review

Genetic factors affecting blood lipoproteins: the candidate gene approach

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Family studies have revealed that individual variations in the levels and structures of lipoproteins in the blood are due in large part to hereditary influences. Since such lipoprotein variations are strongly associated with atherosclerosis, the major cause of heart disease and stroke, the characterization of these hereditary influences provides an opportunity to better understand the genetic components contributing to the disease. Many laboratories have now taken up this challenge (reviewed in ref. 1). The problem is a difficult one, since the lipoprotein variations clearly result from a large variety of hereditary as well as environmental factors. Atherosclerosis is undoubtedly even more complex and may, like cancer, have myriad causes. In this review approaches to the problem and the results obtained to date are discussed. The most powerful strategy involves combined genetic and biochemical studies of candidate genes—genes at which allelic variation is likely to affect lipoprotein phenotypes. Among such candidate genes, many of which have now been characterized, are those encoding apolipoproteins, lipoprotein receptors, and enzymes involved in lipid metabolism. This review focuses on relatively common genetic differences rather than rare mutations. Since animals provide useful models for the genetic control of lipoprotein metabolism, results from animal and human studies have been integrated.

Metabolism

Fig. 1 illustrates the major pathways for the transport of lipids through the circulation (2). Dietary lipids are packaged in intestinal mucosal cells and secreted into the lymph as large, triglyceride-rich particles termed chylomicrons. These contain apolipoproteins B-48, A-I, and A-IV as well as lower amounts of other apolipoproteins. Upon entering the general circulation, chylomicrons are rapidly lipolyzed by the action of endothelial lipoprotein lipase (with apoC-II acting as a required cofactor), thereby delivering fatty acids to tissues such as adipose, for storage, and muscle, for oxidation. During this process, the core remnants acquire other apolipoproteins, including apolipoprotein E (apoE), and surface remnants are transferred to high density lipoproteins (HDL). The core remnants are then taken up by liver via hepatic receptors specific for apoE.

Endogenous synthesis of triglycerides and cholesterol occurs primarily in liver, where the lipids are packaged and secreted as very low density lipoproteins (VLDL). VLDL particles are, like chylomicrons, rich in triglycerides, but contain apoB-100, the C apolipoproteins (C-I, C-II, and C-III) and apoE. VLDL undergo lipolysis in the circulation to give rise to intermediate density lipoproteins (IDL). A significant fraction of IDL is taken up by liver, while the remainder undergoes further lipolysis to produce low density lipoproteins (LDL). The lipolysis of VLDL appears to involve hepatic lipase (HL) as well as lipoprotein lipase (LPL). LDL is greatly enriched in cholesteryl ester and, in humans, contains most of the cholesterol in fasting serum. Its only significant protein component is apoB-100. LDL delivers cholesterol to peripheral tissues, where it is used for membrane and steroid hormone synthesis, as well as to liver. Its cellular uptake is mediated by the LDL (or apoB,E) receptor, which is present on most cells.

High density lipoproteins (HDL) are formed in the circulation from precursors secreted by liver and from

Abbreviations: apo, apolipoprotein; aP2, adipocyte specific protein; CETP, cholesteryl ester transfer protein; CRBP, cellular retinol binding protein; FABP, fatty acid binding protein; FCHL, familial combined hyperlipidemia; FH, familial hypercholesterolemia; FHTG, familial hypertriglyceridemia; HDL, high density lipoproteins; HL, hepatic lipase; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; HMG-CoA synthase, 3-hydroxy-3-methylglutaryl coenzyme A synthase; hyperapoB, hyperapobetalipoproteinemia; IDL, intermediate density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoproteins; Lp[a], lipoprotein[a]; LPL, lipoprotein lipase; RFLP, restriction fragment length polymorphism; VLDL, very low density lipoproteins.

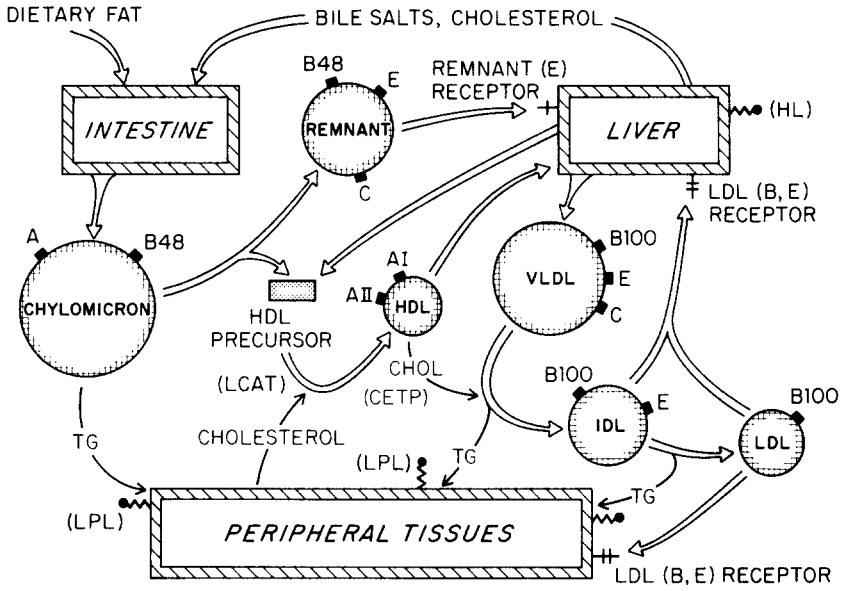

Fig. 1. Schematic illustration depicting pathways for lipid transport.

chylomicron surface remnants. The major proteins of mature HDL are apoA-I and apoA-II. Mature HDL, containing a core of cholesteryl ester, are generated through the action of lecithin:cholesterol acyltransferase (LCAT) by esterification of free cholesterol. HDL facilitates the removal of cholesterol from cells, possibly by a receptor-mediated mechanism. This cholesterol can be transported directly to liver, the major site of HDL catabolism, or to IDL by a mechanism involving cholesteryl ester transfer protein (CETP). Therefore, it appears that HDL may function in "reverse cholesterol transport," a pathway by which excess cholesterol is returned from peripheral tissue to liver, the only organ that is capable of catabolizing and excreting cholesterol.

The levels of lipids in the blood and in cells are controlled by homeostatic mechanisms involving feedback regulation of lipid synthesis and catabolism (3). Such mechanisms are responsible for the relative resistance of lipid levels to environmental influences such as diet, but as discussed below, lipid homeostasis can be perturbed by common or rare mutations of many different genes.

Individual variation: hereditary and environmental influences

The levels and structures of plasma lipoproteins vary widely in the population. For example, **Table 1** shows typical ranges of plasma lipid levels for white American middle-aged males. Significant differences in plasma lipids are also observed between males and females, between different age groups, and between different populations (4, 5). Several percent of the population have plasma lipid levels outside the normal ranges and are termed "dyslipoproteinemic." Some dyslipoproteinemias are due to rare mutations affecting lipoprotein synthesis or catabolism while others appear to represent a continuum of the "normal" variation. Common structural variations of lipoproteins as well as quantitative variations occur in the population. These interindividual variations are due to hereditary and environmental influences.

Environmental influences. The various environmental factors, including diet, exercise, stress, and alcohol consumption, are poorly understood. Of these, diet appears to be the most important. Epidemiological studies and human and animal feeding studies indicate that fat consumption can significantly affect plasma lipid levels (6). In populations where fat intake is very low, such as the Japanese, the levels of plasma cholesterol and the incidence of heart disease are considerably reduced as compared to populations where fat intake is high, such as Americans and Europeans (7). Also, Connor and coworkers (8) found a strong correlation between dietary intake of cholesterol and plasma cholesterol levels within a population (the Tarahumara Indians in Mexico) consuming relatively little

TABLE 1. Individual variation of plasma lipids[a]

Lipid	5th to 95th Percentile Range
	mg/dl
Total cholesterol	146-270
Total triglycerides	54-321
VLDL cholesterol	3-56
LDL cholesterol	81-189
HDL cholesterol	29-62

[a]Values for American white males between the ages 35-39 yr, from studies conducted by the Lipid Research Clinics (4).

dietary cholesterol (20–150 mg/day). However, several studies among Western populations indicate that there is little correlation between cholesterol consumption and plasma cholesterol levels (9, 10). Connor and Connor (6) have proposed that the explanation for this apparent paradox has to do, in part, with the plasma cholesterol response profile at different levels of fat consumption. There appears to be a threshold level of dietary fat consumption, below which dietary fat significantly affects plasma cholesterol levels but above which plasma cholesterol levels plateau. Since fat consumption by nearly all individuals in the Western populations exceeds this threshold, diet does not appear to be the primary factor in the individual variation observed.

Biometrical analyses. Familial associations have been used to examine the importance of genetic as compared to environmental factors in interindividual variations of blood lipoproteins within populations. Various methodologies capable of estimating genetic heritability (h^2) and cultural heritability (c^2) have been developed for analysis of data from nuclear families, extended pedigrees, and twins (11–14). Complex segregation analysis, for example, is used to determine the likelihood that a particular genetic model explains the observed phenotypic data (11, 12). Thus, depending upon the model, it can provide evidence for the effects of major genes as compared to multifactorial inheritance and for allele frequencies. Path analysis, which involves correlations of phenotypes between pairs of related and unrelated individuals, is less powerful but has also been widely applied to estimate genetic and cultural heritability (13). All such statistical modeling approaches involve various assumptions, but, as discussed below, the results provide strong evidence for the genetic determination of quantitative lipoprotein variations. As yet, the conclusions that have emerged from biometrical analyses of lipoprotein variations are rather limited, but these are likely to be extended considerably by the incorporation of data concerning the expression and segregation of various candidate genes.

Dyslipoproteinemias. It appears that most "primary" dyslipoproteinemias, those not secondary to various diseases or drugs, are genetically determined. The best understood is familial hypercholesterolemia (FH), characterized by a selective elevation of plasma LDL and autosomal dominant inheritance with a gene dosage effect (3). Familial clustering also occurs in other common hyperlipidemias, involving elevated plasma cholesterol, triglycerides or both (4, 15, 16). These disorders are poorly understood but in the majority of cases are likely to have polygenic contributions (17). Pedigree analysis suggests that at least some forms of hyperapobetalipoproteinemia, characterized by small, dense LDL, can be explained by a single major gene (18). Extremes in plasma HDL levels, termed "hypoalphalipoproteinemia" (HDL-cholesterol less than the 10th percentile) and "hyperalphalipoproteinemia" (HDL-cholesterol greater than the 90th percentile), also exhibit familial aggregation (19). In kindred studies, Hasstedt, Ash, and Williams (20) found evidence for a dominant major locus as well as polygenic heritability contributing to hypoalphalipoproteinemia, while Rao et al. (21) found evidence for polygenic heritability contributing to hyperalphalipoproteinemia. There are also a variety of rare dyslipoproteinemias, involving in some cases extreme deficiencies of apolipoproteins or enzymes, which clearly have a genetic basis (**Table 2**).

Normolipidemic variations. Family associations have also been used to examine factors contributing to "normolipidemic" variations. A number of studies suggest that about 50 to 60% of the interindividual variability of total cholesterol, LDL cholesterol and HDL cholesterol can be attributed to genetic factors. For example, extensive analyses by Sing and coworkers (22–24) of a population in Tecumseh, Michigan indicate that about 50% of the total normal serum cholesterol variability is associated with polygenic differences. Also, Rao and coworkers (25) obtained the following estimates for genetic heritability (h^2) and cultural heritability (c^2) from a Cincinnati Lipid Research Clinic Family Study: total cholesterol ($h^2 = 0.62$, $c^2 = 0.07$); LDL cholesterol ($h^2 = 0.62$, $c^2 = 0.07$); HDL cholesterol ($h^2 = 0.47$, $c^2 = 0.26$); and VLDL cholesterol ($h^2 = 0.34$, $c^2 = 0.12$). Most other family studies (26, 27) and twin studies (14, 16, 28) have yielded similar results (reviewed in ref. 29). Although heritability data for apolipoprotein concentrations are sparse, most but not all studies suggest that they are also determined in large part by genetic factors (14, 29, 27, 28, 30). For example, in a study of nuclear families in Sweden, Hamsten et al. (27) used path analysis to obtain estimates for genetic heritability of apoA-I (0.43), apoA-II (0.30), and apoB (0.51). In studies of monozygotic versus dizygotic twins in Norway, Berg (14) obtained somewhat higher estimates for genetic heritability of apoA-I (0.53), apoA-II (0.69), and apoB (0.66). Evidence from twin studies also suggests that responses of lipoprotein levels to dietary changes are determined by genetic factors (31). Kuusi et al. (28) obtained high heritability estimates for postheparin plasma hepatic lipase activity but not lipoprotein lipase activity in studies of Finnish twins. In general, the results are consistent with strong hereditary influences on all classes of lipoproteins with the possible exception of VLDL levels.

Animal studies. The poorly understood environmental, sex-related, and age-related effects on plasma lipids can be eliminated in animal studies, providing a less blurred picture of genetic influences than possible from human studies. A number of animal studies, with rabbits, pigs, nonhuman primates, cattle, rats, and mice, indicate strong genetic effects on levels of total cholesterol and particular

TABLE 2. Rare genetic variations affecting lipid transport in humans

Variation	Comments	References
ApoA-I structure	A number of variants have been identified and characterized; several are associated with altered lipid levels, including reduced HDL levels, and three are defective in LCAT cofactor activity.	230, 246, 260
Tangier disease	Autosomal recessive disorder characterized by marked HDL deficiency; primary defect unknown	81, 82, 260
Familial apoA-I and apoC-III deficiencies	Autosomal codominant disorders characterized by marked HDL deficiency, atherosclerosis, and inability to synthesize apoA-I and apoC-III; one variant involves a DNA inversion of the apoA-I and apoC-III genes.	79, 246, 261
Fish eye disease	Autosomal recessive disorder characterized by marked HDL deficiency and corneal opacity; defect unknown.	262
HDL deficiency with planar xanthomas	Autosomal codominant disorder characterized by marked HDL deficiency and xanthomas.	263
Abetalipoproteinemia	Autosomal recessive disorder characterized by absence of apoB in LDL, VLDL, and chylomicrons; primary defect unknown.	108, 128-130
Hypobetalipoproteinemia	Autosomal codominant disorder similar in characteristics to abetalipoproteinemia; some forms result from apoB gene mutations.	128, 131, 132
Abetalipoproteinemia specific for apoB-100	Recessive disorder characterized by the failure to secrete VLDL, although chylomicron secretion is normal; defect unknown.	134
Andersons disease (also called chylomicron retention disease)	Autosomal recessive disorder involving failure of chylomicron secretion; defect unknown	135, 136
ApoC-II deficiency	Autosomal recessive disorder characterized by marked apoC-II deficiency and type I phenotype; some forms due to structural gene mutations.	161-164
ApoE deficiency	Autosomal codominant disorder characterized by marked apoE deficiency and type III phenotype; some forms due to structural gene mutations.	165-168
ApoE structure	In addition to three common alleles, several rare protein variants have been described.	48, 173
Lipoprotein lipase (LPL) deficiency	Autosomal recessive disorder characterized by marked LPL deficiency and type I phenotype; some forms probably due to structural gene mutations.	83, 219
Hepatic lipase (HL) deficiency	Familial disorder characterized by marked HL deficiency in post-heparin plasma and triglyceride-rich HDL and LDL; defect unknown.	227, 228
Combined HL and LPL deficiency	Rare variant that has been only briefly described; probably not due to structural gene defect.	219
LCAT deficiency	Autosomal recessive defect involving marked LCAT deficiency and numerous lipoprotein abnormalities; some forms probably due to structural gene mutations.	85
CETP deficiency	Autosomal codominant disorder associated with marked CETP deficiency and elevated HDL; defect unknown.	84
Wolman disease and cholesteryl ester storage disease	Autosomal recessive disorders characterized by the accumulation of cholesterol esters and neutral lipids in tissues; probably result from deficiencies of certain lysosomal hydrolases.	169
Group C Niemann-Pick disease	Autosomal recessive disorder characterized by the accumulation of sphingomyelin and other lipids in tissues and defective cellular responses to the uptake of exogenous cholesterol.	264

lipoproteins (32-40). The mouse, the classical mammal for genetic studies, affords the clearest view of the kinds of genetic variations affecting plasma lipids that occur naturally. Hundreds of inbred strains of mice, each representing a unique gene pool in which naturally occurring polymorphisms have been fixed by inbreeding, are available. Surveys of different strains maintained on chow or high fat diets have revealed large differences in the levels of all classes of lipoproteins (36, 39). For example, **Fig. 2** shows the levels of plasma cholesterol in 14 inbred strains maintained on chow or high fat diets. In addition to quantitative differences on both diets, genetic factors clearly control the response to a high fat diet challenge (Fig. 2). Different inbred strains of mice also exhibit substantial differences in lipoprotein size and composition (36, 37, 39).

Hereditary influences. From the above studies with humans as well as animals, it is clear that genetic influences have important effects on the levels and structures of all classes of plasma lipoproteins. It appears that the primary dyslipoproteinemias are determined largely by genetic factors. Most studies also suggest that a large fraction of the "normolipidemic" interindividual variation within populations

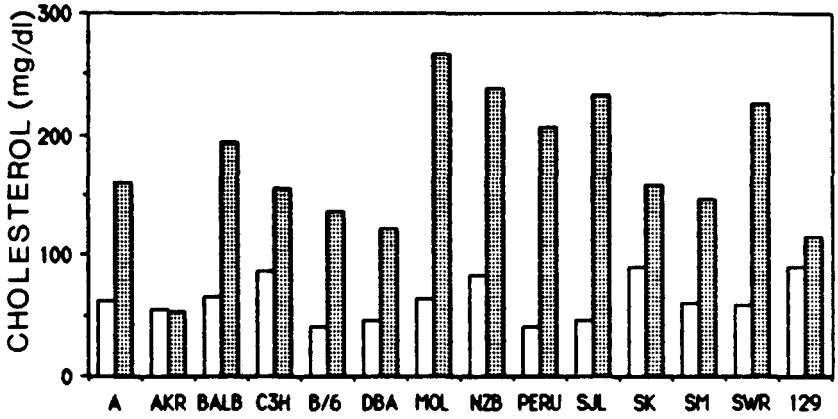

Fig. 2. Genetic control of blood cholesterol levels in mice. The levels of plasma cholesterol in 14 different inbred strains of mice maintained on a chow diet (open bars) or a high fat diet (stippled bars) are shown. Data are plotted from Lusis, et al. (39).

can be explained by hereditary influences. Environmental factors clearly affect blood lipids and are undoubtedly responsible for much of the variation between different populations, but, at least within the Western populations for which the most detailed data are available, they appear to be less important than genetic factors.

Multifactorial control

Since plasma lipid phenotypes are determined by a combination of multiple genetic and environmental factors they are said to be under "multifactorial" control. How complex are the genetic influences contributing to the phenotypic variation? Clearly, there are many genes, probably hundreds, which could affect plasma lipids. These include genes for apolipoproteins, lipoprotein receptors, lipid transfer proteins, and enzymes involved in lipid synthesis, lipid absorption, plasma lipid metabolism, and bile metabolism (Fig. 1). In considering the problem, it is useful to have a sense of the degree of genetic variation in the expression of these genes. Studies with mice suggest that the level of variation among these genes is quite high, as significant differences have been observed among inbred strains for nearly every lipoprotein parameter examined thus far (40). Although a number of the phenotypic differences are undoubtedly secondary, many segregate independently in genetic crosses, suggesting that many gene loci are responsible (A. Lusis, K. Reue, and R. LeBoeuf, unpublished results). Results from human studies also suggest that a large number of genes may be involved in the phenotypic variation. For example, all of the major apolipoprotein loci have been associated with genetic differences affecting plasma lipoproteins (see below). Other studies, such as those of Sing and Orr (41) dealing with the effects of a number of unlinked genetic markers on blood cholesterol levels, are compatible with the concept that many loci in the genome have small effects on cholesterol levels. It is likely that many common hyperlipidemias and other dyslipoproteinemias also result from multigenic inheritance. While certain original studies of hypertriglyceridemias and familial combined hyperlipidemia were interpreted as autosomal dominant inheritance of single major genes, reanalysis of the data using segregation analysis supports multifactorial inheritance (17).

Atherosclerosis probably represents an even more complex phenotype than blood lipoproteins, since it is likely to involve additional local and systemic factors. This is strikingly illustrated by the highly variable severity of atherosclerosis among individuals with FH, all of whom have greatly elevated serum LDL levels. For example, Hobbs et al. (42) recently characterized a defective allele of the LDL receptor that is prevalent among French Canadians. One patient with homozygous FH in the study lived to the age of 33, while another with the same defect died of myocardial infarction at the age of 3.

Identification of genetic factors affecting plasma lipids: the "candidate gene" approach

Direct and indirect approaches. Genetic factors affecting plasma lipids can be identified either directly, by testing for functional differences in the expression of relevant genes, or indirectly, by identifying genetic markers that are correlated with differences in plasma lipids among individuals in the population or in families. The studies of Brown, Goldstein, and colleagues (3) on familial hypercholesterolemia (FH) exemplify the direct approach. They demonstrated that functional differences in the expression of the low density lipoprotein (LDL) receptor are associated with FH, and subsequently they showed that the mutations affecting expression occur in the structural gene for the receptor. Unfortunately, it is often difficult to test for differences in gene expression directly. An impor-

tant limitation in human studies is the inaccessibility of tissues. In the case of the LDL receptor, it was possible to test for expression in fibroblasts, which can be obtained easily and grown in tissue culture. Many of the genes of interest for plasma lipid metabolism, however, are expressed only in inaccessible tissues such as liver or intestine. Therefore, one is generally limited to studies of plasma lipoproteins, representing in most cases a very complex phenotype determined by the interactions of a large number of genes. Because of this problem, most current studies in humans involve the indirect or "candidate gene" approach. The original studies of Utermann, Jaeschke and Menzel (43) as well as important subsequent studies from several other laboratories (44–47) on the role of apolipoprotein E variation in type III hyperlipoproteinemia illustrate this approach. Three common electrophoretic alleles of apolipoprotein E were identified and, subsequently, population studies revealed strong associations between the alleles and type III hyperlipoproteinemia. Studies directed at identifying mechanisms responsible for these associations led to the finding that one of the apoE alleles interacts poorly with the LDL receptor (see ref. 48 for a concise review).

The "candidate gene" approach. This involves, firstly, the identification of genetic markers for a gene likely to be involved in the phenotype of interest. Ideally, one hopes to identify several common polymorphisms of a locus to allow alleles to be traced through most families. These can be either protein markers, such as differences in electrophoretic mobility, or DNA markers, usually involving differences in the size of restriction fragments observed by Southern analysis. DNA markers are more generally useful because they occur considerably more frequently than protein markers. Usually, it is possible to identify restriction fragment length polymorphisms (RFLPs) for a locus by screening DNA from unrelated individuals with a battery of restriction enzymes using Southern blotting (49). Since most base substitutions do not alter restriction enzyme sites, such analyses will identify only a small fraction of the DNA polymorphisms. Other methods have been developed which are capable of detecting a greater fraction of these polymorphisms, including denaturing gradient gel electrophoresis (50) and RNA/DNA hybridization followed by ribonuclease cleavage of mismatched base pairs (51), although these methods are more cumbersome than RFLP analysis.

Second, the markers are correlated in populations or families with the phenotypes of interest (plasma lipids, atherosclerosis, etc.) by statistical likelihood analysis. Usually, the genetic markers will not correspond to polymorphisms affecting function and, therefore, population correlations will depend on the phenomenon of "linkage disequilibrium" (see below). It is crucial that the population studies be well controlled, as differences in sex, age, ethnic origin, and diet between the control and test populations can skew the results and lead to erroneous conclusions.

Third, any correlations must be confirmed by testing for cosegregation of the markers and phenotypes in family studies. Linkage is usually evaluated by the likelihood (lod score) method, although other methods to search for major gene effects have been developed (52). Since it is difficult to fully control population studies, family studies are required to prove associations. In fact, given the uncertainties of population studies, it may in some cases be preferable to proceed directly to family studies. Unlike population studies, associations between silent markers and phenotypic changes observed in family studies do not depend on linkage disequilibrium. Even markers that are millions of base pairs apart cosegregate in a family since the probability of recombination is only about 0.01 per million base pairs per meiosis.

Fourth, if associations are observed in families, the mutant alleles can be characterized with respect to functional differences. An important consideration in such studies is to distinguish silent mutations from mutations affecting function. A recently developed technique to enzymatically amplify DNA corresponding to regions of a gene of interest should simplify the analysis of gene sequences from numerous individuals (53).

Linkage disequilibrium and haplotype analysis. In general, the genetic markers identified in population surveys will be silent with respect to expression. In two random human chromosomes, differences in DNA sequence occur on the average every several hundred nucleotides at many genetic loci. These can be detected as differences in restriction fragment sizes, but the majority of such differences will not affect functional expression since they occur most often in noncoding regions of the gene or at sites within the coding region that do not alter function. The fact that these markers are often associated with functional differences is due to the phenomenon of linkage disequilibrium. This is illustrated in **Fig. 3.** In this example, two silent

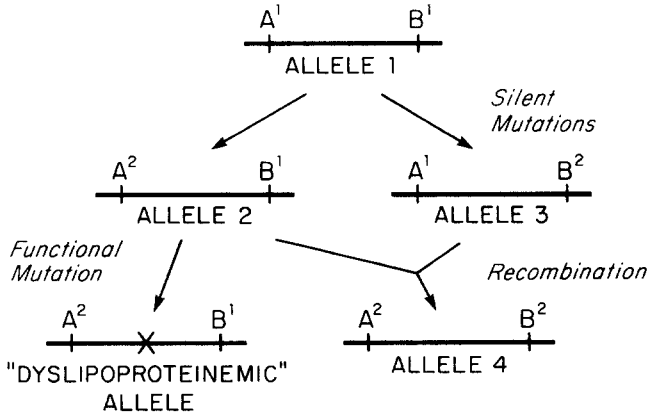

Fig. 3. Schematic illustration depicting the evolution and population genetics of a hypothetical gene. See text for explanation.

polymorphic sites, representing genetic markers, are indicated by A and B. If each of these has two forms, A^1 and A^2 and B^1 and B^2, four possible combinations (alleles 1–4) could occur in the population and, at linkage equilibrium, the A and B polymorphisms would show random associations. If the two marker sites are in close proximity, however, say within several thousand base pairs, certain combinations may occur more frequently than others. This appears to be the result of the evolutionary history of the locus. For example, assume that allele 1 was the primary ancestral allele and that independent mutations of this allele occurred to produce alleles 2 and 3, which became fixed in the population (Fig. 3). Given enough time and assuming the intermingling of populations, allele 4 should eventually be produced by recombination. In fact, however, recombination between such tightly linked sites occurs very infrequently (a probability of roughly 10^{-5} over a distance of 1000 base pairs), and human populations do not freely intermingle. Thus, the frequency of allele 4 could be quite low. If, at some point, a separate mutation affecting function occurs and becomes fixed in the population, it would be expected, due to linkage disequilibrium, to be associated with a particular allele as defined by the silent markers (Fig. 3). As discussed below, substantial linkage disequilibrium has been observed for certain genes involved in plasma lipid metabolism but not others (for example see Table 6). Because of the phenomenon of linkage disequilibrium, "haplotype analysis," in which alleles are defined by examining particular combinations of polymorphisms that occur within and around genes, is useful in establishing correlations between genotypes and phenotypes. For instance, in the example shown in Fig. 3, the association of the "dyslipoproteinemic" mutation would be expected to be higher with the A^2B^1 haplotype than either the A^2 or B^1 polymorphisms alone. Haplotypes are defined by testing for cosegregation of polymorphisms of a locus in families. A classical example of this approach is the study of hemoglobinopathies using DNA polymorphisms of the β-globin gene cluster (54).

Polymorphic markers. In addition to studies of candidate genes, testing for linkage with assorted polymorphic gene markers should prove informative. The standard human phenotypic gene markers cover a relatively small fraction of the genome (about 20%), but additional markers, involving DNA polymorphisms, are rapidly being developed. A complete human linkage map, consisting of highly polymorphic DNA markers evenly spaced throughout the genome (about every 20 centimorgans [cM]) is expected to be completed in the near future (55). This should permit the identification of loci affecting plasma lipoproteins for which the candidate genes have not yet been characterized. Lander and Botstein (55) have reviewed new approaches for mapping complex genetic traits in humans based on such a complete human linkage map.

Phenotypes and subphenotypes

As discussed above, certain phenotypes, such as atherosclerosis or lipid levels, are expected to result in most cases from the effects of numerous gene variations. It appears that in at least some cases such complex phenotypes can be separated into "subphenotypes" which may facilitate analysis of the genetic variations involved. Examples of such subphenotypes include levels of lipoprotein subclasses, ratios of lipoprotein classes, levels of apolipoproteins, lipoprotein densities, lipoprotein compositions, lipoprotein electrophoretic mobilities, and so on.

Such subphenotypes would in most cases be expected to represent less complex genetic influences (involving fewer genes) than phenotypes such as lipid levels. For example, in studies of a single large pedigree, Amos et al. (52) found that the ratio of apoA-I to HDL cholesterol segregated as a single major gene while the total levels of apoA-I and HDL cholesterol were controlled by additional hereditary or environmental influences. This suggests that certain genetic variations may affect primarily the cholesterol content of HDL particles while other genetic variations may affect primarily the number of HDL particles present. Since the total level of HDL-cholesterol is influenced by both types of variations, it represents a more complex phenotype than either the particle cholesterol content or particle concentration subphenotypes. Another, similar, example of a subphenotype that has proved useful is hyperapobetalipoproteinemia, characterized by small dense LDL particles with a high ratio of apoB to cholesterol. In one large pedigree the ratio of LDL apoB to LDL cholesterol appeared to segregate as a single major genetic factor, while total LDL apoB or total LDL cholesterol were influenced by additional genetic factors (18).

Such subphenotyping may also reduce the problem of genetic heterogeneity, in which similar phenotypes result from different genetic influences, and it could provide information about the biochemical nature of the variations. For instance, in the above study of Amos et al. (52), one might suspect that genetic variations of the LCAT or CETP genes could affect HDL cholesterol content, since LCAT controls the formation of the HDL cholesteryl ester core while CETP mediates the transfer of the cholesteryl esters from HDL to other lipoprotein particles.

Finally, subphenotyping could help identify interactions between different gene variations. The association of apoE alleles with type III hyperlipoproteinemia provides a good example, although the other interacting gene (or genes) involved in the disorder are as yet unknown (48). Thus, in the analysis of complex dyslipoproteinemias, it makes sense to examine possible subphenotypes in unaffected family members which could interact to produce the disorder in affected individuals.

Human studies and animal models

There are several major problems in studies of the genetic control of plasma lipids in humans. First, it is nearly impossible to control environmental variables. Many of the variables are unknown and it is difficult to control the ones we partially understand. Second, detailed biochemical studies in humans are frequently not possible. The most important tissues, liver and intestine, are inaccessible, and metabolic kinetic modeling and in vitro expression approaches are rather crude approximations of in vivo regulation. Third, genetic analysis in humans is difficult and limited. For studies of plasma lipids, in particular, pedigree analysis is complicated by substantial age-related and sex-related variations in addition to environmental effects, genetic heterogeneity, and polygenic inheritance. Sophisticated mathematical analyses have been developed to examine major gene determinants (52) but if, indeed, multiple genes contribute to most phenotypic variations, as appears likely, the analysis of variations with relatively subtle effects will be very difficult. **Fig. 4** shows a hypothetical situation where two individuals exhibit different phenotypes resulting from variations at a number of genetic loci. One can imagine the complexity of family studies involving such individuals. Moreover, as illustrated by type III hyperlipidemia (48), complex interactions between genes contribute in some cases to genetic variations of lipoproteins.

These problems can largely be avoided through the use of animal models. Many different species have been examined, but most studies have been purely biochemical or comparative. As discussed below, genetic studies with rabbits and pigs have revealed mutations resulting in hyperlipidemia and atherosclerosis that are of considerable interest. In general, however, genetic studies in these species are limited by the lack of numerous genetic markers and by the difficulty of genetic analysis. Monkeys and baboons are being developed by various groups for genetic and biochemical studies of plasma lipids (56, 57). Nonhuman primates are very similar to humans in terms of lipid metabolism, and they provide advantages in that environmental factors can be controlled and more detailed biochemical studies can be performed. On the other hand, they are expensive to maintain and provide few advantages for genetic analysis. The mouse appears to be the most generally useful mammal for studies of naturally occurring polymorphisms affecting plasma lipids (40). Hundreds of inbred strains have been constructed, many genetic markers covering virtually the entire genome are available, and special tools such as recombinant inbred strains and congenic strains, which are particularly useful in the analysis of complex genetic traits, have been developed. Moreover, recent methodologies, such as the construction of transgenic mice and the isolation of specific null mutants by chemical mutagenesis or viral insertion,

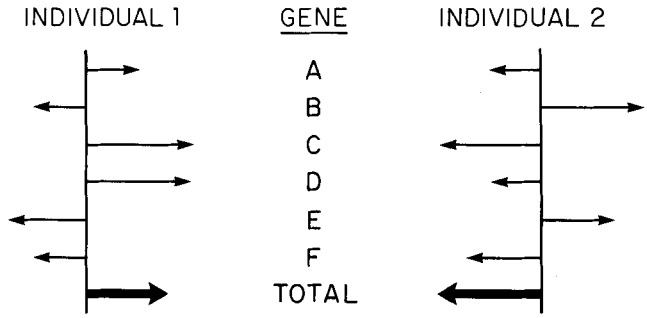

Fig. 4. Illustration depicting multiple hypothetical genetic factors (genes A–F) contributing to the final blood lipid phenotype in two individuals. The incremental effects of the polymorphic loci on plasma lipids are shown with thin arrows and the final phenotypes with thick arrows.

are expanding the utility of mice. However, the mouse model also has important limitations. For example, mice have relatively low levels of LDL and may not be useful for studies of the role of hyperlipidemia in atherosclerosis (39). Moreover, genetic studies in any species are subject to the "luck of the draw" in that they depend largely on the identification of naturally occurring polymorphisms affecting the parameters of interest. Thus, the qualitative and quantitative differences in plasma lipids which occur between species as well as the varieties of genetic variation present in a particular species are important considerations in selecting the best animal model for a particular problem.

Chromosomal organization of candidate genes

Gene clusters. A number of genes involved in lipid metabolism have now been mapped onto chromosomes in humans and mice (**Table 3**) and some noteworthy conclusions have emerged. First, the genes are in some cases clustered. The genes for the major peripheral apolipoproteins (those other than apoB) are clearly members of a family derived from a common ancestral sequence (58, 59). The genes are partly dispersed, on three separate chromosomes, but there is a tight cluster of genes for apoA-I, apoC-III, and apoA-IV on human chromosome 11 (60) and another cluster of genes for apoE, apoC-I, and apoC-II on chromosome 19. The chromosome 19 cluster contains two copies of the apoC-I gene, although one of these could be a pseudogene (61). Similarly, certain members of the gene family encoding low molecular weight proteins that are involved in the uptake or intracellular metabolism of retinol (cellular retinol binding proteins, CRBP), fatty acids (fatty acid binding proteins, FABP) and as yet undefined ligands are clustered (62). Hepatic lipase (HL), lipoprotein lipase (LPL), and pancreatic lipase are also members of a gene family (63), but HL and LPL are unlinked. The genes for CETP and LCAT, on

TABLE 3. Chromosomal organization of genes for lipid metabolism and transport

Gene	Chromosomal Location		References
	Human	Mouse	
ApoA-I	11q13-qter	9	36, 265, 266
ApoA-II	1p21-qter	1	36, 267
ApoA-IV	11q13-qter	9	60, 265; B. Carrasquillo and A. Lusis, unpublished results
ApoB	2p23-p24	12	39, 109
ApoC-I	19q		61, 156, 267
ApoC-II	19q		61, 157
ApoC-III	11q13-qter		265
ApoD	3p14.2-qter		239
ApoE	19q	7	39, 61
CETP	16q12-16q21		64
LDL receptor	19p	9	61, 268; S. Frank, T. Mohandas, B. Taylor, and A. Lusis, unpublished results
LPL	8p22	8	218; C. Heinzmann, T. Mohandas, and A. Lusis, unpublished results
LCAT	16q22		65
HL	15q21	9	218; C. Heinzmann, T. Mohandas, and A. Lusis, unpublished results
HMG-CoA reductase	5q13.1-q14	13	207, 208; D. Quon and A. Lusis, unpublished results
HMG-CoA synthase	5q14-p12		269
CRBP	3	9	62
CRBP-II	3	9	62
aP2		3	270
Liver FABP	2p12-q11	6	271
Intestinal FABP	4q28-q31	3	271

the other hand, reside in close proximity on human chromosome 16 (q21 and q22, respectively), although they exhibit no obvious sequence homology (64, 65). There is no indication that various cholesterol-regulated genes, including those for the LDL receptor, HMG-CoA reductase, or HMG-CoA synthase, are clustered. Although the HMG-CoA reductase and synthase genes are both situated on human chromosome 5 (Table 3), this does not appear to be of functional significance since they occur on separate mouse chromosomes (S. Zollman, D. Quon, and A. Lusis, unpublished results). The clustering and linkage of genes have implications for both population and family studies, since polymorphisms of clustered genes could be in linkage disequilibrium and since clustered or linked genes will cosegregate in families. For this reason, genes present in clusters are considered together in the discussion below.

Linkage conservation. Another important conclusion to emerge from the development of linkage maps in mice and humans is that closely linked loci in one species tend to be linked in the other (66). Thus, the loci for genes involved in lipid metabolism are homologous in mice and humans (and presumably other mammals as well) as judged by the conservation of flanking markers (39). This may prove to be of practical significance; when a gene affecting plasma lipids (or any other phenotype) is mapped in the mouse (or another mammal), it may be possible to predict its location in the human genome. For example, Paigen et al. (67) recently identified a gene in mice which affects both HDL levels and susceptibility to diet-induced atherosclerosis. This gene, called *Ath-1* (for atherosclerosis-1), maps to a region near the structural gene for apoA-II on mouse chromosome 1. Thus, it is likely that the homologous gene is located near the human apoA-II structural gene, and strategies are available to screen for possible polymorphisms of the human gene.

Structural, regulatory and processing genes. The search for gene polymorphisms affecting blood lipids has initially focused on structural genes for receptors, apolipoproteins, and enzymes involved in lipid metabolism (discussed below). This is reasonable, since it is clear that much of the regulatory as well as structural information controlling the expression of a gene resides in its coding and flanking sequences. The final expression of a gene, however, also depends on *trans*-acting regulatory genes and on genes determining protein processing, and these are generally unlinked to the structural locus (68). Some examples of such genes are discussed below.

LDL receptor

Familial hypercholesterolemia (FH) results from mutations that abolish, or nearly abolish, the function of the LDL receptor. Such mutations have a profound effect on plasma LDL levels. Heterozygotes, which occur at a frequency of about 1 in 500 in the population, have two- to threefold elevations of plasma LDL while homozygotes have six- to eightfold elevations. The physiological basis of this increase has been studied extensively (3). These studies have been aided by the identification of a rabbit animal model for FH (69, 70). There appear to be two important factors leading to the LDL accumulation in plasma. First, LDL receptor defects decrease the rate of LDL removal from the circulation. This is due to the fact that receptor-mediated endocytosis of LDL via the receptor is the principal pathway for removal of the lipoprotein from the circulation (Fig. 1). Second, receptor defects result in overproduction of LDL. Normally, a significant fraction of IDL is cleared by the LDL receptor before it is converted to LDL (Fig. 1). This process appears to be mediated by the interaction of the receptor with apoE, which is present on IDL and exhibits higher affinity for the receptor than apoB-100. Thus, reduced levels of receptor result in the further shunting of IDL to LDL (69).

The molecular defects affecting the LDL receptor have been examined in detail by Brown, Goldstein, and colleagues (3, 42, 71, 72). The mutations are extremely heterogeneous, involving various aspects of receptor expression, including receptor synthesis, intracellular transport, binding to LDL, and clustering in coated pits. All of the mutations characterized thus far in humans, and the mutation that occurs in the rabbit animal model, involve the LDL receptor gene locus as judged by molecular analysis, the failure to observe complementation, or cosegregation of FH and genetic markers for the gene. Kingsley and coworkers (73) have utilized a novel selective procedure to isolate mutants affecting receptor function in cells in culture. These studies have revealed *trans*-acting mutations that affect receptor processing and complement receptor structural gene mutations. The results demonstrate that appropriate glycosylation of the receptor is essential for its proper expression, but whether such mutations occur naturally is uncertain. In general, the fairly drastic changes in phenotype observed upon selection in cell culture tend to differ from the more subtle variations found naturally.

Given the fact that null mutations of the LDL receptor have such dramatic effects on plasma LDL levels, one would expect that smaller quantitative variations affecting receptor number or activity would also significantly affect plasma LDL levels. Indeed, the twin studies of Magnus et al. (74) and Maartman-Moe et al. (75) involving measurements of LDL binding and degradation by fibroblasts suggest strongly that genetic differences in receptor activity occur among individuals not having FH. Weight et al. (76) also obtained evidence for heritability of LDL receptor activity using human blood mononuclear cells. Whether such variations result from genetic alterations of the LDL receptor gene and whether they are associated with altered plasma LDL levels are unknown. Given the availability of an LDL receptor gene probe, one approach to test these possibilities would be to correlate genetic markers for the receptor gene with receptor activity or plasma LDL levels in populations or families. To date, several DNA restriction fragment polymorphisms for the human LDL receptor locus, all fairly common, have been reported (**Table 4**).

ApoA-I-C-III-A-IV gene cluster

The genes for these apolipoproteins are present in a tight cluster spanning about 15 kb on the long arm of human chromosome 11 (**Fig. 5**). A similar cluster is present in rats (77). Although the genes have not been studied physically in mice, there is a cluster containing at least the apoA-I and apoA-IV genes on mouse chromosome 9 (Table 3). ApoA-I is the major protein component of HDL, is a cofactor for LCAT, and may serve as a ligand for an HDL receptor. ApoA-IV is a major protein constituent of chylomicrons but rapidly dissociates from the particles during lipolysis; its function is unclear (2). ApoC-III is a minor constituent of VLDL and HDL and inhibits lipoprotein lipase, thus possibly delaying the clearance of triglyceride-rich lipoproteins (78).

Rare disorders. ApoA-I has been studied in most detail. Numerous rare mutations affecting the structure or expression of the protein have been identified (Table 2), including a DNA inversion at the locus that disrupts both the apoA-I and apoC-III genes (79). One particularly interesting rare mutation affecting apoA-I expression occurs in Tangier disease. The disease is characterized by extremely low levels of plasma HDL and its apolipoproteins, apoA-I and apoA-II, due to increased catabolism. In contrast to some earlier reports, recent studies indicate that the apoA-I in Tangier disease is structurally normal (80, 81). In monocyte-derived macrophages from Tangier patients, normal retroendocytosis of HDL appears to be inoperative, resulting in the catabolism of HDL in lysosomes and accumulation of cholesterol in macrophages and other cell types (82).

TABLE 4. DNA polymorphisms of the LDL receptor gene

Enzyme	Frequency[a]	Location	References
PstI	0.4	3' of exon 18	272
PvuII	0.2	intervening sequence 15	273, 274
BstEII	0.25	3' end of gene	275
AvaII	0.44	exon 13	276

[a]The frequency of the rare allele in American or European populations surveyed.

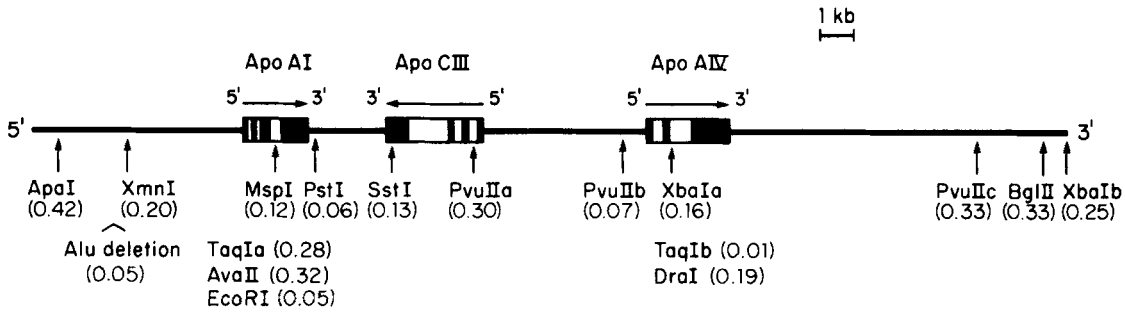

Fig. 5. Restriction fragment length polymorphisms of the human apoA-I–C-III–A-IV gene cluster. The locations of polymorphic sites are indicated by **arrows** or, if locations are not known precisely, sites are placed under probe sequences used. The population frequencies of minor alleles are given in parentheses. References are as follows: Apa I (277); Alu (278); XmnI (279); TaqIa (280); AvaII (280); EcoRI (281); MspI (97, 279, 281, 282); PstI (91, 102, 104, 283, 284); SstI (94, 95, 104, 285); PvuIIa (279); PvuIIb (286); XbaIa (287); TaqIb (287); DraI (288); PvuIIc (286); BglII (289); XbaIb (287, 290).

Common variations. Convincing evidence of family clustering of plasma apoA-I levels has been observed in several studies (27, 29, 30). In particular, the study of Moll et al. (30), in which pedigrees enriched for early coronary artery disease were examined, provided evidence for both a single major locus and polygenic loci which determine apoA-I levels. Whether the major locus corresponds to the apoA-I structural locus is unknown. The major locus does not appear to correspond to any of the rare apoA-I mutations mentioned above since its frequency is too high, but there is accumulating evidence that DNA polymorphisms of the apoA-I locus are associated with certain forms of hypoalphalipoproteinemia (see below). In addition, apoA-I levels may be affected by the expression of various other genes. For example, altered HDL-cholesterol levels have been associated with genetic variations in the activities of lipoprotein lipase (83), hepatic lipase (28), CETP (84), and LCAT (85). As yet, there is little information relating to the genetic control of apoC-III or apoA-IV expression in humans. Two relatively common, as well as at least two rare, structural polymorphisms of apoA-IV occur in the population but there is no evidence that these are associated with altered expression (86, 87).

Animal studies. Studies with mice have revealed that certain strains, but not others, exhibit a decrease in the levels of apoA-I and HDL-lipid in response to a high fat diet challenge (88). The response is determined by a gene, designated *Ath-1,* located on mouse chromosome 1 (67). Its identity and how it acts are unknown. Large differences in apoA-IV mRNA levels in mice fed chow diets as well as differences in the induction of mRNA levels in mice by high fat diets have been observed. Preliminary studies with recombinant inbred strains suggest that basal levels are determined by polymorphisms at the apoA-IV structural locus on chromosome 9, while induction by the high fat diet is controlled by a separate locus (89).

DNA polymorphisms. Several laboratories are utilizing the "candidate gene approach" discussed above to examine the possible involvement of genetic variations at the A-I–C-III–A-IV locus in plasma lipid phenotypes. A large number of DNA polymorphisms of the locus have been identified (Fig. 5). The nucleotide diversity of the region is similar to diversity estimates for a number of other human genes and indicates that about one out of 500 nucleotides will differ in two randomly selected chromosomes (60). The frequencies of the polymorphisms vary considerably among various populations examined, including Northern Europeans, Mediterranean Europeans, American Blacks, Indian Asians, and Japanese (90). Haplotypes for a number of the polymorphisms have been studied in families, and although nonrandom associations of certain polymorphic sites occur, the degree of linkage disequilibrium is low (91–93). The low degree of linkage disequilibrium at the locus suggests that correlations between DNA polymorphisms identified by restriction enzyme analysis and alterations of plasma lipids, presumably resulting from separate mutations, will be attenuated in population studies. Thus, family studies may prove more informative than population studies for this locus. Given the large number of common haplotypes of the cluster, it is possible to trace the inheritance of the genes in most families.

Associations with hyperlipidemias. Several studies suggest that polymorphisms of the locus are associated in populations with diverse hyperlipidemias, although there are conflicting data. Rees et al. (94) found a significant association between the SstI polymorphism (which occurs in the noncoding portion of the last exon of apoC-III) and hypertriglyceridemia (type IV and type V hyperlipidemia) in the United Kingdom population ($P < 0.001$). The finding was confirmed by Ferns et al. (95), who also found that the polymorphism is associated with heart disease. No obvious alterations of HDL, apoA-I or apoC-III, however, were associated with the polymorphism (96). In contrast to this association in Caucasians, a study of a Japanese population, in which the minor SstI allele is

much more frequent, revealed no association with hypertriglyceridemia, although one MspI–SstI haplotype of the locus was significantly increased in hypertriglyceridemic subjects ($P < 0.025$) (97). Henderson et al. (98), in studies of the polymorphism in several different South African populations, found associations with type III hyperlipoproteinemia and hypercholesterolemia as well as hypertriglyceridemia, although Vella et al. (99) failed to find association with type III hyperlipoproteinemia in a United Kingdom study. In contrast to the above studies, Kessling, Hursthemke, and Humphries (91) found no association between the SstI polymorphism and hypertriglyceridemia in the United Kingdom, although a significant association was observed with an XmnI polymorphism of the locus. In a study of a Norwegian population, Kessling et al. (100) failed to find statistically significant associations between the XmnI or SstI polymorphisms and hypertriglyceridemia. Hayden et al. (101) examined the frequencies of the XmnI, PstI, and SstI polymorphisms (Fig. 5) in 53 individuals of Caucasian descent with genetic hyperlipidemias. The rare variant XmnI and SstI sites were found to occur more frequently in individuals with familial combined hyperlipidemia (FCHL) than in controls, and when considered together as a haplotype the results reached statistical significance ($P < 0.03$). The discrepancies between the above results may be due to differences in the populations or to relatively small sample sizes. Taken together, the results support the conclusion that genetic variations of the A-I–C-III–A-IV locus are involved with certain hypertriglyceridemias, although the SstI or XmnI polymorphisms cannot at present be considered useful genetic markers for the disorders.

Associations with hypoalphalipoproteinemia. Several reports also suggest that polymorphisms of the locus may be associated with hypoalphalipoproteinemia. Ordovas et al. (102) reported a striking association between the PstI polymorphism (located 3' to the apoA-I gene) and both coronary artery disease and hypoalphalipoproteinemia. The frequency of the rare allele was less than 0.03 in 123 control individuals and about 0.17 in a group of 88 patients with coronary disease ($P < 0.01$), yielding a relative risk factor greater than 10. The association with HDL-cholesterol in kindreds with familial hypoalphalipoproteinemia (HDL-cholesterol < 10th percentile) was even greater, as 8 of 12 index cases studied carried the rare allele. Anderson et al. (103) studied three polymorphic sites of the locus (SstI, MspI, and PstI) in an American population and observed a significant association between an SstI–MspI haplotype and HDL cholesterol levels in the lowest docile. This population also exhibited significantly higher triglyceride levels. In addition, as observed in the study by Ordovas et al. (102), the frequency of the rare PstI allele was enriched in a series of men with angiographically confirmed coronary artery disease. Sidoli et al. (104) studied a three-generation family with hypoalphalipoproteinemia and found a strong, but not complete, association with apoA-I haplotypes defined by the PstI and SstI (SacI) polymorphisms. The fact that the association was not complete could well be due to environmental or other genetic factors. It is clear from these studies that the associated alleles defined by the DNA polymorphisms are not present in all primary hypoalphalipoproteinemia conditions; this is not unexpected since the disorder is likely a heterogeneous entity. A number of reports indicate significant associations between polymorphisms of the locus and coronary artery disease (discussed below). Since HDL levels are strongly correlated in populations with heart disease, these studies further support the possibility that common alleles of one or more genes of the cluster may affect HDL levels. At this point, it is important to carry out additional family studies and to characterize in detail alleles of the locus that segregate with HDL levels.

ApoA-II

ApoA-II is the second most abundant protein of HDL. In addition to its structural role it appears to activate hepatic lipase and may also affect LCAT activity (105).

Common structural and quantitative polymorphisms of apoA-II occur among inbred strains of mice (36). The quantitative variation, involving about a twofold difference in plasma apoA-II levels between strains, segregates with the apoA-II structural gene on mouse chromosome 1. Examination of mice heterozygous for both structural and quantitative apoA-II variations indicates that plasma levels are regulated in *cis,* providing very strong evidence that plasma levels are determined by structural or regulatory variations of the apoA-II gene itself (R. LeBoeuf and A. Lusis, unpublished results). Among the physiological consequences of altered apoA-II production is a dramatic change in HDL structure. Original studies indicated that an electrophoretic variation of HDL between certain strains of mice segregates with the apoA-II gene (36). This HDL variation (defining the *Hdl-1* gene) is now known to result from the altered levels of apoA-II. The HDL particles in strains exhibiting high levels of apoA-II are larger and have about twice the ratio of apoA-II to apoA-I as compared to strains with low levels of apoA-II (R. LeBoeuf, M. Doolittle, and A. Lusis, unpublished results). Thus, it appears that the stoichiometry for incorporation of apolipoproteins into HDL is determined in part by the relative production of apoA-II and apoA-I. Whether the resulting structural variation of HDL affects functions such as reverse cholesterol transport has not yet been examined.

Scott et al. (106) have identified a hereditary variation for apoA-II levels in humans that appears to be very similar to that observed in mice. Using an MspI polymorphism that occurs in an Alu repeat 3' to the human apoA-II gene,

they were able to distinguish two different alleles of the gene in the population. Individuals that were homozygous for the rare allele (allele frequency about 20%) had significantly higher levels of serum apoA-II levels ($P < 0.005$) and significantly higher apoA-II/apoA-I ratios ($P = 0.02$) than individuals homozygous for the common allele. Ferns et al. (107) utilized the same human apoA-II polymorphism to demonstrate an association ($P = 0.02$) with hypertriglyceridemia (types IV and V) in a United Kingdom population.

ApoB

ApoB is the major protein component of chylomicrons, VLDL, and LDL. It serves at least two crucial functions in lipid transport. First, it is required for the assembly and secretion of chylomicrons and VLDL, since these lipoproteins are absent in individuals with abetalipoproteinemia, a genetic disorder in which apoB is not produced. Second, apoB serves as the ligand for the removal of LDL from the circulation by receptor-mediated endocytosis via the LDL receptor (108). In humans and most other mammals, liver incorporates a 550 kDa species (termed apoB-100) into VLDL, whereas the intestine incorporates a 210 kDa species (termed apoB-48) into chylomicrons. A variety of genetic and molecular studies indicate that both species of apoB are derived from a single gene, located on chromosome 2 in humans and chromosome 12 in mice (39, 109–113). Powell et al. (113) recently reported convincing evidence, in studies with humans and rabbits, that the tissue-specific expression of the two apoB forms occurs by a novel form of RNA processing. This involves the co- or post-transcriptional modification of a single nucleotide in the intestinal but not hepatic transcript, resulting in a translational stop at codon 2153. In addition, an appreciable fraction of the intestinal mRNAs is polyadenylated downstream of the stop codon, suggesting that editing and polyadenylation may be coupled. Differential expression of the two forms is important in directing the metabolism of the lipoproteins, as the apoB-48-containing chylomicron remnants are rapidly cleared from the circulation, while apoB-100-containing particles are partially metabolized to LDL that have a relatively long halflife in the circulation. ApoB-100 but not apoB-48 contains the recognition marker for uptake by the LDL receptor. Other molecular weight species of plasma apoB have also been reported but, with the exception of a rat hepatic species slightly smaller than B-100, these appear to be derived after secretion by proteolysis by B-100 (114).

Polymorphisms. The large size, heterogeneity, and insolubility of apoB peptides have made studies of the protein difficult. However, polymorphisms in human LDL were recognized well before apoB itself was characterized through the use of alloantisera obtained from multiply transfused patients. Characterization of these alloantisera revealed ten Mendelian factors, placed in five antithetical pairs, belonging to what has been termed the "Ag system" (115, 116). It is now clear that these factors represent five distinct allelic variations of the apoB-100 molecule, and a monoclonal antibody capable of distinguishing alleles of apoB has been found to be associated in populations and a family with the Ag(c) epitope (117, 118). Alloantisera have also been used to distinguish apoB alleles in rabbits (119, 120), pigs (121), monkeys (122), and mink (123). ApoB has now been cloned from a variety of species, including rat (124), rabbit (113), and human (125), and the clones have been used to identify a number of DNA polymorphisms in humans (**Table 5**) and mice (39). Recently, associations have been observed between certain DNA polymorphisms and Ag antigenic sites of human apoB (126, 127). From the original studies of Bütler and coworkers (115, 116) on the Ag "factors," it is clear that a total of at least 14 different haplotypes of apoB (from a possible 32 different Ag haplotypes) occur in various populations (**Table 6**). The total number of common apoB alleles will certainly be much larger than 14, since only a fraction of the genetic differences is likely to have been revealed by studies with alloantisera. Thus, apoB is relatively polymorphic and a sufficient number of markers of the gene are now available for population and family studies.

Rare disorders. Several rare disorders involving defective production or secretion of apoB have been described. Abetalipoproteinemia, characterized by the absence of both apoB-100 and apoB-48 and the lipoprotein particles on which they reside, is inherited as an autosomal recessive trait, and heterozygotes have approximately normal levels of apoB (128). A similar disorder, hypobetalipoproteinemia, is characterized by an absence of both apoB species in homozygotes but, unlike abetalipoproteinemia, heterozygotes have about 50% of normal apoB plasma levels (128). Recent studies of abetalipoproteinemic patients

TABLE 5. DNA polymorphisms of the human apoB gene

Probe	Enzyme	Frequency[a]	References
1 kb 5' cDNA	PvuII	0.08	109, 291, 292
1 kb 5' cDNA	HincII	0.12	292
3.5 kb 5' cDNA	MspI	0.15	109
5 kb 5' cDNA	StuI	0.01	293
5 kb 5' cDNA	EcoRV	0.02	293
5.1 kb 3' cDNA	EcoRI[b]	0.2	150, 294
5.1 kb 3' cDNA	XbaI[c]	0.4	150, 295
5.1 kb 3' cDNA	Hypervariable[d]	multiple alleles	296, 297

[a] The frequency of the rare allele in American or European populations surveyed.

[b] The EcoRI site polymorphism alters the apoB protein sequence by substituting a lysine (AAA) for glutamic acid (GAA) at residue 4154 of the mature protein.

[c] The XbaI site polymorphism is due to a silent mutation in the third base of the threonine codon at residue 2488 of the mature protein.

[d] A highly polymorphic sequence downstream of the apoB gene composed of a variable number of AT-rich repeats extending over about 500 bp; detectable using a variety of enzymes including BamHI, EcoRV, and MspI.

TABLE 6. Ag haplotypes of apoB observed in various populations[a]

Haplotype	Ag Factors				
I	x	a_1	c	t	i
II	x	a_1	g	t	i
III	x	a_1	g	z	i
IV	x	d	c	t	i
V	x	d	g	t	i
VI	y	a_1	c	z	i
VII	y	a_1	g	t	h
VIII	y	a_1	g	t	i
IX	y	a_1	g	z	i
X	y	d	c	t	h
XI	y	d	c	t	i
XII	y	d	c	t	i
XIII	y	d	g	t	i
XIV	y	d	g	z	i

[a]Data taken from Bütler and Brunner (115). Each Ag factor represents one of two antithetical epitopes (x/y, a_1/d, g/c, t/z, and i/h) present on apoB. The frequencies of the haplotypes differ between the Swiss, Tibetan, or Senegalese populations studied.

have revealed the presence of a normal-sized apoB mRNA in liver (129) and immunoreactive apoB protein in liver and intestine (130), suggesting that a post-translational defect may be involved. This possibility is also consistent with the recessive nature of the defect. Moreover, the patients examined to date do not have gross rearrangements or deletions of the apoB gene (129). The codominant nature of the defect in hypobetalipoproteinemia, on the other hand, suggests that a structural or regulatory mutation of the apoB gene may be involved. The disorder appears to be quite heterogeneous, as some patients exhibited impaired apoB synthesis, one expressed a truncated form of apoB (131, 132), and another exhibited normal synthesis but increased removal of VLDL remnants (133). Another variant of apoB deficiency in which apoB-100, but not apoB-48, is absent has been described (134). Finally, a recessive defect involving fat-filled enterocytes and the failure to secrete chylomicrons, termed Anderson's disease or chylomicron retention disease, has recently been characterized (135, 136). Immunological studies of intestinal biopsies of patients indicate the presence of apoB-48, suggesting a problem with chylomicron assembly or secretion. Given the numerous apoB gene markers available, it should be possible by pedigree analysis to determine whether the defects in these disorders occur in the gene. Since the assembly of lipoproteins is likely to be a rather complex process, probably involving the products of several genes, it is quite possible that defects of other genes could be responsible for apoB deficiencies.

Hyperlipidemias. Because of its central role in the metabolism of triglyceride-rich lipoproteins, it has been suspected that genetic variations of apoB may contribute to various hyperlipoproteinemias and atherosclerosis. Kinetic modeling studies with labeled lipoproteins suggest that some hyperlipidemias involve overproduction of VLDL-apoB while others involve reduced clearance lipoprotein remnants and LDL (5, 137). The former could be explained by regulatory variations affecting apoB synthesis and the latter by structural mutations of apoB affecting receptor-mediated uptake of lipoproteins. The hyperlipoproteinemias are very heterogeneous and their classification is unclear. The system of classifying hyperlipoproteinemias developed by Fredrickson and coworkers (types I-V) is of limited value since it is based on phenotypes rather than genotypes. For example, familial combined hyperlipidemia (FCHL) is an autosomal dominant disorder that is variably expressed as hypercholesterolemia alone (type IIa), hypertriglyceridemia alone (type IV), or both hypercholesterolemia and hypertriglyceridemia (type IIb) (138). Another disorder, which appears to show overlap with FCHL, is hyperapobetalipoproteinemia (hyperapoB), characterized by an elevated level of plasma LDL-apoB but near normal levels of LDL-cholesterol. Thus, the LDL particles in hyperapoB are smaller, denser, and have a reduced amount of cholesteryl ester as compared to normal particles (139, 140). Both FCHL and hyperapoB appear to exhibit increased VLDL-apoB synthesis (141, 142), and certain FCHL patients have small dense LDL particles similar to those observed in hyperapoB (143, 144). Results from a study of large Amish pedigree were consistent with a Mendelian single locus model for hyperapoB (18). Preliminary pedigree studies to examine the association of apoB DNA polymorphisms with hyperapoB have revealed an association in one family, while in another family there was apparent discordancy (J. Ladias, P. Kwiterovich, C. Heinzmann, A. Lusis, and S. Antonarakis, unpublished results). Familial dysbetalipoproteinemia (type III) may also partially overlap with these disorders. Although a primary defect in type III involves apoE (see below), the disorder becomes manifest only in the presence of other genetic or environmental factors contributing to hyperlipidemia (48). Humphries (145) has reported a study with one family indicating that alleles of apoB were not associated with type III hyperlipidemia. In contrast to FCHL and hyperapoB, familial hypertriglyceridemia (FHTG) does not appear to be associated with significantly elevated VLDL apoB synthesis (142, 146). Studies of pedigrees with excess coronary artery disease have suggested the existence of major loci determining apoB levels as well as LDL-cholesterol to apoB ratios (52, 147).

Associations with cholesterol and triglyceride levels. Population studies utilizing protein or DNA polymorphism have provided some preliminary evidence that alleles of apoB may be associated with altered lipid levels and atherosclerosis. Berg et al. (148) observed higher serum cholesterol and triglyceride levels in Ag(y) than Ag(x) individuals by analysis of the combined data from 10 different populations (P = 0.01–0.02 for cholesterol and $P < 0.001$ for triglyc-

erides). Law et al. (149) utilized an XbaI polymorphism near the middle of the apoB gene coding region to survey middle-aged white men in the United Kingdom. They found that subjects homozygous and heterozygous for the presence of the site had a mean triglyceride 36% higher (P = 0.02) than those homozygous for the absence of the restriction site. Homozygotes lacking the restriction site also had a significantly lower mean total serum cholesterol (P = 0.03). Subsequently, Berg (150) also found that blood cholesterol levels and apoB concentrations were strongly associated with the XbaI polymorphism in a Norwegian population. In these studies, individuals lacking the XbaI site had about 20% lower apoB levels (P = 0.004) and 10% lower cholesterol levels (P = 0.036) than individuals with at least one chromosome containing the site, although no significant associations with triglyceride levels were observed. The associations observed in these latter studies presumably result from linkage disequilibrium, since the XbaI polymorphism results from a silent C to T mutation in the third base of the threonine codon at residue 2488 of the mature protein (150). Berg et al. (127) demonstrated that the XbaI polymorphism exhibits strong linkage disequilibrium with the Ag(x/y) determinant. Hegele et al. (151) reported an association ($P < 0.01$) between polymorphisms of the XbaI, EcoRI, and hypervariable 3' regions of the apoB gene and atherosclerosis in a study of patients who had suffered myocardial infarctions and of matched controls. In this study, however, the apoB alleles were not significantly correlated with altered LDL-cholesterol or apoB levels. On the other hand, Young et al. (152) found no evidence for association of an apoB protein polymorphism, detected by a monoclonal antibody, with either coronary artery disease or elevated cholesterol levels. These results do not contradict the studies with DNA polymorphisms since some apoB polymorphisms may be in linkage disequilibrium with variations affecting apoB expression while others may not.

Animal studies. The elegant studies of Rapacz and coworkers (38, 121), utilizing alloantisera to define lipoprotein polymorphisms in pigs, provide very strong evidence that alleles of apoB are associated with hypercholesterolemia and atherosclerosis. One of a number of apoB alleles defined by alloantisera, termed *Lpb*^{5}, was found to be present in all pigs (out of 14,000 surveyed) exhibiting elevated plasma cholesterol. The degree of hypercholesterolemia of the *Lpb*^{5} pigs was also influenced by two other lipoprotein-borne markers defined by alloantisera, termed *Lpu* and *Lpr*. The *Lpu* marker is tightly linked to the apoB gene (no recombinants in 244 tested progeny from genetic crosses) while the *Lpr* marker is unlinked to the apoB gene. *Lpr* encodes a 23 kDa protein associated with various density classes of lipoproteins while the *Lpu* gene product has not yet been identified. Particular alleles of *Lpr* and *Lpu* result in dramatic elevations of plasma cholesterol when present in combination with the *Lpb*^{5} allele of apoB. The cholesterol elevation in these animals (two- to threefold greater than animals with other alleles) is due primarily to increased LDL and IDL levels. Obviously, the further biochemical and genetic characterization of these interacting genes is of great importance for understanding of the role of apoB in hypercholesterolemia and for the identification of the homologs of *Lpr* and *Lpu* in humans.

Polymorphisms affecting several aspects of apoB expression occur among inbred strains of mice, including differences in plasma levels in mice on both chow and high fat diets, differences in the response to a high fat diet, and differences in the relative levels of the two molecular weight species of apoB. Although high fat diet challenge resulted in considerable changes in the plasma levels of apoB-100 and apoB-48, the levels of apoB mRNA in liver and intestine were not significantly affected by diet. Nor were differences in the plasma levels of apoB between inbred strains of mice correlated with mRNA levels. Thus, the genetic control of apoB expression in mice appears to occur largely at the level of lipoprotein catabolism rather than synthesis (39). Detailed genetic analyses of these variations have not been performed, although genetic studies of LDL/VLDL levels among recombinant inbred strains suggest multigenic inheritance (88; R. LeBoeuf and A. Lusis, unpublished results).

ApoB metabolism has also been examined in selectively bred baboons with low and high levels of LDL (153). Kinetic isotope studies involving the injection of labeled VLDL or LDL indicated that the production of LDL-apoB was greater in high LDL animals. This appeared to result from a lower fraction of IDL removal as well as increased LDL synthesis from a source other than VLDL.

An inherited hypercholesterolemia in a strain of New Zealand White rabbits was associated with increased production rates of VLDL apoB and LDL apoB (154). As in the above studies with baboons, kinetic isotope studies suggested that the LDL apoB was in part derived from sources other than VLDL.

ApoE-C-I-C-II gene complex

These genes are present as a complex in the proximal region of the long arm of human chromosome 19 (61). ApoE has been physically linked to two copies of the apoC-I gene (61, 155, 156), and recombination studies in humans indicate that apoC-II is situated near this cluster (157, 158). ApoE is synthesized primarily by liver but is also produced by a variety of peripheral tissues not associated with lipoprotein synthesis (159, 160). It serves as a ligand for both the LDL (apoB,E) receptor as well as a hepatic remnant (apoE) receptor and it functions in the uptake of chylomicron remnants by liver and also in the uptake of subclasses of VLDL and HDL. ApoC-I and

apoC-II are peptides associated predominantly with triglyceride-rich lipoproteins. The function of apoC-I is unclear although it can activate LCAT in vitro. ApoC-II serves as an activator of lipoprotein lipase (2).

Rare disorders. Rare deficiencies of apoE and apoC-II have been identified and characterized in humans (Table 2). ApoC-II deficiency is characterized by a marked elevation of plasma triglycerides and chylomicrons and decreased HDL and LDL. This type I phenotype results from the inactivity of lipoprotein lipase in the absence of the apoC-II activator. A number of patients with apoC-II deficiency have been characterized, and the results are consistent with heterogeneous structural or regulatory mutations of the apoC-II gene (161–164). ApoE deficiency is characterized by an unusual form of type III hyperlipoproteinemia, xanthomas, and heart disease, probably resulting from delayed clearance of lipoproteins (165). Studies of macrophages from these patients indicate alterations in the levels or size of apoE mRNA, consistent with regulatory or structural mutations of the apoE gene (166, 167). Recently, Cladaras et al. (168) demonstrated that one form of apoE deficiency results from improper splicing at the 3' end of the apoE gene.

ApoE alleles and normolipidemic variations. ApoE has several common structural alleles that show striking associations with altered lipoprotein levels and hyperlipoproteinemias (48, 169, 170). Three common and several rare structural alleles of apoE have been identified and characterized (171–174). The common alleles, designated E2, E3, and E4, occur at frequencies of about 0.07, 0.78, and 0.15, respectively, in white populations. Several studies have shown that the alleles contribute to the variance of lipoprotein levels among normolipidemic individuals (175–178). For example, **Table 7** shows results obtained by Utermann and colleagues (177) for a German population. Similar associations have been observed in studies of other populations, although the apoE allele frequencies and the absolute apolipoprotein levels differ significantly between certain populations (48). Most striking is the association of E2 homozygotes and heterozygotes with reduced cholesterol and apoB levels and elevated apoE levels as comapred to the other alleles. The E4 allele, on the other hand, is associated with slightly elevated cholesterol and apoB levels and reduced apoE levels as compared to the E3 allele. The basis of these associations has been partially revealed by structural and physiological studies of the apoE allelic forms. The E2 form of apoE, although quite frequent in the population, is markedly deficient in binding to the LDL (apoB,E) receptor and hepatic remnant (apoE) receptors (47, 179, 180). This results from an amino acid substitution at a region of apoE involved in receptor binding (47, 181). The explanation for the reduced LDL levels in individuals carrying the E2 allele is still speculative but may have to do with upregulation

TABLE 7. Effects of apoE alleles on plasma lipoprotein levels[a]

Phenotype	Frequency	Mean Cholesterol	Mean ApoB	Mean ApoE
	% population		*mg/dl*	
4/4	2.8	197	89	1.9
4/3	22.9	189	83	2.3
3/3	57.4	184	82	2.4
3/2	4.9	166	69	3.4
2/2	0.2	140	47	5.1

[a]Data from Utermann et al. (177) for a population of 552 Germans.

of hepatic LDL receptors due to reduced delivery of cholesterol to liver from chylomicron remnants and HDL-apoE, thereby enhancing LDL clearance. The E2 form of apoE may also affect the conversion of IDL to LDL (179). Large VLDL particles in hypertriglyceridemia are not a good substrate for LDL formation and may be removed by alternative pathways (182, 183). The E3 and E4 allelic forms of apoE, which differ by a single amino acid substitution, bind to receptors about equally well in vitro. Nevertheless, in vivo kinetic studies suggest that the apoE is metabolized about twice as rapidly in E4 as compared to E3 homozygotes (184). Sing and Davignon (185) have calculated that the apoE gene may be responsible for about 14% of the total polygenic variability (and about 7% of the total phenotypic variability) of plasma cholesterol levels.

Hyperlipidemias. Alleles of apoE also exhibit associations with hyperlipidemias. The strongest association occurs between the E2 allele and type III hyperlipoproteinemia, a relatively rare disorder affecting one in several thousand individuals in the population and characterized by increased plasma triglyceride and cholesterol levels, abnormal lipoproteins (termed β-VLDL), xanthomas, and atherosclerosis (43, 46, 169, 186, 187). More than 90% of the type III patients examined to date are E2 homozygotes, the remainder being E2 heterozygotes or compounds with rare apoE alleles exhibiting defective binding. This association is obviously highly significant since less than 1% of the population are E2 homozygotes. Nevertheless, although the E2 allele is apparently important in type III, other factors are also required for the disease to manifest itself, since the majority of E2 homozygotes are normolipidemic and, in fact, have lower than average plasmic cholesterol levels (Table 7). The nature of these other genetic or environmental factors is unclear, but familial combined hyperlipidemia, diabetes, and obesity frequently coexist in families with type III (48). Preliminary studies with one family suggest that the apoB gene does not represent such a second genetic locus associated with the disease (145). Recently, Klasen et al. (188) reported a common DNA polymorphism of the apoE gene detected with the enzyme HpaI (rare allele frequence 0.38 in normo-

lipidemic individuals). Surprisingly, the frequency of the rare allele in normolipidemic individuals with the E2/E2 phenotype was similar to that of the general population but was 0.97 in 39 individuals with type III hyperlipoproteinemia and the E2/E2 phenotype. The explanation for this is unclear, but these findings raise the possibility that there are genetic variations in or near the apoE gene (in addition to the variation determining the E2 phenotype) that contribute to type III and are in linkage disequilibrium with the HpaI polymorphism (188). An enrichment of the E2 allele also occurs in individuals with primary hypertriglyceridemia (type IV) (189). Among type IV individuals the E2 allele is enriched in the subset with familial hypertriglyceridemia and normal apoB levels but not in the subsets with familial combined hyperlipidemia (189). The E4 allele has been reported to be increased among individuals with "hypercholesterolemia" as defined by cholesterol levels above 280 mg/dl (187) or 260 mg/dl (186) and with type V hyperlipoproteinemia (190). Such "hypercholesterolemia" may represent a continuum of the "normal" variation in which the association results from the incremental effect of the E4 allele on total cholesterol levels. Certain relatively rare alleles of apoE have also been associated with hyperlipidemia and atherosclerosis (173). Two such rare allelic forms of apoE, termed E5 and E7, occur in about 5% of Japanese patients with hyperlipoproteinemia or atherosclerosis (173).

Polymorphisms of apoC-I and apoC-II. Other than the rare deficiencies of apoC-II (Table 2), quantitative polymorphisms of apoC-II and apoC-I have not been reported. A structural variant of apoC-II, differing from the common allelic form by a single amino acid substitution, is present in about 12% of African blacks (191). The protein is normal with respect to ability to activate LPL, and it is not known whether the variation has any physiological effects. Several DNA polymorphisms of the human apoC-II gene have been identified (**Table 8**). The polymorphisms exhibited very marked linkage disequilibrium (158, 192), and did not show significant associations in European populations with serum levels of total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, or with atherosclerosis (193). Two DNA polymorphisms of the human apoC-I gene, a BglI polymorphism with a minor allele frequency of about 0.02 and a DraI polymorphism with a minor allele frequency of about 0.21, have been identified in American and European populations (194, 195).

Animal studies. Approximately twofold variations of apoE plasma levels occur among inbred strains of mice. The variations appear to result from the catabolism of the protein rather than its synthesis, since they do not correlate with hepatic mRNA levels (39). Extensive screens for structural variants of apoE by isoelectric focusing have failed to reveal charge polymorphisms, although DNA polymorphisms have been identified and used to map the gene to the proximal region of mouse chromosome 7 (39). Peritoneal macrophages isolated from different strains of mice exhibit significant genetic variations in both rates of apoE synthesis and the degree of stimulation of synthesis upon cholesterol loading (88).

TABLE 8. DNA polymorphisms of the human apoC-II gene

Enzyme	Frequency[a]	Location	References
TaqI	0.4	3' flanking	192, 298
BglI	0.5	5' flanking	192
BanI	0.3	3' flanking	299
NcoI	0.3	unknown	300

[a] The frequency of the rare allele in American or European populations surveyed.

Apo[a]

Apo[a] is a large glycoprotein that is disulfide linked to apoB-100 in an LDL-like particle termed lipoprotein [a] (Lp[a]). Its normal physiological function is unknown. Lp[a] was first described by Berg in 1963 using absorbed antisera (196). It was initially considered to be a genetic variant of LDL, inherited as a single autosomal dominant trait present in about 30% of Northern European populations (196). Subsequent studies, however, suggested that Lp[a] is present in all individuals, but at very different levels (ranging from less than 1 to more than 100 mg/dl), and that the levels are determined by a single major autosomal locus (197, 198). A variety of studies have established a correlation between high Lp[a] levels and atherosclerosis, with a relative risk factor of about three (14, 199). Since Lp[a] typically carries less than 15% of the cholesterol in plasma, this association does not appear to be due to effects on total cholesterol levels. Recently, there has been considerable progress in the biochemical and genetic characterization of human apo[a] (200). At least six different molecular weight isoforms of the protein, ranging from about 300 kDa to 700 kDa have been observed. These isoforms exhibit both inter- and intra-individual variability, and family studies suggest that all are determined by multiple alleles of a single locus. Interestingly, the size variations are associated with quantitative variations of Lp[a], suggesting that a common gene determines apo[a] structure and Lp[a] levels (201). Partial amino acid sequencing of apo[a] (202) and, subsequently, the cloning and sequencing of apo[a] cDNA (203), have revealed a striking homology to plasminogen, a protease found in plasma. Apo[a] contains two types of plasminogen-like kringle domains, one of which is present in 37 copies, but appears to lack protease activity. It has been proposed that the size isoforms of apo[a] may be due to individual variation in the number of kringle repeats of the apo[a] gene as a result of intragenic recombination within the repeated sequences (203). With the availability

of cloned probes, the answer to this and other questions relating to the genetic control of apo[a] and Lp[a] are likely to be answered in the near future.

HMG-CoA reductase

HMG-CoA reductase is thought to be the rate limiting enzyme in cholesterol synthesis. Its activity is regulated in response to cellular sterol concentrations at multiple levels, including transcription and turnover (204–206). The HMG-CoA reductase gene is located on the long arm of chromosome 5 in humans (207, 208) and on the distal portion of chromosome 13 in mice (D. Quon and A. Lusis, unpublished results); these regions are homologous as judged by the conservation of linked markers.

Animal studies. Genetic variations affecting HMG-CoA reductase activity in mice were reported by Packie and Kandutsch in 1970 (209). They demonstrated that relatively large differences in liver enzyme activity occur among inbred strains of mice and that the high activity strains exhibit correspondingly higher rates of sterol synthesis as judged by acetate incorporation. Rates of sterol synthesis from mevalonic acid were similar in the strains tested, suggesting that the genetic differences in sterol synthesis are due to differences in reductase levels. Numerous DNA polymorphisms of the reductase gene occur among inbred strains of mice, and thus it should be possible to test whether variations of the reductase locus are involved in the differences (D. Quon and A. Lusis, unpublished results). The physiological consequences of this altered sterol production are unclear, but the levels of plasma cholesterol among strains show no obvious correlation with reductase activity. Thus, if some strains do indeed produce much more cholesterol than others, there must be compensatory mechanisms such as differences in bile secretion.

Human studies. The results with mice raise the possibility that similar polymorphisms may occur in humans. Although studies of cholesterol homeostasis in humans have revealed differences in cholesterol synthesis, the many variables affecting synthesis (daily intake of calories, for example) have made it difficult to identify any genetic contributions. However, at least one genetic hyperlipidemia, familial combined hyperlipidemia, appears to be associated with increased cholesterol production (210, 211). In addition, a high fraction of individuals exhibiting the type IV phenotype appear to have enhanced synthesis of cholesterol. A number of studies in humans have attempted to examine associations between hepatic HMG-CoA reductase activity and various disorders involving cholesterol metabolism, including gallstone disease, hyperlipidemia, and obesity (reviewed in ref. 212). The results have been quite variable and often contradictory, although reductase activity appears to be increased in individuals with type IV hyperlipoproteinemia or gross obesity. As yet, no DNA polymorphisms of the human gene have been reported, and one survey with a small number of enzymes failed to reveal polymorphisms (213).

Mutations of cells in culture. A variety of mutants affected in HMG-CoA reductase expression have been isolated in cells in culture (214). These include 25-hydroxycholesterol-resistant mutants selected on the basis of their ability to grow in the presence of oxygenated sterols and in the absence of exogenous cholesterol. Some such mutants are defective in the down-regulation of reductase as well as certain other enzymes of the cholesterol biosynthetic pathway, suggesting that they may involve *trans*-acting elements involved in the coordinate regulation of enzymes for sterol synthesis. Complementation analysis suggests that mutations of at least two separate genes can produce the phenotype (214–216). Although it is unlikely that such mutations occur naturally, less extreme variations of these genes may occur in humans and animals.

Lipoprotein lipase

LPL functions in the hydrolysis of core triglycerides of circulating chylomicrons and VLDL. It is synthesized in parenchymal cells of many tissues but functions on the surface of vascular endothelium, where it is anchored by a membrane-bound glycosaminoglycan chain. In the presence of the cofactor apoC-II, LPL hydrolyzes endogenous triglycerides to monoglycerides and free fatty acids, which are taken up by cells for oxidation or storage. Recently, cDNAs for both mouse (63) and human (217) LPL were cloned and sequenced. The gene for LPL resides on chromosome 8 in humans (218) and on chromosome 8 in mice (Table III).

Human studies. LPL deficiency has now been described in over 50 patients. The disorder is accompanied by the accumulation of triglyceride-rich lipoproteins in plasma as well as decreased LDL and HDL levels (83). The decreased LDL and HDL levels presumably result from the impaired catabolism of triglyceride-rich lipoproteins, thereby decreasing HDL and LDL production. Family studies of LPL deficiency are consistent with an autosomal recessive mode of inheritance. An immunoassay for LPL has been used to show that most deficient patients, while lacking enzyme activity, do have enzyme mass, ranging from low normal to above normal levels (219). Thus, the results are consistent with LPL structural gene mutations in these patients. With the availability of LPL cDNA probes and restriction fragment polymorphisms (**Table 9**), it should be possible to test this in family studies. Brunzell and coworkers (219) have identified a number of variants of the classical LPL deficiency, including individuals exhibiting tissue specific deficiency, combined LPL and HL deficiency, combined LPL and apoC-II deficiency, and transient LPL deficiency. It is clear that

TABLE 9. DNA polymorphisms of the human LPL gene

Enzyme	Frequency[a]	References
HindIII	0.33	301
BamHI	0.27	302
PvuII	0.41	302

[a] Frequency of the rare allele in American populations.

the combined deficiencies do not result from a focal defect involving deletions or rearrangements of these genes in a cluster (as has been seen, for example, with apoA-I and apoC-III in the apoA-I-C-III-A-IV cluster), since the LPL gene is on human chromosome 8, the HL gene is on chromosome 15, and the apoC-II gene is on chromosome 19 (Table 3). Rather, it appears that the mutations affect specific pathways required for the final expression of these proteins, such as modification or transport to the surface of the vascular endothelium.

Animal studies. Inbred strains of mice exhibit quantitative differences in the levels of LPL activity in various tissues. In one genetic cross, the activity differences in heart and adipose tissue segregated independently, indicating that at least two unlinked loci control expression of the enzyme in these tissues (220). Hybridization analyses of the LPL gene suggests that the explanation for this is not the presence of multiple LPL genes which show tissue specific expression (R. LeBoeuf, T. Kirchgessner, M. Schotz and A. Lusis, unpublished results).

One interesting mutation in mice, involving a deficiency of both LPL and HL, was identified in studies of the *t* locus (221, 222). The recessive mutation, termed combined lipase deficiency (cld), results in hyperchylomicronemia (with plasma triglycerides as high as 20,000 mg/dl) and the mice die within 2 days post partum. The *cld* gene is located in the proximal region of mouse chromosome 17, unlinked to the genes for LPL (mouse chromosome 8) or hepatic lipase (mouse chromosome 9). The mutation results in the synthesis of an inactive form of LPL, and thus may involve some aspect of the modification or transport of the enzyme which is also required for the expression of HL.

Recently, Hatanaka et al. (223) observed that hyperlipidemia is associated with mutations of the dominant spotting locus (*W*) on chromosome 5 in mice. W/W^V mice, which are anemic and lack mast cells, showed variable hypertriglyceridemia, hypercholesterolemia, and decreased LPL and HL in plasma after heparin injection. Thus, as in the *cld* mutant, the defect in these mice appears to involve the processing of the enzymes. Hatanaka et al. (223) have speculated that this may involve the anchoring of the enzymes to endothelial cells by heparin sulfate, since heparin is produced by mast cells.

It has been suggested that gene variations affecting LPL expression could be involved in genetic predisposition to obesity. This remains to be determined, but on the basis of the chromosomal location of the mouse LPL gene, it appears that mutations of the gene are not involved with the various obesity syndromes that have been characterized in the mouse, including yellow, obese, diabetes, and tubby (224).

Hepatic lipase

HL appears to function primarily in the metabolism of cholesteryl ester-rich lipoproteins, although its functions remain poorly understood (225). The enzyme is localized on the sinusoidal surfaces of the liver and, like LPL, can be released by injection of heparin. The cDNA for the enzyme has recently been isolated and sequenced, and it is clear that it is a member of a dispersed gene family of lipases that includes LPL and pancreatic lipase (63, 226). Rare deficiencies of HL, characterized by abnormally triglyceride-rich LDL and HDL and the presence of β-VLDL, have been identified in humans (227, 228). The disorder is clearly familial, but the defects involved are unknown. Rare variant defects, described in humans (219) and mice (221), result in a combined deficiency of both HL and LPL. As discussed above for LPL, it is unlikely that such a combined deficiency results from mutations at the LPL and HL structural gene loci. HL activity in postheparin plasma appears to be determined largely by genetic influences and is negatively correlated with plasma HDL cholesterol levels in normal subjects (28).

Lecithin:cholesterol acyltransferase

LCAT is involved in the esterification of HDL-cholesterol. The enzyme is associated with a subclass of HDL, and its substrates, cholesterol and phosphatidylcholine, are derived from the surface of plasma lipoproteins or the plasma membrane of cells. Human LCAT cDNA has recently been cloned and sequenced (229). A number of families with LCAT deficiency have been characterized. The deficiencies, which segregate as autosomal recessive traits, are characterized by abnormalities of all lipoprotein classes and the presence of lamellar HDL particles rich in unesterified cholesterol. Studies in several Norwegian families indicate that the mutation is linked to the serum haptoglobin locus on human chromosome 16 (combined lod score 3.41 at a recombination fraction of 0.00) (85). Since the human LCAT structural gene has recently been localized to this region by somatic cell hybrid studies and in situ hybridization to chromosomes, it is likely that in these families the deficiency results from mutation at the LCAT structural locus (65). ApoA-I is an activator of LCAT, and several structural mutations of apoA-I resulting in defective LCAT activation have been characterized (230).

Cholesteryl ester transfer protein

CETP, which appears to be identical with lipid transfer protein-one (LTP-1), functions in the transfer of cholesteryl esters among lipoproteins and is thought to play an important role in the regulation of plasma cholesterol homeostasis (231, 232). Human CETP cDNA has recently been isolated and characterized (232). Rare deficiencies of CETP activity have been reported and appear to be associated with unusually large HDL particles and hyperalphalipoproteinemia (84). The basic defect in such families is unclear since the CETP deficiency could be secondary to other changes in plasma lipids and since an inhibitor of CETP is present in serum (233). It is interesting, however, that certain mammals that apparently lack CETP activity, such as mice and rats, have relatively high HDL levels (231). Three DNA polymorphisms of the human CETP locus have been reported (**Table 10**), and these should be useful for testing, by family studies, whether mutations of the CETP gene are involved in CETP deficiency or in other alterations of plasma lipoprotein metabolism. The gene for CETP is located in the q12-q21 region of human chromosome 16 (64) in close proximity to the gene for LCAT, located in the q22 region (65). The significance of this is unclear, since they exhibit no obvious sequence homology. This linkage will, nevertheless, have to be considered in future family studies directed at examining the effects of polymorphisms of the two genes on lipoprotein phenotypes, since the genes may cosegregate. Recently, Amos et al. (52) observed apparent linkage between the haptoglobin locus (located in the q22 region of human chromosome 16) and HDL-cholesterol/apoA-I ratios in a single large pedigree (lod score 1.72 at a recombination fraction of 0.05). It is interesting to speculate that this may have resulted from linkage with the CETP or LCAT genes.

Other candidate genes

The search for genetic factors affecting blood lipids is only beginning. In addition to the genes discussed above, the products of a number of other genes that play very central roles in plasma lipid metabolism have now been biochemically characterized and will probably be examined for genetic variations in the next few years. Among these the following appear to be of particular interest: hepatic receptors involved in the apoE-mediated uptake of lipoprotein remnants (234, 235); an HDL receptor, which may facilitate uptake or transfer of cholesterol from cellular membranes to HDL (236); the "scavenger" receptor, which recognizes certain modified lipoproteins and may be involved in foam cell formation (237); apoD, a protein complexed with LCAT and comprising about 5% of HDL protein (238, 239); and lipid transfer protein-2, which is involved in phospholipid exchange between lipoprotein particles (231). In addition, a large number of intracellular proteins involved in lipid synthesis, binding, transport, and catabolism may be involved in the genetic control of blood lipids. For example, Angelin, Hershon, and Brunzell (146) have recently shown that patients with monogenic familial hypertriglyceridemia have significantly elevated rates of bile acid synthesis as compared to normal individuals or individuals with FCHL, suggesting a defect in the regulation of bile acid synthesis.

TABLE 10. DNA polymorphisms of the human CETP gene[a]

Enzyme	Frequency
TaqIa	0.12
TaqIb	0.48
MspI	not determined

[a] Data from Drayna and Lawn (303).

Atherosclerosis

Atherosclerosis is a disease of the large arteries that is responsible for coronary artery disease and stroke. The early lesions of atherosclerosis, referred to as "fatty streaks," consist of subendothelial accumulations of "foam cells," cholesterol-engorged macrophages that have penetrated the arterial wall. Subsequently, these lesions develop into fibrous plaques characterized by the accumulation of matrix components and the proliferation of smooth muscle cells (240). A small fraction of the population is likely to develop atherosclerosis irrespective of lifestyle and diet (for example, individuals with FH) and a small fraction is probably unusually resistant, but for the vast majority of individuals the interaction of environment and genetics determines the development of the disease. Since blood lipids are among the major risk factors for atherosclerosis that have been identified through epidemiological as well as experimental studies (others include hypertension, cigarette smoking, diabetes, and obesity) the studies of genetic variations affecting blood lipids offer an opportunity to identify some of the genetic factors contributing to the disease.

The mechanisms by which the various risk factors affect the disease are unclear, although several hypotheses to explain the development of atherosclerotic lesions have been proposed. The "lipid infiltration hypothesis" proposes that atherogenesis is initiated as a consequence of penetration of lipids into the arterial wall from the blood, while the "endothelial injury hypothesis" proposes that lesions result from a variety of cellular and molecular interactions (involving platelets, macrophages, smooth muscle cells, and growth factors) that occur upon damage to the endothelial lining of the artery (240, 241). Benditt and Benditt (242) provided evidence that human atherosclerotic plaques are monoclonal in origin and proposed a "monoclonal hypothesis" to explain the proliferation of smooth muscle

cells; this hypothesis recently received support from the finding of a transforming gene in DNA extracted from human plaques (243). Most likely, each hypothesis has some validity.

The epidemiological and experimental evidence linking blood lipids with atherosclerosis, in both humans and animal models, is very strong (reviewed in 10, 29, 241, 244). The lipoprotein alterations associated with the disease can be grouped into four types: *1*) hyperlipidemias, *2*) hypoalphalipoproteinemia, *3*) normolipidemic variations, and *4*) structural alterations of lipoproteins. Each of these is discussed in turn below.

Hyperlipidemias. A number of studies have revealed that coronary artery disease and atherosclerosis are strongly correlated with various hyperlipidemias, in particular, those involving elevated LDL levels, and that familial hyperlipidemias aggregate in families of individuals with myocardial infarction. For example, Goldstein et al. (138) found that about 30% of all survivors of premature myocardial infarction (under 55 years of age) exhibited some form of familial hyperlipidemia. Other similar studies have recently been reviewed (29). Hyperlipidemias could contribute to the disease by promoting lipid infiltration into arterial walls or by damaging endothelial cells lining artery walls. Thus far, the studies reviewed above have led to the identification of two major genes contributing to moderately common hyperlipidemia (**Table 11**). The best understood is FH, which occurs at a frequency of about one in 500 and involves defects of the LDL receptor. The other is type III hyperlipoproteinemia, which occurs with a frequency of one in several thousand and involves a major allele of apoE as well as other, as yet unidentified, genetic or environmental factors. In addition, genetic factors contributing to some rare hyperlipidemias are known (Table 2). Other common hyperlipidemias, including familial combined hyperlipidemia (FCHL), primary polygenic hypercholesterolemia, and primary hypertriglyceridemia, are not well understood in terms of either genetics or biochemistry (discussed above). Undoubtedly, the genetic factors affecting "normolipidemic" variation (Table 11) contribute incrementally to certain multigenic hyperlipidemias. At present, it is obviously of great interest to identify major genes involved in other relatively common familial hyperlipidemias. This will require the detailed characterization of large families with markers for as many candidate genes as possible, and given the likely heterogeneity of hyperlipidemias, a large number of families will have to be studied.

Hypoalphalipoproteinemia. A large number of epidemiological studies have revealed a strong inverse correlation between levels of HDL-cholesterol (as well as the HDL proteins apoA-I or apoA-II) and atherosclerosis (reviewed in 245, 246). Some studies have suggested that over 50% of patients with premature coronary artery disease have an HDL cholesterol level below the 10th percentile (246). The association presumably reflects the role of HDL in "reverse cholesterol transport." As discussed previously, extremes of HDL concentrations (hyperalphalipoproteinemia or hypoalphalipoproteinemia) show familial aggregation with evidence for polygenic heritability (20, 21). Several studies have now revealed associations between polymorphisms of the apoA-I-C-III-A-IV locus and hypoalphalipoproteinemia and heart disease (Table 11). It is tempting to speculate that polymorphisms of the locus affect the development of atherosclerosis as a result of effects on HDL levels, although the biochemical mechanisms involved are unknown and further family studies are required to confirm the associations. However, since hypoalphalipoproteinemia has multigenic contributions,

TABLE 11. Summary of common gene polymorphisms associated with plasma lipid phenotypes or coronary artery disease in humans

Gene Locus	Phenotype	References
LDL receptor	Familial hypercholesterolemia and atherosclerosis	3, 304
ApoA-I-C-III-A-IV	Hypertriglyceridemia (type IV and type V)	91, 92, 94, 98
ApoA-I-C-III-A-IV	Hypoalphalipoproteinemia	102–104
ApoA-I-C-III-A-IV	Coronary artery disease	95, 102, 103, 305
ApoA-I-C-III-A-IV	Familial combined hyperlipidemia	101
ApoA-II	HDL size and apolipoprotein composition	36, 106
ApoA-II	Hypertriglyceridemia (type IV and V)	107
ApoB	Blood cholesterol and apoB levels	148–150
ApoB	Coronary artery disease	151
ApoB	Triglyceride levels	148, 149
ApoE-C-I-C-II	Type III hyperlipoproteinemia and other hyperlipidemias	48, 169, 173, 186–188, 306
ApoE-C-I-C-II	Levels of blood cholesterol, apoE, and apoB	48, 185
ApoE-C-I-C-II	Coronary artery disease	173, 175, 307–309
Haptoglobin (linked to CETP, LCAT)	Ratio of HDL-cholesterol to apoA-I	52

other loci must be involved in some forms of the disorder. Possible candidate genes include LPL, HL, CETP, apoA-II, apoD, LCAT, and the HDL receptor. Some extreme deficiencies of HDL, such as those involving apoA-I and apoC-III gene rearrangements, result in severe premature atherosclerosis (Table 2). Just as FH provides strong presumptive evidence for the atherogenic potential of LDL, these deficiencies support the concept that HDL have antiatherogenic potential. There are exceptions, however; for example, patients with Tangier disease have less than 10% of the normal levels of HDL but show only moderately increased atherosclerosis. How can this be explained? There may, of course, be other hereditary or environmental factors affecting atherosclerosis in these patients, or the various HDL deficiencies may result in different alterations of atherogenic lipoprotein particles. Another possibility is that the important parameter with respect to atherosclerosis is not the absolute levels of HDL but rather the functional capacity of HDL. While HDL levels may in general correlate with HDL functional capacity, this need not be so in all cases. For example, CETP deficiency may result in increased HDL levels while, at the same time, adversely affecting reverse cholesterol transport.

Normolipidemic variations. Recent studies of the four apolipoprotein loci using the "candidate gene" approach have revealed associations with normolipidemic variations of blood lipid levels and with coronary artery disease (Table 11). Whether the effects on blood lipid levels explain the associations with coronary artery disease is unclear; they could also be due to associations with altered lipoprotein structures, as discussed below. The significance of the associations with triglyceride levels is unclear, since there is no strong evidence that elevated triglycerides contribute directly to the disease, although high triglyceride levels may well signal the presence of other disorders. With the exception of apoE, the mechanisms involved in the associations with altered lipid levels are unknown and, in fact, the involvement of the loci with altered blood lipids should be considered provisional until confirmation by family studies.

Structural variations. In addition to altered lipoprotein levels, certain heritable structural alterations of lipoproteins are associated with atherosclerosis. Heterogeneity of LDL, as revealed by analytical ultracentrifugation and polyacrylamide gel electrophoresis, has been recognized by many investigators (247, 248). One relatively common structural variation of LDL associated with atherosclerosis is hyperapobetalipoproteinemia (hyperapoB), characterized by small dense LDL particles and elevated apoB levels (138, 144, 249). Another lipoprotein variation which can be considered structural is Lp[a]. Lp[a] appears to contribute to atherosclerosis by a mechanism independent of increased blood cholesterol levels (14). Since important progress in characterizing the apo[a] component of Lp[a] has recently been made (203), it will soon be possible to test whether levels of the protein result from polymorphisms of the apo[a] gene locus. How such variations affect atherosclerosis is unknown, but it has been speculated that structural alterations of lipoproteins could affect infiltration of the artery wall or uptake by macrophages to produce foam cells (237, 248, 250). Of interest with respect to this question is the preliminary report that an allele of apoB in the pig is associated with enhanced LDL uptake by macrophages and atherosclerosis but not with elevated LDL cholesterol levels (251). A common polymorphism of the apoA-II locus, associated with altered HDL apolipoprotein composition (Table 11), was not associated with atherosclerosis in one study (107).

Animal models. Genetic studies involving various animal models are also proving extremely useful for analysis of aspects of atherosclerosis. The rabbit animal model for FH (the WHHL rabbit) has helped to define biochemical mechanisms contributing to the hyperlipidemia (69) and to the pathogenesis of the disease (240). Also, Rapacz and coworkers (38) have identified polymorphisms with striking effects on LDL levels and atherosclerosis in the pig animal model. Detailed characterization of the nature of the polymorphisms is likely to provide important information about mechanisms involved in hyperlipidemias. In particular, molecular studies of various alleles of pig apoB (one of the loci clearly involved in the hyperlipidemias) should allow identification of regions of the molecule important in controlling LDL expression.

Strong evidence for a relationship between HDL levels and atherosclerosis has recently been obtained in mice (67). Strains of mice exhibit striking differences in susceptibility to aortic intimal lipidosis when fed high fat diets (37), and strain surveys have revealed that susceptible but not resistant strains tend to exhibit decreases in HDL levels when fed such diets (88). Genetic crosses among certain resistant and susceptible strains indicate that aortic intimal lipidosis and high density lipoprotein levels cosegregate as a single Mendelian gene (designated *Ath-1*) which maps about 6 centimorgans proximal to the apoA-II structural gene on mouse chromosome 1 (67). The identity of this gene, and how it acts to affect HDL levels, are unknown, but it is located in a linkage group which is conserved between humans and mice, and, therefore, a homologous gene is probably linked to apoA-II in humans. Although large (several-fold) genetic variations of LDL/VLDL levels occur among inbred strains of mice, these do not appear to be associated with aortic intimal lipidosis. The explanation for this may have to do with the relatively low levels of these lipoproteins in mice as compared to a number of other mammals (39).

Prospects. The identification and analysis of genetic factors affecting blood lipids have important clinical implications for the problem of atherosclerosis. The association

of alleles of apoE with type III hyperlipoproteinemia has already demonstrated the utility of genetic markers for disease diagnosis. Similarly, with the identification of major defective alleles of the LDL receptor in various populations (42, 71), it should be possible to rapidly screen potentially affected individuals from those populations for FH. In addition, genetic markers may well prove helpful in targeting therapy for patients; for example, the therapeutic efficacy of the lipid-lowering drug probucol depends in part on apoE structural phenotypes (252). Clearly, additional useful markers will be forthcoming. Whether it will be possible to reliably predict individuals at risk for atherosclerosis using a panel of different genetic markers, as has been speculated, is uncertain. It seems unlikely that genetic markers will replace the need for quantitative lipoprotein determinations; rather they will probably be used in combination to assess risk or to target therapy. Certainly, one important result of the work will be a better understanding of mechanisms and interactions involved in plasma lipid transport, which will facilitate the development of new therapies.

Conclusions

Human and animal studies have demonstrated that the individual variations of plasma lipoprotein structures and levels are determined in large part by hereditary influences. Genetic variations at numerous loci appear to be responsible. The work reviewed here is directed at identifying and characterizing these variations. For reasons discussed above, a strategy involving studies of "candidate genes" is likely to prove most useful in identifying genetic factors contributing to phenotypic alterations of lipoproteins in both humans and animal models. At present, a large number of relevant candidate genes have been characterized, while many others are as yet poorly understood.

The "candidate gene" approach has now been applied to the four loci encoding the major apolipoproteins. Common polymorphisms of all four loci have been associated with alterations of plasma lipoproteins or with coronary artery disease (Table 11). Some of the associations are quite convincing, but most require further confirmation. Moreover, family studies are necessary to prove that the loci are involved in the phenotypic alterations of blood lipids. Only in the case of the LDL receptor and apoE is there any information concerning the molecular mechanisms involved in common lipoprotein variation.

The associations identified thus far account for only a fraction of the observed phenotypic variations of plasma lipoproteins. However, it is likely that further population and family studies utilizing the candidate gene approach will result in the identification of numerous additional genetic factors affecting blood lipids. With the development of a complete human linkage map based on polymorphic DNA markers (253), it will also be possible to map genes that affect lipoproteins but have not as yet been characterized. Such studies should lead to the identification of certain genetic factors with relatively gross effects on plasma lipoproteins, although the detailed analysis of complex dyslipoproteinemias, involving multiple gene contributions or gene interactions, represents a very formidable challenge unlikely to be achieved in the near future. Also, whether such studies will allow most of the "normal" interindividual genetic variation of plasma lipoproteins to be explained is unclear. In particular, genetic and biochemical analyses of gene variations with relatively subtle effects will be difficult, given the problems resulting from environmental effects and from multifactorial inheritance.

Because of the difficulties with human studies, animal models will probably be crucial in understanding mechanisms contributing to genetic variations of blood lipids. The important information that has been derived from studies of the FH rabbit illustrates the advantages of an animal model for both physiological and clinical questions. The mutations identified by Rapacz and colleagues (38) in pigs will probably be at least as important. Because of tremendous advantages for genetic analysis, the mouse is likely to be the most important animal model for studies of variations with relatively subtle effects on plasma lipids. Experimental approaches in animals should also permit the testing of hypotheses. At present, several laboratories are utilizing transgenic mice to examine questions pertaining to the regulation and function of genes involved in plasma lipid metabolism. In the future, it will likely be possible to introduce specific genetic mutations into the germ line of animals by means of targeted mutagenesis (254) in combination with transgenesis by embryonic stem cell lines (255).

These efforts will have important consequences for the problem of atherosclerosis, and they may also be of interest for other clinically important problems such as gallstone disease (212), obesity (256), disorders involving amyloidosis (257), and aspects of host defense (258, 259). Moreover, the combination of biochemical and genetic analyses provides a very powerful approach for examining basic aspects of lipid metabolism and homeostasis.

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