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APOLIPOPROTEIN E AND THE APOLIPOPROTEIN E–DEFICIENT MOUSE

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Abstract

Apolipoprotein E (apoE) is one of several lipoprotein transfer genes. A primary function of this protein is the mediation of receptor-mediated lipoprotein removal from the blood. Several studies have demonstrated that genetic variation at the apoE locus is associated with an increased risk of developing atherosclerosis, and recent studies implicate this same genetic variation in determining susceptibility to Alzheimer's disease. An apoE-deficient mouse has been created to further understand the role of apoE in these areas. This review briefly discusses the biological and clinical importance of this protein and describes the early experiments performed in the apoE-deficient mouse.

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INTRODUCTION

The mouse has become one of the most powerful investigatory tools of molecular biologists and geneticists. For more than a decade, transgenic mice have been used to study the consequences of overexpression of countless genes. More recently, through gene targeting in embryonic stem (ES) cells, scientific investigators have been able to create mice devoid of any desired gene. These techniques have become indispensable for studying gene function because they enable us to dissect the genetics, pathophysiology, and therapy of human disease. Over the past 6–8 years, the mouse has replaced the rat as the organism of choice for lipoprotein biologists. Recently, a mouse deficient in apolipoprotein E (apoE) was created by gene targeting in ES cells (74, 78, 109). This animal develops severe hypercholesterolemia and atherosclerosis. It is the first small animal model for atherosclerotic heart disease and has already been used to demonstrate the importance of high-density lipoprotein (HDL) in preventing atherosclerosis. The apoE-deficient mouse exemplifies the power of mouse genetics and is the focus of this review.

apoE

A thorough understanding of the apoE-deficient mouse requires a working knowledge of apoE, a protein that has been extensively studied for 25 years. Investigators first described apoE as a lipoprotein constituent in 1973 (89). It is a member of the evolutionarily conserved apolipoprotein multigene family that includes apoA-I, apoA-II, apoA-IV, apoC-I, apoC-II, apoC-III, and the apoC-I pseudogene (8, 9, 52). Human apoE is composed of 317 amino acids, with an 18–amino acid signal peptide sequence that when cleaved generates the 299–amino acid mature apoE found in plasma. It is highly glycosylated and is found in plasma at 3–5 mg/dl, mainly as a 34-kDa protein, although its size varies with the level of glycosylation. Additionally, apoE is the primary ligand for low-density lipoprotein (LDL) receptor–mediated removal of lipoprotein remnants from the circulation. The LDL receptor–binding domain of the protein is located in an arginine- and lysine-rich segment between amino acids 136 and 150. A number of natural and synthetic mutations, together with recent structural information, have established this region as the receptor-binding domain and have demonstrated its crucial role in lipoprotein metabolism (56). The human apoE gene was cloned in 1985 (21, 71). The 3.6-kb gene is located on human chromosome 19 and contains 4 exons, with the leader sequence in the first exon, the translation start and signal peptide in the second exon, and most of the structural codons in the third and fourth exons. The cDNA contains 1163 base pairs (bp).

apoE in Lipoprotein Metabolism

Investigators have implicated apoE in the three lipoprotein metabolic pathways: dietary fat transport, endogenous fat transport, and reverse cholesterol transport (36). This protein acts as a ligand for removal of chylomicron remnant and intermediate density lipoprotein (IDL) particles from the circulation and may also act as a component of the reverse cholesterol transport pathway. A role for apoE in reverse cholesterol transport at the level of both the peripheral cell and the liver has been postulated. Although the extent of its involvement in reverse cholesterol transport remains to be determined, in its primary function as a ligand for the receptor-mediated removal of chylomicron remnant and IDL particles from the circulation, apoE is recognized by two distinct membrane-bound receptors, the LDL receptor and the chylomicron remnant receptor [probably the LDL receptor–related protein (LRP)]. Recent studies in the apoE-deficient and the LDL receptor–deficient mouse have demonstrated unequivocally that apoE is recognized by two distinct receptors (44). This protein is the most potent known physiological ligand for the LDL receptor (43). Lipoprotein particles containing apoB100, the other physiological ligand for the LDL receptor, bind at only 5% of the affinity of those that contain apoE. Larger, apoE–enriched lipoprotein particles, such as very low-density lipoprotein (VLDL) and β-VLDL, are cleared by the LDL receptor (31, 104), and smaller chylomicron remnant and IDL particles, also enriched in apoE, can be cleared either by the LDL receptor or by the chylomicron remnant receptor, which also recognizes apoE (41, 57).

In vivo and in vitro studies have clearly defined the role of apoE in the receptor-mediated uptake of remnant particles. Less conclusive evidence suggests that apoE is involved in reverse cholesterol transport as well. Two areas of interest in this regard are the role of apoE in the efflux of cholesterol from peripheral cells to acceptor molecules in the plasma and the function of this protein in the eventual uptake of cholesterol esters by the liver (i.e. the initial and final stages of reverse cholesterol transport). In the first phase of reverse cholesterol transport, cholesterol is secreted from cells such as the macrophage that are located in the arterial subintima to HDL in the plasma. The acquisition of secreted tissue cholesterol by HDL is facilitated by apoE, and studies conducted over the past several years have further implicated apoE in the efflux of cholesterol from the arterial macrophages (91). Other studies, however, suggest that apoE is not directly involved in this process (6). Although apoE levels and rate of secretion increase severalfold in cholesterol-loaded macrophages (5, 61), studies in tissue culture have yielded conflicting evidence as to whether apoE and cholesterol secretion from macrophages occur in a coordinated fashion.

Although the role of apoE in the acquisition of tissue cholesterol by HDL

is uncertain, apoE can assist in the delivery of tissue cholesterol to the liver. Once in HDL, cholesterol derived from tissue is esterified by the enzyme lecithin:cholesterol acyltransferase (LCAT) and delivered to the liver via one of three pathways: (*a*) cholesterol ester transfer protein (CETP)–mediated transfer to VLDL, IDL, and LDL followed by LDL receptor uptake of IDL and LDL; (*b*) particulate uptake of the HDL particle; or (*c*) selective uptake of HDL cholesterol ester (25). In the pathway in which the CETP-mediated exchange of cholesterol ester from HDL to apoB-containing particles takes place, apoE acts as the primary ligand in the removal of apoB-containing lipoproteins (14, 102). It may also serve some function in the selective and particulate uptake pathways. Several studies have indicated that in the selective uptake pathway, apoE is required to approximate the HDL particle with the hepatocyte so that cholesterol ester can diffuse from HDL to the hepatocyte (32, 33). By interacting specifically with receptors and nonspecifically with extracellular matrix heparan sulfate, apoE may bridge HDL and the hepatocyte and thus allow exchange of cholesterol ester. In the particulate uptake pathway, HDL as a whole is thought to be internalized by the hepatocyte (32, 33), and apoE may mediate this process through specific binding to known hepatic receptors such as the LDL receptor or through an unknown HDL receptor.

The biology of apoE is complicated by the fact that expression is not limited to the intestine and liver as is the case for most other apolipoprotein genes. Although the majority of plasma apoE is derived from liver [liver transplant studies in humans in which apoE isoforms can be traced suggest that 90–95% of plasma apoE is of hepatic origin (48)], most differentiated tissues appear to express at least low levels of apoE (24, 26, 57). In addition to its well-established function in plasma, apoE has been postulated to act in several cells, such as the macrophage, the astrocyte, the cortical cell of the adrenal gland, the Leydig cell of the testis, the granulosa cell of the ovary, and the T cell (57). Because apoE is widely expressed and can scavenge cholesterol from cells, investigators have made a concerted effort to pinpoint a role for this protein in the local redistribution of cholesterol. Much of the existing evidence for this paracrine-type function is indirect, and no convincing in vivo evidence exists to suggest a necessary or partial role for apoE in local cholesterol redistribution.

Clinical Importance of apoE

From a clinical perspective, the apoE gene has been implicated in a growing number of processes. Genetic studies of apoE point to causal relationships between several clinical conditions and apoE structural variants and deficiency. Population studies further support an association between a common apoE allelic variation and both atherosclerosis and Alzheimer's disease.

In three clinical situations, apoE is involved in the development of dys-

Table 1 Common genetic variation in apo E

Designation	Protein change	Allele frequency[a]
E2	Cys^{112}, Cys^{158}	4–13%
E3	Cys^{112}, Arg^{158}	73–85%
E4	Arg^{112}, Arg^{158}	14–23%

[a] Frequency depends on country and study (9, 56).

lipidemia and atherosclerosis: a common genetic variation; a less common disease, type III hyperlipoproteinemia; and the rare apoE deficiency syndrome. In the general population, apoE exists as three major structural isoforms: E2, E3, and E4 (58, 106) (Table 1). Each variant differs from the next by a 1–amino acid change at residue 112 or 158 of the full-length protein. E2 is cys112, cys158 and has an allele frequency of 4–13% (depending on the population studied); E3 is cys112, arg158 with an allele frequency of 73–85%; and E4 is arg112, arg158 with an allele frequency of 14–23%.

Studies have suggested that the apoE phenotype may help explain the variability in plasma total cholesterol levels and in plasma LDL cholesterol levels observed in the general population (22). On average, individuals with the apoE4 allele have total cholesterol levels 20 mg/dl higher than those found in individuals with the apoE2 allele (22, 105). More importantly, postmortem studies have shown that coronary artery disease occurs to a significantly greater extent in individuals with the apoE4 isoform (39). The mechanism behind this variability is poorly understood but may reflect the relative affinity of the various alleles for the chylomicron remnant and LDL receptor. The affinity of the apoE alleles for these receptors is apoE4 = E3 > E2; apoE2 has <2% of the binding affinity for the LDL receptor of either apoE3 or E4 (101). The fact that individuals with apoE4 have higher plasma cholesterol levels than those with the apoE2 allele seems paradoxical. The allele with high receptor-binding affinity is associated with the highest levels of plasma cholesterol, whereas the allele with the lowest affinity is associated with the lowest plasma cholesterol levels. To understand this apparent paradox, one must note that the majority of plasma cholesterol increase in the individuals with apoE4 is in the LDL fraction and not in the apoE–containing VLDL and IDL fractions. This phenomenon has three possible explanations. First, increased uptake of remnant and IDL particles may lead to increases in intracellular cholesterol levels in hepatocytes and subsequent downregulation of LDL receptor activity through a series of well-defined transcriptional and posttranscriptional mechanisms (34). Because LDL has a longer half-life than remnant particles, this downregulation in receptor activity can cause a cascade in which fasting LDL levels are affected but levels of the more rapidly cleared remnant particles are

not. Second, the apoE4 allele may allow more IDL to be converted to LDL than does the apoE2 allele. Third, the apoE4 allele may increase dietary cholesterol uptake either by direct regulation of intestinal absorption or by indirect effects, such as may occur with intracellular hepatic cholesterol stores that could promote increased absorption through increased bile production.

The second clinical scenario in which apoE plays an important role in determining susceptibility to atherosclerosis is type III hyperlipoproteinemia, also known as dysbetalipoproteinemia. Approximately 1 in 5000 individuals develops this disease, and those affected often do not exhibit signs or symptoms until adulthood (58). The disorder typically occurs in those individuals homozygous for the apoE2 allele (~1% of the population). A secondary environmental or genetic insult is necessary for expression of the disorder in these individuals, and only ~1 in 50 apoE2 homozygotes develops obvious clinical manifestations. The secondary factors are poorly understood. Certain diseases, such as diabetes or hypothyroidism, can predispose the apoE2 homozygote to type III hyperlipoproteinemia. In patients who do not have a precipitating disorder, a secondary genetic locus has been hypothesized but has yet to be identified.

Type III hyperlipoproteinemia is characterized by abnormal triglyceride and cholesterol metabolism. Individuals with the disorder typically have fasting plasma cholesterol levels between 300 and 600 mg/dl (58). Fasting triglyceride levels are often equal to or greater than plasma cholesterol levels. The disease is distinguished from other hyperlipidemias with fasting hypertriglyceridemia by an associated elevation in plasma VLDL cholesterol levels. A VLDL cholesterol:triglyceride ratio above 0.3 in the setting of hypercholesterolemia and hypertriglyceridemia is diagnostic. In addition to combined hyperlipidemia, premature and accelerated atherosclerosis as well as cholesterol deposition in histiocytes of the dermis develop in patients with type III disease, resulting in the formation of xanthomas. *Xanthoma striata palmaris* or palmar crease xanthomas are pathognomonic of type III hyperlipidemia.

The final clinical scenario in which apoE is involved in determining susceptibility to atherosclerosis is apoE deficiency. In four kindreds, a proband or a group of affected individuals with this rare disorder has been defined (30, 49, 54, 55, 84). The most extensively studied family was the first apoE-deficient kindred identified in 1983. Several follow-up studies in this family have addressed the molecular nature of the apoE deficiency (18, 107) and the metabolic abnormalities associated with this genotype (29, 84). The phenotype of the apoE-deficient patient is remotely similar to that of the type III hyperlipidemic patient. Deficiency of apoE is more severe in terms of cholesterol metabolism and less severe in terms of triglyceride metabolism. Moreover, the manifestations of cholesterol deposition in skin and artery appear more extensive. In apoE-deficient individuals, the VLDL cholesterol:triglyceride ratio can

be 1:1, threefold higher than the 1:3 ratio needed for a diagnosis of type III hyperlipoproteinemia. Compared with type III hyperlipidemics, apoE-deficient patients have slightly lower plasma triglyceride and slightly higher plasma cholesterol levels.

Recent evidence has further implicated genetic variation in the apoE gene in determining susceptibility to sporadic and late-onset Alzheimer's disease (20, 93). Several population studies have demonstrated that individuals with one copy of the apoE4 gene can have as much as a threefold increase in susceptibility to Alzheimer's disease. Individuals with two copies of this allele can have a sixfold increased risk of developing the disease. Although the exact mechanism by which the apoE4 allele predisposes to Alzheimer's disease is uncertain, evidence suggests that the genetic changes that distinguish apoE4 from apoE3 are not markers but causative mutations. Investigators initially thought that apoE4 was harmful. Although this scenario is possible, more recent speculation centers around a protective role for apoE3. In the latter scenario, the presence of apoE4 is harmful because it signifies less or no apoE3. The effect of allelic variation in the apoE gene on susceptibility to Alzheimer's disease might involve amyloid plaque and/or neurofibrillary tangle formation (85, 93–95). Recent biochemical studies indicate at least an association, if not a causation, between apoE and the formation of both these structures.

In two multifactorial diseases that preferentially afflict elderly people, apoE genetic variation is important in determining susceptibility. In Alzheimer's disease, a common allelic variation in the apoE gene has become the most potent predictor of disease in the general population. Genetic variability at the apoE locus can also account for significant variability in lipid metabolism and susceptibility to atherosclerosis. These clinical scenarios, together with a large body of experimental evidence, suggest that apoE is important in lipoprotein physiology and in determining susceptibility to disease. In the current studies, we used gene targeting to create apoE deficiency syndrome in the mouse. We reasoned that a mouse lacking apoE should resemble humans with apoE deficiency in that it should manifest severe lipid abnormalities and, possibly, susceptibility to atherosclerosis.

THE apoE-DEFICIENT MOUSE

The first of the lipoprotein transport genes to be disrupted by gene targeting in mice was apoE. This knockout mouse is also among the most extreme of the viable phenotypes observed in lipoprotein transport transgenic and gene knockout mice (for review see 10–12, 70). Investigators in two laboratories used gene targeting in ES cells to create two independent lines of apoE null mutants (2, 74). The apoE mutation segregated in a Mendelian fashion in each study, suggesting 100% viability of animals lacking apoE. Although some

humans have apoE deficiency, these individuals are few in number, and it is possible that in each of these families small amounts of apoE are produced. The observation that complete apoE deficiency is viable in mice was thus important in demonstrating the overall viability of an apoE-deficient organism.

Abnormalities in Lipoprotein Metabolism

As would be expected, the ability of apoE-deficient mice to clear plasma lipoproteins is severely impaired. When fed a low-fat chow diet that contains only 4% fat by weight and <0.01% cholesterol by weight, these animals exhibit plasma cholesterol levels of 500 mg/dl, whereas control mice have levels of 75 mg/dl (78, 109) (Table 2). This massive elevation in plasma cholesterol levels in apoE-deficient mice is primarily due to an increase in VLDL-sized particles. In control mice, 10–20 mg/dl of their plasma cholesterol is located in the VLDL fraction, whereas in apoE-deficient mice, >300 mg/dl of their plasma cholesterol is found in this density. The amount of cholesterol that appears in the IDL and LDL fractions is also elevated in these mice. Normal mice have no IDL and 5–10 mg/dl LDL, whereas apoE-deficient mice have combined IDL and LDL levels of >100 mg/dl.

The nature of the elevated VLDL, IDL, and LDL cholesterol levels in the apoE-deficient mouse is of interest from a metabolic perspective. The most exaggerated abnormality in this mouse is in the VLDL fraction, and it has been an area that we have actively studied (75). The predominant VLDL particle is an apoB48-containing, cholesterol ester–enriched sphere that appears to be a remnant of intestinal chylomicrons. It is similar in size to control VLDL (both

Table 2 Plasma cholesterol levels in apolipoprotein E–deficient mice and humans[a]

	TC (mg/dl)	VLDL-C (mg/dl)	HDL-C (mg/dl)
Mouse			
apo E +/+	100	20	65
apo E −/−	600	400	50
apo E −/−, hapo A-I	600	400	100
Human			
apo E +/+	200	15	50
apo E −/−	500	250	50

[a] Values in this table represent approximate total plasma cholesterol (TC), VLDL cholesterol (VLDL-C), and HDL cholesterol (HDL-C) levels in control, apo E–deficient, and apo E–deficient mice that overexpress a human apo (hapo)A–I transgene (77). Mice were fed a low-fat, chow diet. For comparison, approximate plasma cholesterol and lipoprotein levels are given for normal and apo E–deficient humans (84).

are ~50 nm spherical particles) but is distinct in its apolipoprotein and chemical composition. Compared with control particles, apoE-deficient VLDL is relatively enriched in free and esterified cholesterol and depleted in triglyceride. In addition, it has more apoA-I and apoA-IV on its surface. The apoE-deficient VLDL particle is similar to the β-VLDL particle found in the plasma of type III hyperlipoproteinemic and apoE-deficient humans. By definition, a β-VLDL particle has a cholesterol:triglyceride ratio of >0.3. In humans with type III hyperlipoproteinemia, the ratio is typically between 0.3 and 0.7. In apoE-deficient humans, however, this ratio is around 1.0. In apoE-deficient mice, the ratio is 3.0. The differences between the relative cholesterol and triglyceride contents of the type III hyperlipoproteinemic patient, the apoE-deficient patient, and the apoE-deficient mouse probably can be explained by the differences in relative roles of apoE in the human and mouse in mediating lipoprotein clearance.

Why do type III hyperlipoproteinemic humans have higher levels of triglyceride than apoE-deficient humans or mice? The obvious difference between these groups is the presence or absence of apoE, even though the apoE2 phenotype typically present in apoE-deficient humans is an isoform defective in binding to its receptors. Type III hyperlipoproteinemia is apparently characterized by a defect in clearance of the large apoB-containing particle, which accounts for the hypercholesterolemia. In addition, diminished hydrolysis accounts for the hypertriglyceridemia. Diminished particle clearance is probably secondary to the reduced affinity of both the LDL receptor (101) and, to a lesser extent, the LRP (47) for apoE2. The diminished lipolysis is apparently due to an inability of apoE2–containing particles to associate with lipoprotein lipase on the basement membrane of adipose and skeletal muscle capillary beds (46). In the case of apoE deficiency, receptor-mediated uptake is further diminished, but lipolysis is apparently less affected.

The second question is why such a significant difference exists between apoE-deficient humans and apoE-deficient mice. Plasma cholesterol levels in apoE-deficient mice fed a low-fat diet are slightly higher than those in humans who eat high-fat diets. This observation suggests that either small amounts of residual apoE are found in apoE-deficient humans (i.e. they are not true null mutants) or apoE is more involved in mediating the clearance of lipoproteins in mice than in humans. This latter hypothesis is consistent with the fact that mice have a greater percentage of apoB48 in their circulation. In humans, apoB48, a truncated form of apoB100 created by mRNA editing, acts as a structural protein in chylomicrons and chylomicron remnants (62). It is not produced by the liver. In rodents, apoB48 acts as a chylomicron structural protein as well but is also synthesized in the liver (16, 40, 79). It does not contain the carboxy-terminal LDL receptor–binding domain found in apoB100 (62). Particles that contain apoB48 as their primary structural protein require

apoE for receptor-mediated uptake. The mouse has a greater percentage of apoB48 in the plasma and a greater need for apoE–mediated receptor clearance. This finding may partially explain why hypercholesterolemia is more exaggerated in apoE-deficient mice than in apoE-deficient humans.

The presence of hepatic apoB mRNA editing does not fully explain the exaggerated defect in the apoE-deficient mouse. Several lines of indirect but strong evidence indicate that the VLDL particles are derived from the intestine and not the liver (75). Theoretically, apoE-deficient VLDL can originate from intestine, liver, or both. If it originates from the liver, a cholesterol ester–enriched particle can form in one of two ways. The first is by hepatic secretion of a normal 50-nm, triglyceride-rich particle that undergoes remodeling in plasma by CETP and/or LCAT. The second is via direct hepatic secretion of a cholesterol ester–enriched particle. The mouse does not express CETP (35), and apoE-deficient mouse plasma has very low levels of cholesterol ester transfer activity. VLDL cholesterol is a poor substrate for LCAT, and free cholesterol in apoE-deficient VLDL undergoes esterification at a very slow pace. The lack of CETP and the slow rate of esterification of VLDL free cholesterol by LCAT suggest that a nascent triglyceride-rich hepatic particle of 50 nm cannot become a cholesterol ester–rich particle of 50 nm. Hydrolysis of the triglyceride core of nascent hepatic-derived particles may account for the smaller cholesterol ester–rich particles such as the 20–35-nm particles found in the IDL and LDL fractions of apoE-deficient mice.

If CETP or LCAT does not account for the formation of the VLDL particles in these mice, the remaining possibilities are that a nascent cholesterol ester–enriched particle is secreted directly from the liver or that the particles are remnants of very large chylomicrons derived from the intestine. Primary hepatocyte cultures from control and apoE-deficient mice have been incubated in the presence of radiolabeled cholesterol and triglyceride precursors (75). Nascent VLDL particles collected from the medium of these cultures have been studied. The VLDL particles from control and apoE-deficient mice have a similar ratio of triglyceride to cholesterol. This observation suggests that livers from control and apoE-deficient mice secrete a similar triglyceride-rich particle.

Having excluded the liver, we turn to the intestine as the probable source of apoE-deficient VLDL. This source is consistent with the results of a vitamin A–fat tolerance test performed in these animals (78). Vitamin A (retinol) is normally absorbed in the intestine, esterified in intestinal epithelial cells, and packaged in chylomicrons (7). In the absence of plasma CETP, vitamin A cannot be removed from these intestinal particles until they are cleared from the circulation by the liver. After uptake in the liver, the retinyl esters are cleaved to retinol, which is either stored or secreted with retinol-binding protein. Retinyl esters are not repackaged in hepatic-derived lipoprotein par-

ticles. Vitamin A is thus a marker of intestinal absorption, chylomicron synthesis, and chylomicron remnant clearance. In apoE-deficient mice, clearance of vitamin A from the plasma after an enteral bolus is greatly diminished compared with that observed in control mice. Control mice reach peak plasma levels 2–4 h after a vitamin A load and after 12 h have no detectable plasma levels. Mice deficient in apoE have almost 20 times more vitamin A than control mice at peak, i.e. 2–4 h after bolus delivery, and at 12 h show no detectable reduction in plasma levels. Although this study cannot differentiate uptake from clearance and does not exclude the liver as a source, the results clearly indicate a defect in the metabolism of intestinal lipoprotein particles.

Dietary Responsiveness in the apoE-Deficient Mouse

When fed a low-fat diet of mouse chow, the apoE-deficient mice develop significant hypercholesterolemia. This outcome suggests that apoE deficiency in the absence of an environmental stimulus is sufficient to cause massive changes in lipoprotein metabolism. In addition to causing this independent genetic effect, the lack of apoE results in increased sensitivity to dietary fat and cholesterol. The best example of this increased sensitivity is the phenotype of apoE-deficient mice fed a Western-type diet (the origin of the diet is described in 64). This diet contains 21% fat by weight (1:13 polyunsaturates to saturates) and 0.15% cholesterol by weight. When control mice are fed this diet for several weeks, their total plasma cholesterol levels double. This effect results primarily from an increase in HDL cholesterol levels caused by a mechanism that increases translational efficiency of apoA-I (N Azrolan, H Odaka, EA Fisher & JL Breslow, submitted). In apoE-deficient mice fed this diet, a fourfold increase in total plasma cholesterol was observed (78). Most of this increase is due to an increase in VLDL cholesterol.

Heterozygous apoE-deficient mice do not exhibit elevated plasma cholesterol levels on the chow or Western-type diet, suggesting that when mice are fed a physiological diet, a 50% decrease in apoE is not sufficient to influence fasting plasma lipids. Although extensive studies examining heterozygous apoE-deficient mice in the postprandial state have not been performed, reduced apoE does appear to affect intestinal lipoprotein clearance, as indicated by an abnormal vitamin A–fat tolerance test. After a vitamin A bolus feed, all retinyl ester is cleared from the plasma within 12 h, but peak levels are two- to threefold higher than in control mice. Other studies similarly have shown that when fed an atherogenic diet that contains 1.0% cholesterol, 15% saturated fats, and 0.5% cholic acid, homozygous apoE-deficient mice have exaggerated levels of plasma cholesterol between 3000 and 4000 mg/dl (98, 108). The heterozygous apoE-deficient mice fed this diet also have elevated plasma cholesterol levels. The relevance of these studies to physiological diets is

uncertain, but they clearly demonstrate the extreme effect of partial or complete apoE deficiency in determining response to diet.

Studies in apoE transgenic mice also point to a role for apoE in determining plasma cholesterol levels in response to diet. Transgenic mice that express rat apoE at levels three- to fourfold higher than those observed in control mice have almost 50% lower fasting cholesterol levels (88). When transgenic mice were fed a diet containing 1.0% cholesterol, their plasma cholesterol levels rose from 55 to 94 mg/dl; in control mice fed the same diet, cholesterol levels rose from 95 to 194 mg/dl. In mice overexpressing apoE following high cholesterol intake, the absolute increase in plasma cholesterol was diminished relative to that in wild-type mice. In the mouse, extreme underproduction or overproduction of apoE can influence dietary responsiveness.

Although a large body of evidence suggests that apoE is involved in determining human dietary responsiveness, the precise function of this protein is not well established. Humans with type III hyperlipoproteinemia or apoE deficiency have a brisk hypocholesterolemic response to a low-fat diet. Allelic variation of the apoE gene can also lead to differences in plasma lipoprotein levels and in cholesterol absorption (reviewed in 2). Individuals with apoE4 have significantly higher plasma cholesterol levels, and individuals with apoE2 have significantly lower plasma cholesterol levels, than those with apoE3 (96). Whether these differences relate to differences in diet responsiveness is not entirely clear, but studies have shown that individuals with the E4 isoform absorb significantly more dietary cholesterol than individuals with the E2 isoform. These studies in humans and results from the apoE-deficient mice indicate a role for this gene in determining both basal and diet-induced plasma cholesterol levels.

ATHEROSCLEROSIS IN THE MOUSE

The mouse is the best mammalian system for the study of genetic contributions to disease. In this model, investigators can perform genetic manipulation using transgenic and gene-targeting technology. Other advantages are easy breeding, a short generation time, and the availability of inbred strains, many of which have interesting heritable phenotypes. Additionally, groups in the United States and England have created a refined genetic map of the mouse genome using polymorphic repeats of simple sequences that can be assessed relatively easily with polymerase chain reaction (PCR) (19, 23). These markers can be used to map genetic elements associated with specific phenotypes known as quantitative trait loci (QTL). If a phenotype is well-defined, the associated QTL can be mapped to <4 centimorgans on average.

Unfortunately, the mouse is highly resistant to atherosclerosis. The benefits of using the mouse as a system for studying complex genetic diseases have

Table 3 Mouse models of the lipoprotein disorders associated with coronary artery disease in humans

Lipoprotein pattern	Mouse	Reference
↑ LDL cholesterol	LDL receptor deficient	42
	apo B transgenic	15, 53
↓ HDL cholesterol and	apo A–I–deficient	51, 76, 103
↑ VLDL triglyceride	apo CIII transgenic	1, 45
	apo CI transgenic	90
	apo CII transgenic	87
	CETP transgenic	3, 59, 60
	apo A-I, CETP transgenic	38
	apo A–I, apo CIII, CETP transgenic	37
↑ IDL & chylomicron remnant cholesterol	apo E deficient	74, 78, 109
	apo $E_{3\text{-Leiden}}$ transgenic	97, 99
	apo $E_{4\text{-Arg142Cys}}$ transgenic	27, 28
↑ Apolipoprotein(a)	apo(a) transgenic	17
	apo(a), apo B transgenic	15, 53

nevertheless prompted substantial efforts to alter environment and genes in order to create an atherosclerosis-sensitive species. To date, these efforts have focused on altering the mouse's lipoprotein profile to create more atherogenic lipoprotein patterns (Table 3).

The mouse offers an array of desirable attributes for studying genetic diseases and has been used in the past as a model for atherogenesis. Early studies have taken advantage of the power of mouse genetics by using environmental insults to overcome the organism's resistance to atherosclerosis. When fed an atherogenic diet that contains 15% fat, 1.25% cholesterol, and 0.5% cholic acid, certain inbred mouse strains, such as C57BL/6, will develop atherogenic changes in the arterial intima of the proximal aorta (65, 67, 68). This diet contains 10–20 times the amount of cholesterol found in a typical human diet and an unnatural dietary constituent, cholic acid, that causes hepatotoxicity in mice when fed for long periods of time. When fed this diet, mice can exhibit four- to fivefold higher cholesterol levels than animals fed a low-fat chow diet. Plasma cholesterol levels can reach 200–300 mg/dl, with the majority of the increase in plasma cholesterol levels occurring in the non-HDL fractions. When fed this diet for 3–4 months, susceptible strains of mice will develop foam cell lesions at the base of the aorta in the region of the aortic valves. After extensive feeding, these mice may develop small fatty-streak lesions in the abdominal aorta. Crosses between resistant and susceptible mouse strains

have been used to identify three loci that can modify susceptibility to the diet-induced fatty-streak formation (66, 69, 92).

Transgenic techniques have been used extensively in the mouse model to assess the atherogenicity of many of the lipoprotein transport genes. Several studies have assessed the susceptibility of C57BL/6 mice that contain one or more of the various lipoprotein transport genes to diet-induced atherosclerosis. In the first of these studies, overexpression of a human apoA-I transgene, which can elevate HDL cholesterol levels, blocked atherogenesis in response to the atherogenic diet (83), suggesting that apoA-I expression with its attendant increase in HDL cholesterol levels can protect against atherogenesis. In a related study, apoA-I transgenic mice that coexpress human apoA-II had significantly less protection against this disease than apoA-I only mice (86) despite the fact that their HDL cholesterol levels were identical. In these animals, the difference in the level of protection lies not in the quantity, of HDL cholesterol but in the quality of the particle. In transgenic mice overexpressing both apoA-I and apoA-II, the predominant HDL particle contains both apoA-I and apoA-II, whereas in the apoA-I only mice, the predominant HDL is an apoA-I only–containing particle.

When overexpressed in mice that do not have a human apoA-I transgene, human apoA-II does not affect HDL cholesterol levels, a result consistent with reports in humans that apoA-II plasma levels do not correlate independently with HDL cholesterol levels (13). Other studies have examined the role of mouse apoA-II in susceptibility to atherosclerosis (100). The transgenic studies point to obvious species differences in the function of mouse and human apoA-II. For example, mice that overexpress mouse apoA-II have higher HDL cholesterol levels than mice overexpressing human apoA-II. Interestingly, the elevated HDL cholesterol levels in these mice do not protect against atherosclerosis and appear to predispose the mice to more severe atherosclerotic disease. The finding that HDL cholesterol levels alone are not sufficient to determine susceptibility to atherosclerosis was confirmed in apoA-I-deficient mice as well (51, 76, 103). These mice have 80% lower HDL cholesterol levels than control mice but do not develop atherosclerosis on a chow, high-fat, or high-cholesterol diet. The general conclusion from these studies is that HDL composition is as important as HDL cholesterol levels in determining susceptibility to atherosclerosis.

In addition to HDL cholesterol levels and HDL protein composition, other factors can determine the ability of HDL to influence susceptibility to atherosclerosis. The ratio of non-HDL to HDL cholesterol in humans is an excellent predictor of coronary artery disease. An altered ratio was created in a line of simian CETP transgenic mice made in the C57BL/6 inbred strain (60). CETP activity was almost 20-fold greater in these mice than in humans. With this high level of CETP activity, a reciprocal increase in non-HDL cholesterol

levels and a decrease in HDL cholesterol levels were observed. When challenged with the atherogenic high-cholesterol diet, these mice developed slightly more fatty streaks than control C57BL/6 animals. Of greatest interest in this study was the fact that the degree of atherosclerosis could be correlated with the ratio of non-HDL to HDL cholesterol.

The observation that increased non-HDL cholesterol levels can increase susceptibility to atherosclerosis was further demonstrated in human apoB transgenic mice (15, 53, 80). These mice exhibit 50% higher non-HDL cholesterol levels and a significantly greater degree of proximal aorta atherosclerosis than control mice in response to the atherogenic diet.

The plasma level of lipoprotein(a) [Lp(a)], an LDL-like particle containing a second protein of variable size [apo(a)] that has homology to the fibrinolytic plasma protein plasminogen, is another risk factor for the development of atherosclerosis in humans. The atherogenic diet has been used to assess the atherogenicity of apo(a) (50). When fed the high-cholesterol diet, mice expressing a human apo(a) transgene develop fatty-streak lesions at the base of the aorta. This result is striking in light of the observation that the majority of apo(a) in the plasma of these mice is free and not lipid associated. The conclusion drawn from this study was that the atherogenicity of Lp(a) does not rely fully on the association of apo(a) with the lipoprotein particle. In future studies, investigators will need to determine whether transgenic mice that make Lp(a)—mice that coexpress human apoB and human apo(a)—(15, 53) are more susceptible to diet-induced fatty streaks than the apo(a) only mice.

The diet-induced model of atherosclerosis has been of further use in another set of mice that resemble the apoE-deficient animals. Two lines of mice that mimic human type III hyperlipoproteinemia have been created by overexpressing *trans*-dominant forms of apoE, apoE(Arg 112, Cys 142) (97) and $apoE_{3\text{-Leiden}}$ (27). In human families that harbor either of these two mutations, a hyperlipidemia resembling that seen in type III hyperlipoproteinemia segregates with the mutant allele with high penetrance. When either mutation is overexpressed in mice, a similar phenotype is observed. These transgenic animals develop hyperlipidemia characterized by moderately elevated plasma cholesterol and triglyceride levels and by the appearance of β-VLDL. In both groups of mice, an atherogenic diet can induce significant atherosclerosis (28, 99).

ATHEROSCLEROSIS IN THE apoE-DEFICIENT MOUSE

Studies in the C57BL/6 diet-induced model of atherosclerosis have been valuable in assessing the atherogenicity of many genes. Unfortunately, the model is not an accurate representation of human disease. The apoE-deficient mouse has provided a new model of atherosclerosis that is superior to the diet-induced model in several respects. This mouse develops widespread fibroproliferative

atherosclerosis when fed a low fat, low-cholesterol chow diet (63, 82). Lesions are dispersed throughout the arterial tree forming at the base of the aorta, in the proximal coronary arteries, and along the entire length of the aorta, with predisposition at the branch points of major vessels leaving the aorta. These vessels include the carotids, the intercostals, the mesenterics, the renal arteries, and the iliac arteries. Lesions also form in the carotid, femoral, subclavian, and brachiocephalic arteries. In terms of anatomical localization and quality of lesion, this model more closely resembles humans than did the previous mouse atherosclerosis model.

A chronological analysis of atherosclerosis in the apoE-deficient mouse has shown that the sequential events involved in lesion formation in this model are strikingly similar to those in well-established larger animal models of atherosclerosis and in humans (63). Animals as young as 5–6 weeks of age have monocytic adhesions to the endothelial surface of the aorta that can be appreciated readily with electron microscopy (EM). EM also has demonstrated transendothelial migration of blood monocytes in similarly aged mice. By 6–10 weeks of age, most apoE-deficient mice have developed fatty-streak lesions comprised primarily of foam cells with interspersed ellipsoid cells that are probably migrating smooth muscle cells. These fatty-streak lesions rapidly progress to advanced lesions, which are heterogeneous but are typically comprised of a necrotic core surrounded by proliferating smooth muscle cells and varying amounts of extracellular matrix, including collagen and elastin. These lesions have well-formed fibrous caps made up of smooth muscle cells and extracellular matrix that often have groups of foam cells at their shoulders. It is not uncommon for the inflammatory lesion to erode deep into the medial wall of the aorta, and some of these animals develop aortic aneurysms. Many of the lesions found in older mice develop calcified foci (81).

Other characteristics of the lesions in the apoE-deficient mouse, such as indications of oxidative change, merit attention as well (72). The atherosclerotic lesions in this mouse contain oxidation-specific epitopes. In young lesions these epitopes are predominantly localized in macrophage-rich areas, whereas in advanced lesions they are localized in necrotic regions. In addition, high titers of antibodies against the oxidized epitopes are present in the plasma of the apoE-deficient mice. Although these data do not differentiate a causal from an effectual relationship of oxidation with atherosclerosis, they are consistent with a large body of evidence that links oxidative insults and the process of atherogenesis. Furthermore, the data suggest that this model may be useful for assessing the benefit of antioxidant therapy.

Dietary Control of Atherosclerosis in the apoE-Deficient Mouse

The complexity of lesions in the apoE-deficient mouse, together with the benefits of using the mouse as a model of human disease, makes it a desirable

system in which to study both environmental and genetic determinants of atherosclerosis. Initial studies examined the effects of grossly different diets on susceptibility to atherosclerosis in this animal. These studies confirmed the validity of this mouse as a model of human atherosclerotic disease and laid the groundwork for future dietary studies.

The apoE-deficient mouse responds appropriately to a human-like Western-type diet (63, 78). On this diet, lesion formation is greatly accelerated and lesion size is increased. In 10-week-old animals fed this diet for only 5 weeks, lesions are 3–4 times the size of those observed in mice fed a low-fat diet. In addition, monocytic adhesions and advanced lesions develop at a significantly earlier age. The results of this dietary challenge demonstrate that the mouse model responds in an appropriate manner, i.e. increased fat leads to increased plasma cholesterol, which in turn leads to increased atherosclerosis. Moreover, the data suggest that in addition to its histological similarity to humans, the mouse model exhibits a response to environmental cues resembling that of humans. Other studies have shown that the apoE-deficient mouse is very sensitive to the atherogenic high-cholesterol, cholic acid–containing diet (98, 108) that has been used with the C57BL/6 model. As would be expected, homozygous apoE-deficient mice develop massive lesions on this diet, with plasma cholesterol levels approaching 3000 mg/dl. Of greater interest in these studies is the fact that heterozygous apoE-deficient mice fed this diet have a higher incidence of atherosclerosis than control mice. This observation suggests that with an extreme dietary insult, the absolute level of apoE in plasma becomes important in determining susceptibility to atherosclerosis.

Because first-generation experiments already have demonstrated that the mouse model is very sensitive to dietary changes, second-generation experiments should address the role of various dietary constituents, such as saturated and unsaturated fats, both alone and in combination, in determining atherosclerosis resistance or susceptibility.

Genetic Control of Atherosclerosis in the apoE-Deficient Mouse

The true power of the apoE-deficient mouse lies in its potential for genetic analysis. No existing larger model of atherosclerosis lends itself to genetic studies. Although the C57BL/6 diet-induced model has been helpful in assessing the relative atherogenicity of many genes, the conclusions drawn from these studies are suspect. The toxic diet required to induce lesions and the immature quality of the lesions raise questions as to the applicability of these studies to humans. The apoE-deficient mouse model should help answer many of these questions as well as offer a substrate for testing other genes that may modify development of atherosclerosis.

Recently, two studies demonstrated that overexpression of human apoA-I can elevate HDL cholesterol levels and decrease the incidence of atherosclerosis in

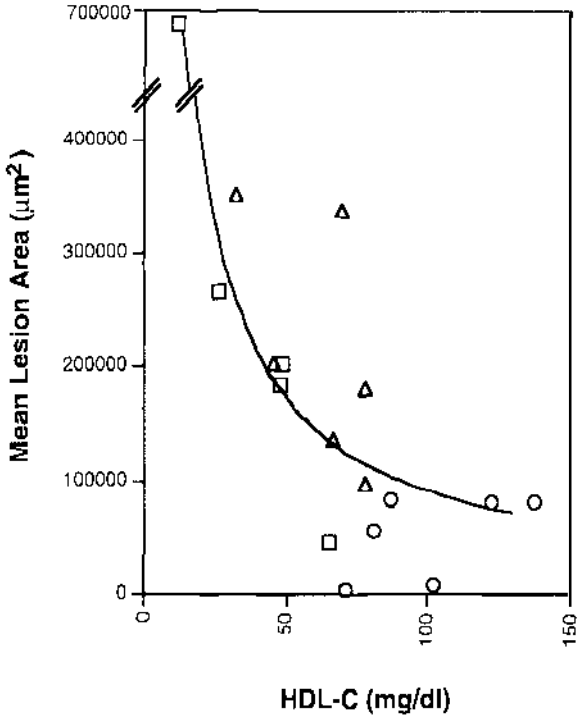

Figure 1 Correlation of mean lesion area and HDL cholesterol levels in apoE-deficient mice and apoE-deficient mice that coexpress a human apoA-I transgene. Squares indicate apoE −/− mice; triangles signify apoE −/− mice expressing low levels of human apoA-I; and circles represent apoE −/− mice expressing high levels of human apoA-I. (Taken from 102.)

the apoE-deficient mouse (73, 77). Overexpression of human apoA-I in apoE-deficient mice increased HDL cholesterol levels twofold (Table 2) and substantially decreased fatty-streak and advanced fibroproliferative lesion formation. By 4 months of age, all but 3–5% of apoE-deficient mice have detectable fatty streaks that vary considerably in size; some are barely detectable, whereas others occlude as much as 8% of the aortic lumen. In apoE-deficient mice that overexpress human apoA-I, more than 50% of animals have no lesions by 4 months of age, and the animals that do develop atherosclerosis have lesions that are barely detectable. By 8 months of age, apoE-deficient mice have lesions that are highly organized and that occlude on average 25% of the aortic lumen. Those apoE-deficient mice that overexpress human apoA-I have mainly immature fatty-streak lesions that occlude on average only 5% of the aortic lumen. Collectively, these data suggest that overexpression of apoA-I can diminish lesion size and slow the initiation of fatty-streak formation.

The apoE-deficient mice and the apoE-deficient mice that overexpress human apoA-I have offered further insight into the role of HDL as an antiatherogenic lipoprotein. The role of HDL in atherosclerosis was established in the mid-1970s, when several population studies demonstrated a strong inverse correlation between HDL cholesterol levels and clinical sequelae of arterial disease. Several theories have attempted to explain the mechanism by which HDL is protective. Possible mechanisms include the role of HDL in reverse cholesterol transport, the ability of HDL to provide direct protective effects to the vessel wall or against lipoprotein oxidation, and the possibility that HDL levels are mere inverse indicators of the level of atherogenic apoB-containing lipoproteins. No definitive evidence exists to support or disprove any of these hypotheses. In the older mice used in the human apoA-I study, a strong inverse correlation was found between HDL cholesterol levels and lesion size (73) (Figure 1). The correlation was reciprocal, with variation in HDL cholesterol levels accounting for more than 75% of the variability in lesion area. Lesion area could not be correlated with non-HDL cholesterol, and non-HDL cholesterol could not further enhance the predictive value of HDL cholesterol on lesion area. In sum, these observations demonstrate that HDL is directly involved in mediating its own protective effect and is not simply a marker of non-HDL cholesterol. These data are among the most convincing to date in demonstrating a direct protective role for HDL in atherosclerotic vascular disease.

SUMMARY AND FUTURE PROSPECTS FOR THE apoE-DEFICIENT MOUSE

The single genetic lesion causing apoE deficiency leads to severe hypercholesterolemia that is sufficient to make the mouse, which normally is very resistant to atherosclerosis, highly susceptible to this disease. Because the atherosclerosis in the apoE-deficient mouse is remarkably similar to that of humans, the mouse model should greatly improve our understanding of the genetics of atherosclerosis. The model has already proved useful in assessing the role of apoA-I and HDL in mediating atherosclerosis resistance. The list of candidate genes that are being tested or have yet to be tested in this model is extensive and includes other lipoprotein transport genes, cell adhesion molecules, growth factors, immune system mediators, and any gene that may play a role in atherogenesis. This model should be useful in identifying new genes that may modify development of atherosclerosis through positional cloning techniques. On the present hybrid genetic background, atherosclerosis in the mouse model is highly heterogeneous in size and quality. By creating congenic strains of the apoE mutation on several genetic backgrounds, one should be able to identify new loci involved in atherosclerosis. Although promising from

many perspectives, the model is young. Future studies will determine the true usefulness of the apoE-deficient mouse

Literature Cited

1. Aalto-Setälä K, Fisher EA, Chen X, Chajek-Shaul T, Hayek T, et al. 1992. Mechanism of hypertriglyceridemia in human apoCIII transgenic mice: diminished VLDL fractional catabolic rate associated with increased apoCIII and reduced apoE on the particles. *J. Clin. Invest.* 90:1889–900
2. Abbey M. 1992. The influence of apolipoprotein polymorphism on the response to dietary fat and cholesterol. *Curr. Opin. Lipidol.* 3:12–16
3. Agellon LB, Walsh A, Hayek T, Moulin P, Jiang XC, et al. 1991. Reduced high density lipoprotein cholesterol in human cholesteryl ester transfer protein transgenic mice. *J. Biol. Chem.* 266:10796–801
4. Deleted in proof
5. Basu S, Brown M, Ho Y, Havel R, Goldstein J. 1981. Mouse macrophages synthesize and secrete a protein resembling apolipoprotein E. *Proc. Natl. Acad. Sci. USA* 78:7545–49
6. Basu S, Goldstein J, Brown M. 1983. Independent pathways for secretion of cholesterol and apolipoprotein E by macrophages. *Science* 219:871–73
7. Blomhoff R, Green MH, Berg T, Norum KR. 1990. Transport and storage of vitamin A. *Science* 250:399–404
8. Breslow J. 1985. Human apolipoprotein molecular biology and genetic variation. *Annu. Rev. Biochem.* 4:163–84
9. Breslow J. 1988. Apolipoprotein genetic variation and human disease. *Phys. Rev.* 68:85–132
10. Breslow JL. 1993. Transgenic mouse models of lipoprotein metabolism and atherosclerosis. *Proc. Natl. Acad. Sci. USA* 90:8314–18
11. Breslow JL. 1994. Insights into lipoprotein metabolism from studies in transgenic mice. *Annu. Rev. Physiol.* 56: 797–110
12. Breslow JL. 1994. Lipoprotein metabolism and atherosclerosis susceptibility in transgenic mice. *Curr. Opin. Lipidol.* 5:175–84
13. Brinton EA, Eisenberg S, Breslow JL. 1994. Human HDL cholesterol levels are determined by apoA-I fractional catabolic rate, which correlates inversely with estimates of HDL particle size. Effects of gender, hepatic and lipoprotein lipases, triglyceride and insulin levels, and body fat distribution. *Arterioscler. Thromb.* 14:707–20
14. Brown ML, Inazu A, Hesler CB, Agellon LB, Mann C, et al. 1989. Molecular basis of a lipid transfer protein deficiency in a family with increased high density lipoproteins. *Nature* 342: 448–51
15. Callow MJ, Stoltzfus LJ, Lawn RM, Rubin EM. 1994. Expression of human apolipoprotein B and assembly of lipoprotein(a) in transgenic mice. *Proc. Natl. Acad. Sci. USA* 91:2130–34
16. Chen S-H, Habib G, Yang C-Y, Gu ZW, Lee BR, et al. 1987. Apolipoprotein B-48 is the product of a messenger RNA with an organ-specific in-frame stop codon. *Science* 238:363–66
17. Chiesa G, Hobbs HH, Koschinsky ML, Lawn RM, Maika SD, Hammer RE. 1992. Reconstitution of lipoprotein(a) by infusion of human low density lipoprotein into transgenic mice expressing human apolipoprotein(a). *J. Biol. Chem.* 267:24369–74
18. Cladaras C, Hadzopoulou-Cladaras M, Felber B, Pavlakis G, Zannis V. 1987. The molecular basis of a familial apoE deficiency: an acceptor splice site mutation in the third intron of the deficient apoE gene. *J. Biol. Chem.* 262:2310–15
19. Copeland NG, Jenkins NA, Gilbert DJ, Eppig JT, Maltais, et al. 1993. A genetic linkage map of the mouse: current applications and future prospects. *Science* 262:57–66
20. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, et al. 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261:921–23

21. Das H, McPherson J, Burns G, Karathanasis S, Breslow JL. 1985. Isolation, characterization, and mapping to chromosome 19 of the human apolipoprotein E gene. *J. Biol. Chem.* 260:6240–47
22. Davignon J, Gregg R, Sing C. 1988. Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis* 8:1–21
23. Dietrich W, Katz H, Lincoln SE, Shin H-S, Friedman J, et al. 1992. A genetic map of the mouse suitable for typing intraspecific crosses. *Genetics* 131:423–47
24. Driscoll D, Getz G. 1984. Extrahepatic synthesis of apolipoprotein E. *J. Lipid Res.* 25:1368–79
25. Eisenberg S, Oschry Y, Zimmerman J. 1984. Intravascular metabolism of cholesterol ester moiety of rat plasma lipoproteins. *J. Lipid Res.* 25:121–28
26. Elshourbagy N, Liao W, Mahley R, Taylor J. 1985. Apolipoprotein E mRNA is abundant in the brain and adrenals, as well as the liver, and is present in the other peripheral tissues of rats and marmosets. *Proc. Natl. Acad. Sci. USA* 82:203–7
27. Fazio S, Lee Y, Sheng Z, Rall SC Jr. 1993. Type III hyperlipoproteinic phenotype in transgenic mice expressing dysfunctional apolipoprotein E. *J. Clin. Invest.* 92:1497–1503
28. Fazio S, Sanan DA, Lee Y-L, Ji Z-S, Mahley RW, Rall SC Jr. 1994. Susceptibility to diet-induced atherosclerosis in transgenic mice expressing a dysfunctional human apolipoprotein E(arg 112, cys 142). *Arterioscler. Thromb.* 14: 1873–79
29. Gabelli C, Gregg R, Zech L, Manzato E, Brewer H. 1986. Abnormal low density lipoprotein metabolism in apolipoprotein E deficiency. *J. Lipid Res.* 27: 326–33
30. Ghiselli G, Schaeffer E, Gascon P, Brewer H. 1981. Type III hyperlipoproteinemia associated with apolipoprotein E deficiency. *Science* 214:1239–41
31. Gianturco S, Bradley W, Gotto AM Jr, Morisett J, Peavy D. 1982. Hypertriglyceridemic very low density lipoprotein induces triglyceride synthesis and accumulation in mouse peritoneal macrophages. *J. Clin. Invest.* 70:168–78
32. Glass C, Pittman R, Civen M, Steinberg D. 1985. Dissociation of tissue uptake of cholesterol ester from that of apoprotein A-I of rat plasma high density lipoproteins: selective delivery of cholesterol ester to the liver, adrenal, and gonad. *J. Biol. Chem.* 260:744–50
33. Glass C, Pittman R, Weinstein D, Steinberg D. 1983. Uptake of high density lipoprotein–associated apoprotein A-I and cholesterol esters by 16 tissues of the rat in vivo and by adrenal cells and hepatocytes in vitro. *Proc. Natl. Acad. Sci. USA* 80:5435–39
34. Goldstein J, Brown M. 1989. Familial hypercholesterolemia. See Ref. 86a, pp. 1215–50
35. Ha YC, Barter PJ. 1982. Differences in plasma cholesteryl ester transfer activity in sixteen vertebrate species. *Comp. Biochem. Physiol.* 71:265–69
36. Havel J, Kane J. 1989. Introduction: structure and metabolism of plasma lipoproteins. See Ref. 86a, pp. 1129–38
37. Hayek T, Azrolan N, Verdery RB, Walsh A, Chajek-Shaul T, et al. 1993. Hypertriglyceridemia and cholesteryl ester transfer protein interact to dramatically alter high density lipoprotein levels, particle sizes, and metabolism. *J. Clin. Invest.* 92:1143–52
38. Hayek T, Chajek-Shaul T, Walsh A, Agellon LB, Moulin P, et al. 1992. An interaction between the human cholesteryl ester transfer protein (CETP) and apolipoprotein A-I genes in transgenic mice results in a profound CETP-mediated depression of high density lipoprotein cholesterol levels, particle sizes, and metabolism. *J. Clin. Invest.* 90:505–10
39. Hixon J, PDAY Research Group. 1991. Apolipoprotein E polymorphisms affect atherosclerosis in young males. *Arterioscler. Thromb.* 11:1237–44
40. Hospattanker A, Law S, Meglin N, Brewer H. 1987. Identification of a novel in-frame translational stop codon in human intestine apoB mRNA. *Biochem. Biophys. Res. Commun.* 148:279–85
41. Hui D, Innerarity T, Mahley R. 1981. Lipoprotein binding to canine hepatic membranes—metabolically distinct apo-E and apo-B,E receptors. *J. Biol. Chem.* 256:5646–55
42. Ishibashi S, Brown MS, Goldstein JL, Gerard RD, Hammer RE, Herz J. 1993. Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. *J. Clin. Invest.* 92:883–93
43. Innerarity T, Mahley R. 1978. Enhanced binding by cultured human fibroblasts of apo-E-containing lipoproteins as compared with low density lipoproteins. *Biochemistry* 17:1440–47
44. Ishibashi S, Herz J, Maeda N, Goldstein JL, Brown MS. 1994. The two-receptor model of lipoprotein clearance: tests of the hypothesis in "knockout" mice lacking the low density lipoprotein receptor,

apolipoprotein E, or both proteins. *Proc. Natl. Acad. Sci. USA* 91:4431–35
45. Ito Y, Azrolan N, O'Connell A, Walsh A, Breslow JL. 1990. Hypertriglyceridemia as a result of human apolipoprotein CIII gene expression in transgenic mice. *Science* 249:790–93
46. Ji ZS, Fazio S, Mahley RW. 1994. Variable heparan sulfate proteoglycan binding of apolipoprotein E variants may modulate the expression of type III hyperlipoproteinemia. *J. Biol. Chem.* 269:13421–28
47. Kowal RC, Herz J, Weisgraber KH, Mahley RW, Brown MS, Goldstein JL. 1990. Opposing effects of apolipoprotein E and C on lipoprotein binding to low density lipoprotein receptor–related protein. *J. Biol. Chem.* 265:10771–79
48. Kraft H, Menzel H, Hoppichler F, Vogel W, Utermann G. 1989. Changes of genetic apolipoprotein phenotypes caused by liver transplantation: implications for apolipoprotein synthesis. *J. Clin. Invest.* 83:137–42
49. Kurosaka D, Teramoto T, Matsushima T, Yokoyama T, Yamada A, et al. 1991. Apolipoprotein E deficiency with a depressed mRNA of normal size. *Atherosclerosis* 88:15–20
50. Lawn RM, Wade DP, Hammer RE, Chiesa G, Verstuyft JG, Rubin EM. 1992. Atherogenesis in transgenic mice expressing human apolipoprotein(a). *Nature* 360:670–71
51. Li H, Reddick RL, Maeda N. 1993. Lack of apoA-I is not associated with increased susceptibility to atherosclerosis in mice. *Arterioscler. Thromb.* 13: 1814–21
52. Li W-H, Tanimura M, Luo C-C, Datta S, Chan L. 1988. The apolipoprotein multigene family: biosynthesis, structure, structure-function relationships, and evolution. *J. Lipid Res.* 29:245–71
53. Linton MF, Farese RV Jr, Chiesa G, Grass DS, Chin P, et al. 1993. Transgenic mice expressing high plasma concentrations of human apolipoprotein B100 and lipoprotein(a). *J. Clin. Invest.* 92:3029–37
54. Lohse P, Brewer HB, Meng MS, Skarlatos SI, LaRosa JC. 1991. Familial apolipoprotein E deficiency and type III hyperlipoproteinemia due to a premature stop codon in the apolipoprotein E gene. *J. Lipid Res.* 33:1583–90
55. Mabuchi H, Itoh H, Takeda M, Kajinami K, Wakasugi T, et al. 1989. A young type III hyperlipoproteinemic patient associated with apolipoprotein E deficiency. *Metabolism* 38:115–19
56. Mahley R. 1988. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 240:622–30
57. Mahley R, Hui D, Innerarity T, Weisgraber K. 1981. Two independent lipoprotein receptors on hepatic membranes of dog, swine, and man. Apo-B,E and apo-E receptors. *J. Clin. Invest.* 68:168–78
58. Mahley R, Rall S. 1989. Type III hyperlipoproteinemia (dysbetalipoproteinemia): the role of apolipoprotein E in normal and abnormal lipoprotein metabolism. See Ref. 86a, pp. 1195–213
59. Marotti KR, Castle CK, Murray RW, Rehberg EF, Polites HG, Melchior GW. 1992. The role of cholesteryl ester transfer protein in primate apolipoprotein A-I metabolism. Insights from studies with transgenic mice. *Arterioscler. Thromb.* 12:736–44
60. Marotti KR, Castle CK, Boyle TP, Lin AH, Murray RW, Melchior GW. 1993. Severe atherosclerosis in transgenic mice expressing simian cholesteryl ester transfer protein. *Nature* 364:73–74
61. Mazzone T, Gump H, Diller P, Getz GS. 1987. Macrophage free cholesterol content regulates apolipoprotein E synthesis. *J. Biol. Chem.* 262:11657–62
62. Myant N. 1990. *Cholesterol Metabolism, LDL, and the LDL Receptor.* San Diego: Academic
63. Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. 1994. Apo E–deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler. Thromb.* 14:133–40
64. National Health and Nutrition Examination Survey: Dietary Intake and Cardiovascular Risk Factors. Part II. Serum urate, serum cholesterol, and correlates 1987. (Survey No. 017-022-00821-1). US Dept. Health Hum. Serv.—Natl. Cent. Health Stat., Off. Health Res. Stat. Tech.
65. Nishina P, Verstyft J, Paigen B. 1990. Synthetic low and high fat diets for the study of atherosclerosis in the mouse. *J. Lipid. Res.* 31:859–69
66. Paigen B, Mitchell D, Reue K, Morrow A, Lusis AJ, LeBoeuf RC. 1987. Ath-1, a gene determining atherosclerosis susceptibility and high density lipoprotein levels in mice. *Proc. Natl. Acad. Sci. USA* 84:3763–67
67. Paigen B, Morrow A, Brandon C, Mitchell D, Holmes P. 1985. Variation in susceptibility to atherosclerosis among inbred strains of mice. *Atherosclerosis* 57:65–73
68. Paigen B, Morrow A, Holmes P, Mitchell D, Williams R. 1987. Quantitative assessment of atherosclerotic le-

sions in mice. *Atherosclerosis* 68:231–40

69. Paigen B, Nesbitt MN, Mitchell D, Albee D, LeBoeuf RC. 1989. Ath-2, a second gene determining atherosclerosis susceptibility and high density lipoprotein levels in mice. *Genetics* 122:163–68
70. Paigen B, Plump AS, Rubin EM. 1994. The mouse as a model for human cardiovascular disease and hyperlipidemia. *Curr. Opin. Lipidol.* 5:258–64
71. Paik Y-K, Chang DJ, Reardon CA, Davies GE, Mahley RW, Taylor JM. 1985. Nucleotide sequence and structure of the human apolipoprotein E gene. *Proc. Natl. Acad. Sci. USA* 82:3445–49
72. Palinski W, Ord VA, Plump AS, Breslow JL, Steinberg D, Witzum JL. 1994. Apolipoprotein E–deficient mice are a model of lipoprotein oxidation in atherogenesis: demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum. *Arterioscler. Thromb.* 14:606–16
73. Paszty C, Maeda N, Verstuyft J, Rubin EM. 1994. Apolipoprotein AI transgene corrects apolipoprotein E deficiency–induced atherosclerosis in mice. *J. Clin. Invest.* 94:899–903
74. Piedrahita JA, Zhang SH, Hagaman JR, Oliver PM, Maeda N. 1992. Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. *Proc. Natl. Acad. Sci. USA* 89:4471–75
75. Plump AS, Forte TM, Eisenberg S, Breslow JL. 1993. Atherogenic β-VLDL in the apoE-deficient mouse: composition, origin, and fate. *Circulation* 88(4):I-2 (Abstr.)
76. Plump AS, Hayek T, Walsh A, Breslow JL. 1993. Diminished HDL cholesterol ester flux in apoA-I-deficient mice. *Circulation* 88(4):I-2 (Abstr.)
77. Plump AS, Scott CJ, Breslow JL. 1994. Human apolipoprotein A-I gene expression increases high density lipoproteins and suppresses atherosclerosis in the apolipoprotein E–deficient mouse. *Proc. Natl. Acad. Sci. USA* 91:9607–11
78. Plump AS, Smith JD, Hayek T, Aalto-Setälä K, Walsh A, et al. 1992. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E–deficient mice created by homologous recombination in ES cells. *Cell* 71:343–53
79. Powell L, Wallis SC, Pease RJ, Edwards YH, Knott TJ, Scott J. 1987. A novel form of tissue-specific RNA processing produces apolipoprotein B48 in intestine. *Cell* 50:831–40
80. Purcell-Huynh DA, Farese RV Jr, Flynn LM, Pierotti V, Newland D, et al. 1994. Transgenic mice expressing high levels of human apolipoprotein B develop severe atherosclerotic lesions in response to a high-fat diet. *Circulation* 90:II–134 (Abstr.)
81. Qiao J-H, Xie P-Z, Fishbein MC, Kreuzer J, Drake TA, et al. 1994. Pathology of atheromatous lesions in inbred and genetically engineered mice. Genetic determination of arterial calcification. *Arterioscler. Thromb.* 14: 1480–97
82. Reddick RL, Zhang SH, Maeda N. 1994. Atherosclerosis in mice lacking ApoE. *Arterioscler. Thromb.* 14:141–47
83. Rubin EM, Krauss RM, Spangler EA, Verstuyft JG, Clift SM. 1991. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. *Nature* 353:265–67
84. Schaefer E, Gregg RE, Ghiselli G, Forte TM, Ordovas JM, et al. 1986. Familial apolipoprotein E deficiency. *J. Clin. Invest.* 78:1206–19
85. Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, et al. 1993. Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc. Natl Acad. Sci. USA* 90:9649–53
86. Schultz JR, Verstuyft JG, Gong EL, Nichols AV, Rubin EM. 1993. Protein composition determines the anti-atherogenic properties of HDL in transgenic mice. *Nature* 365:762–64

86a. Scriver C, Beaudet A, Sly W, Valle D, eds. 1989. *The Metabolic Basis of Inherited Disease.* New York: McGraw Hill. 6th ed.

87. Shachter NS, Hayek T, Leff T, Smith JD, Rosenberg DW, et al. 1994. Overexpression of apolipoprotein CII causes hypertriglyceridemia in transgenic mice. *J. Clin. Invest.* 93:1683–90
88. Shimano H, Yamada N, Katsuki M, Shimada M, Gotoda T, et al. 1992. Overexpression of apolipoprotein E in transgenic mice: marked reduction in plasma lipoproteins except high density lipoprotein and resistance against diet-induced hypercholesterolemia. *Proc. Natl. Acad. Sci. USA* 89:1750–54
89. Shore V, Shore B. 1973. Heterogeneity of human plasma very low density lipoproteins. Separation of species differing in protein components. *Biochemistry* 12:502–7
90. Simonet WS, Bucay N, Pitas RE, Lauer SJ, Taylor JM. 1991. Multiple tissue-specific elements control the apolipo-

protein E/C-I gene locus in transgenic mice. *J. Biol. Chem.* 265:8651–54
91. Smith JD, Schmookler E, Grigaux C, Plump AS. 1993. Apolipoprotein E secretion in the presence of cAMP mediates cholesterol efflux from a macrophage cell line. *Circulation* 88(4):I-1 (Abstr.)
92. Stewart-Phillips JL, Lough J, Skamene E. 1989. Ath-3, a new gene for susceptibility to atherosclerosis in mice. *Clin. Invest. Med.* 12:121–26
93. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Anghild J, et al. 1993. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc. Natl. Acad. Sci. USA* 90:1977–81
94. Strittmatter WJ, Weisgraber KH, Goedert M, Saunders AM, Huang D, et al. 1994. Hypothesis: Microtubule instability and paired helical filament formation in the Alzheimer disease brain are related to apolipoprotein E genotype. *Exp. Neurol.* 125:163–71
95. Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, et al. 1993. Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. *Proc. Natl. Acad. Sci. USA* 90:8098–102
96. Tikkanen M, Huttunen J, Ehnholm C, Pietinen P. 1990. Apolipoprotein E4 homozygosity predisposes to serum cholesterol elevation during high fat diet. *Arteriosclerosis* 10:285–88
97. van den Maagdenberg AMJ, Hofker MH, Krimpenfort PJA, De Brujin I, van Vlijmen B, et al. 1993. Transgenic mice carrying the apolipoprotein $E_{3\text{-Leiden}}$ gene exhibit hyperlipoproteinemia. *J. Biol. Chem.* 268:10540–45
98. van Ree JH, van den Broek W, Dahlmans V, Groot P, Vidgeon-Hart M, et al. 1994. Diet-induced hypercholesterolemia and atherosclerosis in heterozygous apolipoprotein E-deficient mice. *Atherosclerosis* 11:25–37
99. van Vlijmen BJ, van den Maagdenberg, Gijbels MJ, van der Boom H, HogenEsch H, et al. 1994. Diet-induced hyperlipoproteinemia and atherosclerosis in apolipoprotein E3–Leiden transgenic mice. *J. Clin. Invest.* 93:1403–10
100. Warden CH, Hedrick CC, Qiao J-H, Castellani LW, Lusis AJ. 1993. Atherosclerosis in transgenic mice overexpressing apolipoprotein A-II. *Science* 261:469–72
101. Weisgraber K, Innerarity T, Mahley R. 1982. Abnormal lipoprotein-receptor binding activity of the human E apolipoprotein due to cysteine-arginine interchange at a single site. *J. Biol. Chem.* 257:2518–21
102. Whitlock M, Swenson TL, Ramakrishnan R, Leonard MT, Marcel YL, et al. 1989. Monoclonal antibody inhibition of cholesteryl ester transfer protein activity in the rabbit. *J. Clin. Invest.* 84: 129-37
103. Williamson R, Lee D, Hagaman J, Maeda N. 1992. Marked reduction of high density lipoprotein cholesterol in mice genetically modified to lack apolipoprotein A-I. *Proc. Natl. Acad. Sci. USA* 89:7134–38
104. Windler E, Chao Y, Havel R. 1980. Regulation of the hepatic uptake of triglyceride-rich lipoproteins in the rat: opposing effects of homologous apolipoprotein E and individual C apoproteins. *J. Biol. Chem.* 257:14642–47
105. Xhingese M, Lussier-Cacan S, Sing C, Kessling A, Davignon J. 1991. Influences of common variants of apolipoprotein E on measures of lipid metabolism in a sample selected for health. *Arteriosclerosis* 11:1100-10
106. Zannis V, Breslow JL, Utermann G, Mahley RW, Weisgraber KH, et al. 1982. Proposed nomenclature of apoE isoprotein genotypes and phenotypes. *J. Lipid Res.* 23:911–14
107. Zannis V, Ordovas JM, Cladaras C, Cole FS, Forbes G, Schaefer EJ. 1985. mRNA and apolipoprotein E synthesis abnormalities in peripheral blood monocyte macrophages in familial apolipoprotein E deficiency. *J. Biol. Chem.* 260:12891–94
108. Zhang SH, Reddick RL, Burkey B, Maeda N. 1994. Diet-induced atherosclerosis in mice heterozygous for apolipoprotein E gene disruption. *J. Clin. Invest.* 94:937–45
109. Zhang SH, Reddick RL, Piedrahita JA, Maeda N. 1992. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science* 258:468–71