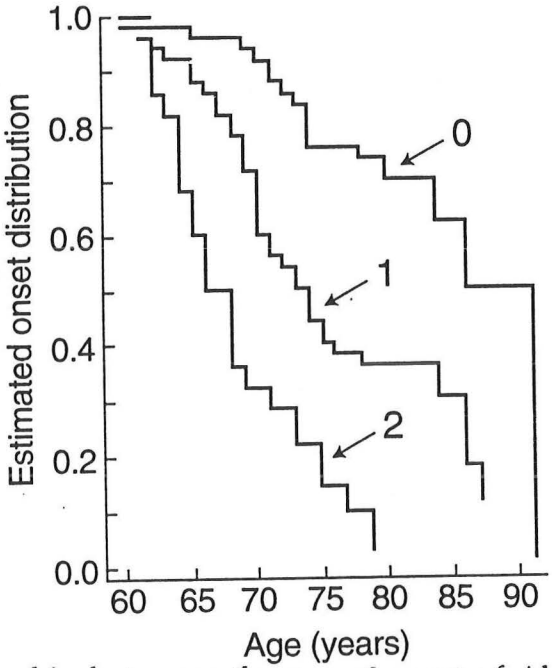


Figure 16. The relationship betweem the age of onset of Alzheimer's disease and the number of ε4 alleles (102).

Not only did the ε4/ε4 homozygotes develop the disease at an earlier age, but they also had a decreased lifespan. In individuals with one or two ε4 alleles the mean survival was 78 years and in those with no ε4 allele, it was almost eleven years longer (84.3 years).

Exactly how much the apoε4 allele increases the risk of Alzheimer's disease in the general population can not be estimated from these studies. It would be of interest to compare the prevalence of Alzheimer's disease in populations which have either very high (Bushman - 0.36) or a very low (Chinese - 0.05) frequency of the ε4 allele to see if there is a correlation with disease prevalence, as has been proposed (104) [Figure 17].

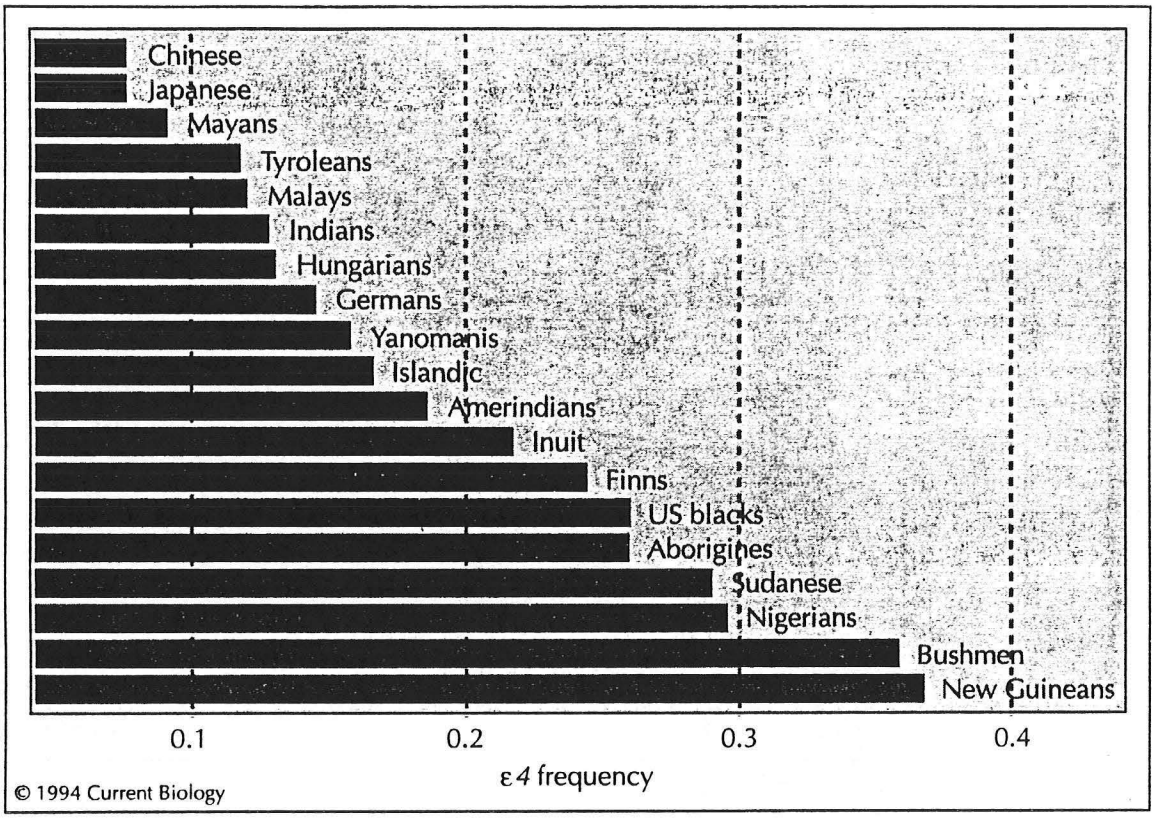


Figure 17. The frequency distribution of apoε alleles in different populations (104).

Despite the strength of the association between the ε4 allele and late-onset Alzheimer's disease, there are important exceptions. In one study, nineteen of the 95 family members who had well-documented late-onset Alzheimer's disease did not have a single ε4 (98). In the other family study, 12 of the 42 late-onset disease families had affected individuals who did not have an ε4 allele (102). Thus, it is clear that genes other than apoE, as well as yet-to-be identified factors play important etiological roles in the pathogenesis of Alzheimer's disease.

WHY IS THERE A HIGHER FREQUENCY OF ε4 IN ALZHEIMER'S DISEASE?

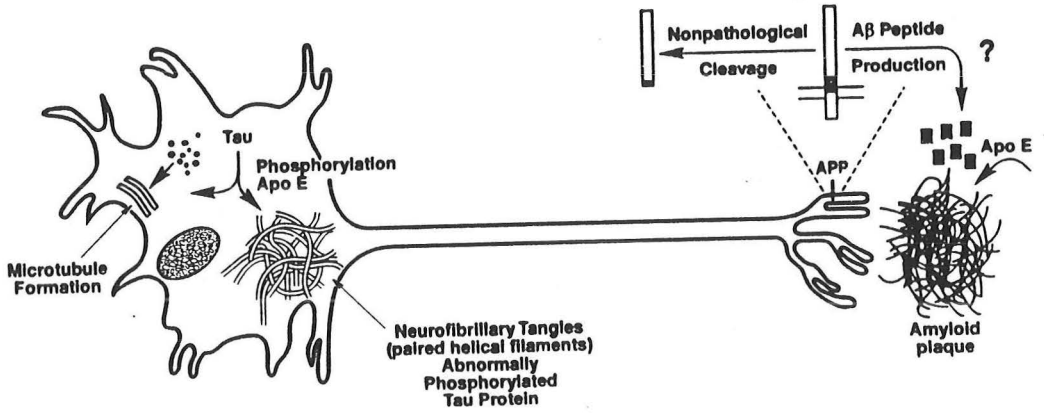
The association between the ε4 allele and Alzheimer's disease may be due to linkage disequilibrium with the gene that is actually responsible for Alzheimer's disease. However, this appears increasingly unlikely given that the association is not limited to familial cases and has been described in different populations (104).

The two leading hypotheses as to how apoε4 and Alzheimer's are causally linked are 1) that apoE4 promotes the deposition of ßA4-amyloid and 2) that it is not the presences of apoE4, but rather the absence of apoE3 that is causative [Figure 18, from reference 106].

Both the biochemical and pathological data support the former hypothesis; ApoE4 binds tightly to ßA4 and is associated with an increase in the deposition of ßA4 in the brain. The observation that apoE associates with soluble βA4 fibers and not just the amyloid deposits suggests that perhaps the binding of apoE4 initiates a nucleation event that precipitates the formation of amyloid.

Recently, it has also been proposed that apoE3 may stabilize the tau protein inhibit the formation of neurofibrillary tangles. Highly phosphorylated tau proteins are a major protein component of the neurofibrillary tangles . ApoE3 has been shown to bind to the tau protein with higher affinity than apoE4. It has been proposed that if apoE3 binds to the tau protein, the protein is protected from phosphorylation so does not form neurofibrillary tangles. The main problem with this hypothesis is that apoE is a secreted protein and is not present in the cytoplasm of the normal cell. It is difficult to imagine that apoE3 has an important physiological role in a cellular compartment in which it cannot be detected.

Figure 18



CONCLUSIONS

ApoE has risen in esteem over the last 20 years after a rather ignoble entrance onto the scientific stage. It was originally given a singularly undistinguished name - the "arginine-rich" apolipoprotein. Its importance in lipoprotein metabolism was readily appreciated by lipidologists, but not by the general medical community. The association of particular isoforms with Type III HLP and atherosclerosis broadened interest in the apolipoprotein. The new-found connection between apoE and Alzheimer's disease has finally given this versatile, ubiquitous, multifunctional protein the attention it deserves.

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