review

Genetics of LDL particle heterogeneity: from genetic epidemiology to DNA-based variations

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Abstract **Substantial evidence exists suggesting that small, dense LDL particles are associated with an increased risk of coronary heart disease. This disease-related risk factor is recognized to be under both genetic and environmental influences. Several studies have been conducted to elucidate the genetic architecture underlying this trait, and a review of this literature seems timely. The methods and strategies used to determine its genetic component and to identify the genes have greatly changed throughout the years owing to the progress made in genetic epidemiology and the influence of the Human Genome Project. Heritability studies, complex segregation analyses, candidate gene linkage and association studies, genome-wide linkage scans, and animal models are all part of the arsenal to determine the susceptibility genes. The compilation of these studies clearly revealed the complex genetic nature of LDL particles. This work is an attempt to summarize the growing evidence of genetic control on LDL particle heterogeneity with the aim of providing a concise overview in one read.**—Bossé, Y., L. Pérusse, and M-C. Vohl. **Genetics of LDL particle heterogeneity: from genetic epidemiology to DNA-based variations.** ***J. Lipid Res.*** **2004.** 45: **1008–1026.**

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LDL cholesterol is a well-known risk factor for coronary heart disease (CHD) and is now recognized as the primary target of lipid-lowering therapy (1). However, it is known that LDL particles are heterogeneous in terms of size, density, chemical composition, and electric charge (2–4). Data from case-control (5) and prospective (6–9) studies have suggested that small, dense LDL particles are associated with increased risk of CHD. The atherogenicity of these particles is attributed to several possible biological mechanisms, including greater susceptibility to oxidation (10–14), decreased affinity for the LDL receptor (15–19), increased binding to the arterial wall (20–23), and greater facility to cross the arterial wall (24, 25) as well as having negative effects on the endothelium function (26). Additional evidence for the relevance of the small, dense LDL on atherosclerotic lesion development and CHD progression is derived from an animal model (27) and lipid-lowering trials in humans (28, 29). On the other hand, recent findings from the Cholesterol and Recurrent Events trial (30) support earlier case-control (31–34) and prospective (35) studies showing that small, dense LDLs are not risk factors for CHD. In fact, some of these studies have shown that larger LDL particles are associated with CHD. Although these studies disagree on which LDL particle size (small or large) is related to CHD risk (36), defining the genetic and environmental factors that modulate LDL particle properties may be helpful in understanding its relationship with CHD.

Multiple approaches have been used to determine the genes involved in complex human diseases and disease-related risk factors. Through the years, methods and strategies have evolved following the progress made in genetic epidemiology and the completion of the Human Genome Project. Genetic studies on LDL particles represent a perfect example of this phenomenon. Several studies have investigated the genetics of LDL particle heterogeneity. Heritability studies, complex segregation analyses, linkage and association studies with candidate genes, and genome-wide linkage scans are all part of the arsenal used to

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Abbreviations: apoB, apolipoprotein B; BMI, body mass index; CAD, coronary artery disease; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; DGU, density gradient ultracentrifugation; DZ, dizygotic; FATP1, fatty acid transport protein-1; FCHL, familial combined hyperlipidemia; GET, Genetic Epidemiology of Hypertriglyceridemia; GGE, gradient gel electrophoresis; HDL-C, high density lipoprotein cholesterol; LDL-PPD, low density lipoprotein peak particle diameter; LDLR, low density lipoprotein receptor; LDL-Rf, low density lipoprotein flotation rate; LOD, logarithm of the odds; MTP, microsomal triglyceride transfer protein; MZ, monozygotic; QFS, the Québec Family Study; QTL, quantitative trait locus; RFLP, restriction fragment length polymorphism; SOD2, manganese superoxide dismutase; SR-BI, scavenger receptor class B type I.

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dissect the genetic architecture of this trait. Cumulative evidence is growing rapidly, and a review of these studies seems timely.

Several studies have shown that small, dense LDLs are associated with a constellation of other well-recognized lipoprotein-related risk factors, including increased plasma triglyceride and apolipoprotein B (apoB) levels as well as decreased HDL cholesterol concentrations. Furthermore, small, dense LDL particles coexist in the same subjects as part of multifaceted phenotypes, including the metabolic syndrome, the atherogenic lipoprotein phenotype (LDL subclass pattern B), and familial combined hyperlipidemia (FCHL) (37). Thus, small, dense LDL may be a qualitative trait representing a common atherogenic lipoprotein/metabolic profile, and the proposed genetic loci responsible for small, dense LDL may in fact be responsible for a more extensive syndrome. However, throughout this review, we have chosen to adopt a more narrow view on the phenotypes that characterize LDL particle heterogeneity. These phenotypes are the central focus of this paper, and we summarize the published genetic studies surrounding them.

A number of analytical techniques are available for characterizing LDL heterogeneity, and it is beyond the scope of the present paper to describe them in detail. However, some technicality must be addressed before going through genetic ground. LDL heterogeneity was first described using analytical ultracentrifugation (38). Over the years, this technique was replaced by others, including density gradient ultracentrifugation (DGU), gradient gel electrophoresis (GGE), and, more recently, NMR spectroscopy. The phenotypes derived from these techniques are those used in the genetics studies performed to date. Based on GGE, a continuous variable can be defined as LDL peak particle diameter (LDL-PPD), reflecting the size of the major LDL subclass in an individual subject. A dichotomous classification can also be defined based on GGE; it is referred to as LDL subclass patterns, or phenotypes, A and B. LDL subclass phenotype A is characterized by a predominance of large LDL particles and skewing of the densitometric scan toward small particles, whereas LDL subclass phenotype B is characterized by a predominance of small LDL particles and skewing of the curve toward large particles (39). Other phenotypes can be constructed using GGE, including LDL score, which is calculated using the migration distance (in millimeters) of each peak multiplied by its respective relative area (40), and LDL type, which is a weighted average of seven possible categories of LDL, resulting in a variable ranging from 1 (largest) to 7 (smallest) (41). For a detailed description of these techniques, the reader is referred to previously published reviews (5, 42, 43).

GENETIC EPIDEMIOLOGY

Familial aggregation

The first evidence for a genetic determination of LDL properties was reported by Fisher et al. in 1975 (44). Five families, including 11 couples and 16 offspring, were examined for their LDL molecular weights. Only subjects having monodisperse LDL (i.e., LDL that is found to be present as a single, essentially homogeneous population of macromolecules) were included in the study. Correlation coefficients between pairs of relatives revealed a significant parent-offspring correlation (0.82; $P < 0.01$) but absence of correlation between fathers and mothers (0.32; P = NS). These results provided evidence for the genetic contribution of LDL molecular weight. To further determine the degree of resemblance of the offspring to their parents, a regression coefficient of the mean molecular weight of the offspring on the mean parental molecular weight was calculated. The regression coefficient was 0.30 ($P < 0.01$), which made the authors conclude that ~30% of the observed LDL molecular weight variance is attributable to additive gene action. In addition, based on the five families, the authors postulated a model consistent with a single-gene (two-allele) locus genetic mode of inheritance without dominance. Although the sample size used in this study was relatively small, it demonstrated for the first time that LDL characteristics segregate within families.

Since this earlier report, accumulating evidence of familial and ethnic aggregation of LDL subclasses has emerged in the literature. Haffner et al. (45, 46) demonstrated a significant difference between ethnic groups in LDL size among 1,571 subjects from the Insulin Resistance Atherosclerosis Study and 466 subjects from the San Antonio Family Heart Study. These studies cannot distinguish the effect of the genetic background from the effect mediated by the difference in lifestyles between ethnic groups, but they clearly motivated genetic studies in the field. More recently, the familial resemblance of LDL-PPD was evaluated in 681 individuals participating in the Québec Family Study (QFS) (47). An ANOVA comparing between-family and within-family variance indicated that there was approximately two times more variance between families than within families. Thus, results from the QFS suggested that the family lines accounted for close to 50% (47–49% depending on covariate adjustment) of the variance in LDL-PPD phenotype. In addition, the pattern of familial correlations revealed no spouse correlation but significant parent-offspring and sibling correlations for the LDL-PPD phenotypes, suggesting that genetic factors are the major determinants of the familial aggregation. The same pattern of correlations was observed among Finnish families with FCHL (48).

Heritability analysis

Twin studies. Studies using identical [monozygotic (MZ)] and fraternal [dizygotic (DZ)] twins have been used to assess the heritability of LDL size (**Table 1**). The first study on this issue was based on 119 MZ and 113 DZ twin pairs participating in the third examination of the National Heart, Lung, and Blood Institute Twin Study (49). In this study, the LDL subfractions were separated by GGE and the heritability analysis used LDL type. The LDL type in-

TABLE 1. Heritability analyses on LDL particle characteristics

Reference	Study	Subject Characteristics	Phenotype	Heritability		
				Methods	Covariates or Assortment	Results
Lamon-Fava et al. (49)	The third examination of the National Heart, Lung, and Blood Institute Twin Study	119 MZ and 113 DZ male twin pairs aged 59–70 years	LDL type	ANOVA (among component		0.52 ($P = 0.12$)
				ANOVA (among component)	BMI, alcohol consumption, cigarette smoking, and physical activity	0.39 ($P = 0.39$)
Austin et al. (51)	The second examination of the Kaiser Permanente Women Twins Study	203 MZ and 145 DZ female twin pairs with a median age of 51 years; 90% were white	LDL-PPD	Classic	All pairs	0.54 ($P < 0.001$)
					Postmenopausal pairs	0.55 ($P < 0.003$)
					Nondiabetic pairs	0.35 ($P < 0.016$)
					Non β-blocker-user pairs	0.45 ($P < 0.002$)
					Caucasian pairs	0.51 ($P < 0.001$)
				ANOVA (within pair)	All pairs	0.48 ($P < 0.001$)
					Postmenopausal pairs	0.34 ($P < 0.021$)
					Nondiabetic pairs	0.44 ($P < 0.001$)
					Non β-blocker-user pairs	0.52 ($P < 0.001$)
					Caucasian pairs	0.43 ($P < 0.001$)
Edwards et al. (52)	The GET Study	85 families at high risk for cardiovascular disease including 780 individuals, primarily white	LDL-PPD	Maximum likelihood-based approach	Age and sex	0.34 ($P < 0.001$)
Bossé et al. (47)	The Québec Family Study	681 French Caucasians	LDL-PPD	Familial correlations under the most parsimonious model	Age, age^2, and age^3	0.59 (95% CI 0.43–0.75)
					Age, age^2, age^3, and BMI	0.58 (95% CI 0.42–0.75)
					Age, age^2, age^3, BMI, and triglyceride	0.52 (95% CI 0.46–0.58)
Barzilai et al. (53)	The Longevity Genes Project	429 Ashkenazi Jews with exceptional longevity	LDL size (NMR)	Linear regression	Men	0.60 ($P = 0.006$)
					Women	0.46 ($P = 0.003$)
Rainwater, Martin, and Comuzzie (54)	The San Antonio Heart Study	1,157 Mexican Americans	ΔLDL[a]	Maximum likelihood-based approach	Sex, age, age^2, diabetes status, contraceptive use, and hypertension medications	0.44 ($P < 0.001$)
					Sex, age, age^2, diabetes status, contraceptive use, hypertension medications, and triglyceride	0.30
Austin et al. (81)	The GET Study	140 subjects, members of 26 kindreds	LDL-PPD	Maximum likelihood-based approach	Sex, age, oral contraceptive use, menopausal status, and hormone replacement therapy	0.26 ($P = 0.025$)
		Primarily Caucasians			+ triglyceride	0.12 ($P = 0.168$)
					+ HDL-C	0.15 ($P = 0.121$)
					+ triglyceride and HDL-C	0.10 ($P = 0.213$)

BMI, body mass index; CI, confidence interval; DZ, dizygotic; GET, Genetic Epidemiology of Hypertriglyceridemia; HDL-C, HDL cholesterol; LDL-PPD, low density lipoprotein peak particle size; MZ, monozygotic.

[a] ΔLDL is a metrics for particle size phenotype to optimally reflect the size correlations between LDL and HDL particles.

traclass correlation coefficient in MZ twins was significantly higher than the correlation coefficient in DZ twins (0.58 vs. 0.32; $P < 0.005$), with a heritability of 0.52 before controlling for covariate effects. After adjustment for body mass index (BMI), alcohol consumption, cigarette smoking, and physical activity, the heritability decreased to 0.39. Despite being of great magnitude, these estimates were not statistically significant, suggesting the lack of heritability for LDL type. Similar results were obtained when only the major LDL band (LDL-PPD) was used as a variable. Thus, the authors concluded that LDL particle size is not greatly influenced by genetic factors within this population. It is noteworthy that the authors used the more conservative among-component (50) estimate of heritability because there was some indication of unequal total variance between zygosities. Although this procedure is considered more suitable in such cases, the power to detect significant heritability is substantially reduced.

The heritability estimates were also analyzed based on 203 MZ and 145 DZ pairs of adult female twins who participated in the second examination of the Kaiser Permanente Women Twins Study (51). The classic heritability estimate for LDL-PPD was 0.54, and the within-pair estimate was 0.48. These estimates were not changed substantially when the analyses were restricted to postmenopausal, nondiabetic, non-β-blocker users or Caucasian pairs, with

heritability ranging from 0.34 to 0.5. Thus, the authors suggested that between one-third and one-half of the variability in LDL size appears to be attributable to genetic influences in this sample of female twins.

Family studies. Heritability estimates of LDL-PPD were also evaluated using family data. The first family study on this issue was based on 780 individual members of 85 families participating in the Genetic Epidemiology of Hypertriglyceridemia (GET) Study (52). The GET Study is based on two family studies, one ascertained through hyperlipidemic probands surviving a myocardial infarction and the second through hypertriglyceridemic probands without CHD. After accounting for age and sex effects, results suggested that approximately one-third of the residual variance in LDL-PPD (heritability = 0.34) was attributable to additive genetic effects. The heritability estimate for LDL-PPD from the QFS was slightly higher (47). In this study, three LDL-PPD phenotypes based on three different adjustment procedures were constructed: LDL-PPD1 adjusted for age, LDL-PPD2 adjusted for age and BMI, and LDL-PPD3 adjusted for age, BMI, and triglyceride levels. Heritability estimates for the three phenotypes were 58.8, 58.4, and 52.0%, respectively. The high heritabilities obtained may be explained by the design of the study. Indeed, in this case, heritability is defined as the proportion of variance attributable to additive familial effects, including both genetic and nongenetic sources of variance. Although the pattern of familial correlations in the QFS suggested that the familial resemblance is mostly attributable to genetic factors, heritability estimates derived from this cohort may be considered as the upper bound estimates for LDL-PPD. High heritability coefficients were also observed for LDL size in Ashkenazi Jewish families ascertained for exceptional longevity (53). In this study, LDL size was characterized by NMR, and heritability was estimated at 0.46 in women and 0.60 in men. These results demonstrated that LDL size is highly heritable irrespective of the analytical methods used to characterize the particles and suggested that the measurement error inherent in each technique does not mask the genetic signal.

By means of a new metric representing coordinated size variation between HDL and LDL particles, Rainwater, Martin, and Comuzzie (54) conducted an original study to test the hypothesis that there are "lipoprotein size genes." The new metric, named ΔLDL, is a metric for LDL particle size phenotype that optimally reflects the size correlation between LDL and HDL particles. ΔLDL was subjected to quantitative genetic analyses using 1,157 Mexican Americans participating in the San Antonio Family Heart Study. The heritability of ΔLDL was highly significant and indicated that nearly half (44%) of the residual variance (after adjustment for sex, age, diabetes status, contraceptive use, and hypertension medications) in ΔLDL was explained by additive gene effects. After including triglyceride levels in the model as a covariate, the heritability estimate decreased from 0.44 to 0.30, indicating gene(s) common for both traits. These data indicate that particle size phenotypes are under substantial genetic control.

Taken together, the above studies suggested that 30% to 60% of the variance in LDL particle size is attributable to genetic factors, with the remainder attributable to nongenetic influences. Accordingly, these genetic studies also indicated the importance of nongenetic factors on LDL subclasses, because ~50% of the variance is attributable to nongenetic factors. A number of environmental influences have been identified, including dietary factors (55), physical activity (56), abdominal obesity (57), and insulin resistance and hyperinsulinemia (58). The combination of genetic and environmental influences provides opportunities to develop prevention strategies to reduce CHD risk among genetically susceptible individuals (59).

Inheritance of the LDL heterogeneity: testing for a single-gene effect

Heritability estimates obtained from twin and family studies reinforced the interest in finding gene(s) underlying that genetic effect. Below, we describe the different lines of evidence that proved the existence of a single gene with a major effect on the phenotype, including commingling analyses, segregation analyses, and complex segregation analyses.

Commingling analyses. Commingling analyses are often used as a preliminary method to screen for the presence of a single gene with a major effect (60). In the presence of such a gene, the distribution of a quantitative phenotype is better characterized by a mixture of distributions than by a single distribution. An analysis of the LDL-PPD distribution in the Kaiser Permanente Women Twins Study identified three distinct subgroups of subjects (51). Indeed, a trimodal model provided a better fit to the data compared with a bimodal or a unimodal model. A trimodal model was also reported for the age- and gender-adjusted LDL-PPD distribution in a sample of healthy families from a Mormon community (61). Finally, in 373 family members of Israel origin, the mixture of two normal distributions for age- and gender-adjusted LDL-PPD fit the data significantly better than a single distribution (62). However, the trimodal model could not be rejected over the bimodal model. Taken together, results from commingling analyses are compatible with the presence of a major gene effect affecting LDL-PPD. However, this pattern of distribution may also arise as a result of nongenetic factors. Thus, any inference drawn from these studies needs to be interpreted with caution.

Segregation analyses. The first studies that investigated the inheritance of LDL heterogeneity were derived from fitting the data into pedigrees under a hypothetical genetic mode (**Table 2**). Fisher et al. (44) were the first to provide evidence for a single-gene, two-allele system locus affecting LDL heterogeneity. Using pedigrees from five families, they proposed a model of two alleles, one a determinant for high, the other for low, LDL molecular weight. A decade later, Austin and Krauss (63) evaluated the lipoprotein subclasses (pattern A/B) by GGE in 79 healthy members of 16 nuclear families living in a local Mormon community. Their data proposed a genetic model consistent with a single-locus, two-allele system as well. The esti-

mated frequency of the allele leading to the phenotype characterized by a predominance of small, dense LDL subclasses (pattern B) was ~15% under a dominant mode of inheritance. However, in contrast to the observation of Fisher et al. (44), expression of the phenotype appears to be age dependent in that most affected subjects in the population were older than 40 years. Although different techniques were used to detect LDL properties between these two studies, it is possible that the LDL pattern reported in the latter study and the molecular weight reported in the former study represent the same trait. Although these studies were limited by their sample size, they provided additional evidence in favor of a single gene affecting LDL density and size.

Complex segregation analyses. The presence of a major gene effect in addition to its mode of inheritance also has been investigated using complex segregation analyses. The results of these studies are summarized in Table 2. Two years after proposing a single-gene, two-allele system locus affecting LDL patterns, Austin et al. (64) confirmed their results on an enlarged sample of the same Mormon community containing 61 healthy families with 301 members. The model providing the best fit to the data included a single gene with a dominant mode of inheritance and a frequency of 25% and reduced penetrance for men younger than 20 years and for premenopausal women. It should be noted, however, that both recessive and additive modes of inheritance could not be rejected. Similar results were observed for 234 individuals in 78 nuclear families with FCHL (65). In this sample, complex segregation analyses suggested that LDL subclass pattern B is controlled by a single major genetic locus (with either a dominant or an additive mode of inheritance) and a small, but significant, multifactorial inheritance component. The prevalence of the LDL subclass pattern B allele was also common in these families (~0.30), suggesting that the proposed allele for pattern B is just as likely to occur in families with FCHL as in healthy families. Again, reduced penetrance of the pattern B allele in FCHL families was observed for men younger than 20 years and for women younger than 50 years.

The two later complex segregation studies were based on the dichotomization of the LDL subfraction into two discrete phenotypes. It is possible that this dichotomous definition oversimplifies the biochemical heterogeneity of LDL particles. de Graaf et al. (66) were concerned by such a procedure because much information is lost, i.e., we do not know whether an individual is close to or far from the LDL size threshold for the pattern A/pattern B classification, which results in a loss of power (67). Accordingly, they constructed a continuous variable, named parameter K, that reflects LDL subfraction profile and that is characterized by the relative contribution of the three major LDL subfractions (LDL-1, LDL-2, and LDL-3) determined by DGU. Analysis for this quantitative trait was performed on 19 healthy Dutch families including 159 individuals. Results indicated that the LDL subfraction profile is controlled by a major autosomal, highly penetrant recessive allele with a population frequency of 19% and an additional multifactorial inheritance component. The penetrance of the more dense LDL allele increases with age for both sexes but was higher for men than for women. Furthermore, it appeared that oral contraceptive use was associated with a high penetrance of the more dense LDL subfraction profile. Also concerned by the possibility that the dichotomous trait may not provide the best reflection of LDL size distribution, Austin et al. (61) reanalyzed their healthy subjects living in a Mormon community, this time using LDL-PPD instead of the dichotomous classification reported earlier (64). The model providing the best fit to the data consisted of a single major gene effect with Mendelian inheritance and with no additional multifactorial inheritance component. However, the available sample was not sufficient to distinguish dominant versus recessive mode of inheritance. Thus, analysis of the continuous LDL-PPD variable was not superior to the dichoto-

TABLE 2. Inheritance of LDL particle heterogeneity

Phenotype	Family Status	n Kindreds (n Members)	Major Gene	Mode of Inheritance	Allele Frequency	Polygenic Component	Reference
Segregation analysis							
Pattern A/B	Healthy	8 (79)	Yes	Dominant	0.15	NA	Austin and Krauss (63)
				Recessive	NA		
Molecular weight	Normal and hyperlipidemic	5[a] (38)	Yes	Codominant	NA	NA	Fisher et al. (44)
Complex segregation analysis							
Pattern A/B	Healthy	29 (301)	Yes	Dominant	0.25	0%	Austin et al. (64)
Pattern A/B	FCHL	7 (234)	Yes	Dominant	0.32	1%	Austin et al. (65)
				Codominant	0.34	33%	
LDL-PPD	Healthy	29 (301)	Yes	Undetermined	NA	0%	Austin et al. (61)
LDL-PPD	Healthy	80 (373)	Yes	Codominant	0.24	74%	Friedlander et al. (62)
LDL-PPD	FCHL	48 (553)	Women, yes Men, no	Recessive Polygenic	0.06	0%	Vakkilainen et al. (48)
Parameter K[b]	Healthy	19[a] (159)	Yes	Recessive	0.19	12%	de Graaf et al. (66)
Parameter K	FCHL	40 (623)	Yes	Recessive	0.42	25%	Bredie et al. (68)

FCHL, familial combined hyperlipidemia; NA, not applicable.
[a] Nuclear family.
[b] A continuous quantitative trait estimating the relative contribution of each LDL subfraction.

mous LDL subclass pattern classification in determining the mode of inheritance of LDL subclasses in this healthy sample of families. The mode of inheritance of parameter K was also investigated in a large sample of Dutch families with FCHL (68). The genetic basis of LDL subfraction profile in these families was best described by a common, major autosomal gene effect with a population frequency of 42% and a recessive mode of inheritance with a polygenic heritability component of 25%. Subsequently, the mode of inheritance of LDL-PPD was investigated in 373 subjects from 80 kindreds residing in kibbutz settlements in Israel (62). Complex segregation analyses of sex- and age-adjusted LDL-PPD were inconclusive in this study, because both the mixed recessive genetic model and the mixed environmental model could not be rejected. However, when the regression model for sex and age allowed coefficients to be ousiotype (genotype class) specific, the mixed environmental model was rejected and a major Mendelian model was not. Indeed, a major additive gene (codominant) model for LDL-PPD with an allele frequency of 24% for small LDL particles could not be rejected. In addition, this model contains a large polygenic component (74%). The authors postulated that the ethnic homogeneity and the lifestyle similarity of the sample may explain the high contribution of polygenic factors to LDL-PPD. More recently, the genetic influence of LDL-PPD was modeled in 48 Finnish FCHL families (48). Complex segregation analyses in these families suggested that the trait is the result of the additive effects of multiple genes in which a recessive major gene effect of low frequency (6%) may contribute to large LDL-PPD in women. For men, the authors could not establish that LDL-PPD follows a strictly polygenic model, but the results indicated that LDL size is unlikely to be influenced by a major gene effect in this population.

With the exception of the latter study, results from complex segregation analyses support the concept of a major gene effect involved in LDL size and density. However, some dissimilarities were found between the studies with regard to the mode of inheritance, allele frequency, and the presence or absence of a multifactorial inheritance component. This discrepancy could be explained by differences in family structures, criteria for proband ascertainment, and the use of different techniques to characterize LDL heterogeneity. Nevertheless, these studies unanimously provided evidence about the contribution of a major gene effect and clearly motivate the race to find it.

DNA-BASED VARIANTS

Linkage studies

Many investigators have used linkage analyses to identify the genes underlying the genetic contribution of LDL particle characteristics. The early studies were performed using candidate gene strategies by studying genetic variations located within or in proximity to genes coding protein products known to be involved in lipoprotein/lipid metabolism. On the other hand, recent studies have used a genome-wide scan approach to identify chromosomal regions influencing LDL size-related phenotypes. **Table 3** presents a summary of the loci and genes, ordered by chromosome number, that have provided evidence of linkage using these two strategies. It should be noted that only positive findings are provided in this table, and careful examination of the literature might in fact show significant evidence against linkage for certain loci presented. It is also worth mentioning that opposite results for the same gene may not necessarily imply controversy, given the different study samples.

The APOB gene was of particular interest because it is the principal protein component of LDL particles. Using the classic logarithm of the odds (LOD) score-linkage method, the first two linkage studies clearly rejected the involvement of this locus with LDL subclass pattern B after obtaining LOD scores of −13.3 and −7.5 (69, 70). In addition, no evidence of linkage to the APOB locus was observed for LDL-PPD in families ascertained for coronary artery disease (CAD) (71). However, a subsequent study performed in DZ twin pairs indicated, for the first time, a positive linkage between LDL-PPD and the APOB locus (72). Thus, it is possible that the APOB locus has an effect on LDL size in particular subgroups of the population, perhaps in women. Because low density lipoprotein receptor (LDLR) is responsible for the clearance of apoB-containing lipoproteins, the LDLR locus on chromosome 19p was also a reasonable candidate gene for linkage analyses. Using parametric linkage analyses with reduced penetrance of pattern B, Nishina et al. (73) obtained evidence of linkage to the LDLR locus (LOD = 4.27). This finding was confirmed by a subsequent study using quantitative sib-pair linkage analyses in CAD families (71). Borderline significant evidence of linkage was also observed between the LDLR locus and LDL-PPD in DZ twin pairs from the Kaiser Permanente Women Twins Study ($P = 0.082$) (72). On the other hand, results from the Dutch FCHL families (74) and from families identified through hyperlipidemic probands (75) showed no evidence of linkage between the LDLR locus and either the LDL-PPD or the dichotomized pattern A/B phenotype. It is also worth mentioning that a follow-up study of the original families in which linkage to this locus was demonstrated (73) found no mutation in the coding sequence of the LDLR gene, suggesting that a nearby gene was responsible for the linkage (76). Using the parametric linkage method and adjusting the phenotype for the within-genotype variance, Hokanson et al. (77) found in heterozygous LPL-deficient families a highly significant LOD score of 6.24 between LDL-PPD and the LPL gene, which encodes a rate-limiting enzyme in the formation of LDL particles. However, two other studies were unable to confirm this linkage in different samples (71, 75). To assess whether the hepatic lipase (HL) gene was linked to LDL size, Allayee et al. (78) conducted sib-pair analyses among the FCHL Dutch families using two microsatellite markers located near the HL gene (D15S643 and D15S148). In the quantitative analysis (LDL-PPD), both markers yielded evidence of linkage, and in the qualitative analysis (pattern A/B), only marker D15S643 reached the level of significance. Finally, two

TABLE 3. Evidence for the presence of linkage with LDL particle characteristics

Genes and/or Markers	Location (Mb)[a]	Chromosome Band[a]	Samples	Phenotype	*P* or LOD Values	Reference
D1S203–D1S198	**60.7–66.4**	**1p32.1–p31.2**	681 subjects; 236 nuclear families	LDL-PPD	LOD = 2.6	Bossé et al. (80)
APOB	21.2	2p24.1	119 DZ twin pairs	LDL-PPD	$P = 0.014$	Austin et al. (72)
D2S1384–IRS1	**205.4–227.8**	**2q33.3–q36.3**	681 subjects; 236 nuclear families	LDL-PPD	LOD = 2.3	Bossé et al. (80)
D3S2387–D3S2403	**1–13.1**	**3p26.3–p25.2**	470 subjects; 10 pedigrees	LDL-3	LOD = 2.6	Rainwater et al. (82)
D3S1754–D3S1311	**174.4–193**	**3q26.32–q29**	470 subjects; 10 pedigrees	LDL-3	LOD = 4.1	Rainwater et al. (82)
D4S2397–D4S3248	**27–60**	**4p15.2–q13.1**	681 subjects; 236 nuclear families	LDL-PPD	LOD = 2.2	Bossé et al. (80)
D4S1647–D4S1644	**99.7–142.6**	**4q23–q31.21**	470 subjects; 10 pedigrees	LDL-3	LOD = 4.1	Rainwater et al. (82)
CART–D5S1501	**71.1–78.6**	**5q13.2–q14.1**	681 subjects; 236 nuclear families	LDL-PPD	LOD = 2.4	Bossé et al. (80)
D6S1009–D6S1277	**137.3–164.2**	**6q23.3–q26**	140 subjects; 26 families	LDL-PPD	LOD = 2.1	Austin et al. (81)
D6S1003–D6S1277	**144.3–163.7**	**6q24.2–q27**	470 subjects; 10 pedigrees	LDL-3	LOD = 2.9	Rainwater et al. (82)
SOD2	160	6q25.3	55 sib-pairs	LDL-PPD	$P = 0.001$	Rotter et al. (71)
SOD2 (D6S1008)	163.5	6q26	481 subjects; 18 families	Pattern A/B	$P = 0.020$	Allayee et al. (74)
LPL	19.8	8p21.3	120 subjects; 5 kindreds	LDL-PPD	LOD = 6.2	Hokanson et al. (77)
APOAI-CIII-AIV	116.2	11q23.3	481 subjects; 18 families	Pattern A/B	$P = 0.005$	Allayee et al. (74)
			65 sib-pairs	LDL-PPD	$P = 0.06$	Rotter et al. (71)
D14S592–D14S617	**59.4–90.2**	**14q23.1–q32.12**	681 subjects; 236 nuclear families	LDL-PPD	LOD = 2.8	Bossé et al. (80)
D15S659	**44.1**	**15q21.1**	240 subjects; 18 families	LDL-PPD	LOD = 2.2	Allayee et al. (78)
LIPC (D15S148)	56.8	15q21.3	498 subjects; 18 families	LDL-PPD	$P = 0.008$	Allayee et al. (78)
LIPC (D15S643)	57.4	15q22.2	498 subjects; 18 families	Pattern A/B	$P = 0.035$	Allayee et al. (78)
			498 subjects; 18 families	LDL-PPD	$P = 0.019$	Allayee et al. (78)
CETP (D16S313)	27.1	16p12.1	87 sib-pairs	LDL-PPD	$P = 0.03$	Rotter et al. (71)
CETP	56.8	16q13	119 DZ twin pairs	LDL-PPD	$P = 0.001$	Talmud et al. (79)
CETP/LCAT (D16S496)	68.7	16q22.1	481 subjects; 18 families	LDL-PPD	$P = 0.035$	Allayee et al. (74)
D17S1290-ACE	**56.8–62**	**17q23.2–q23.3**	681 subjects; 236 nuclear families	LDL-PPD	LOD = 6.8	Bossé et al. (80)
LDLR	11.1	19p13.2	102 sib-pairs	LDL-PPD	$P = 0.008$	Rotter et al. (71)
			51 subjects; 9 families	Pattern A/B	LOD = 4.3	Nishina et al. (73)
D19S714–D19S433	**16.1–31**	**19p13.12–q12**	470 subjects; 10 pedigrees	LDL-1	LOD = 2.3	Rainwater et al. (82)
D19S587–D19S178	**35.8–45.1**	**19q13.1–q13.31**	470 subjects; 10 pedigrees	LDL-2	LOD = 1.9	Rainwater et al. (82)
D19S246	**55.6**	**19q13.33**	240 subjects; 18 families	LDL-PPD	LOD = 1.6	Allayee et al. (78)

Status as of December 2003. Chromosomal locations in boldface indicate quantitative trait loci (QTLs) from genome-wide linkage scans. When one marker per line is shown, the marker is the one defining the peak. When two markers per line are shown, they indicate a conservative location interval for the QTL and the LOD score corresponds to the highest peak observed in the region. For candidate genes, the location of the gene is provided if the tested marker is located within the gene. However, if the marker is not within the gene but is located close to it, the specific tested marker is given in parentheses and the location provided corresponds to the marker. ACE, angiotensin-converting enzyme; APO, apolipoprotein; CART, cocaine- and amphetamine-regulated transcript; CETP, cholesteryl ester transfer protein; DZ, dizygotic; LIPC, hepatic lipase; IRS1, insulin receptor substrate 1; LDL-1, -2, and -3, cholesterol concentration in LDL fractions of 26.4–29.0 nm, 25.5–26.4 nm, and 24.2–25.5 nm, respectively; LDLR, low density lipoprotein receptor; LOD, logarithm of the odds; SOD2, manganese superoxide dismutase.

[a] The physical and genetic locations of markers and genes are from the genome browser of the University of California, Santa Cruz (http://genome.ucsc.edu).

other studies excluded the hypothesis of linkage with the HL locus (72, 75). The cholesteryl ester transfer protein (CETP) mediates the transfer of cholesteryl ester from HDL to apoB-containing lipoproteins in exchange for triglyceride and thus constitutes an excellent candidate gene. Three independent studies using all sib-pair linkage analysis have shown consistent evidence of linkage for LDL-PPD at this locus (71, 74, 79). It should be noted, however, that the LCAT gene, which is responsible for the esterification of free cholesterol within HDL particles, is located near (~10 Mb) the CETP locus and might be responsible for the signal. The APOAI-CIII-AIV gene cluster is also an interesting genetic locus potentially affecting LDL size. Rotter et al. (71) originally suggested linkage to this locus with LDL-PPD ($P = 0.06$). A subsequent study was unable to confirm this linkage with the quantitative phenotype but did so with the qualitative phenotype (74). However, two other studies rejected the hypothesis of linkage to the APOCIII locus (72, 75). Finally, the manganese superoxide dismutase (SOD2) gene was also linked to LDL size more than once. Although the influence of this candidate gene on plasma lipoproteins is less obvious, it was linked to LDL-PPD (71) and the atherogenic lipoprotein pattern A/B (74). However, a subsequent study provided significant evidence against linkage (LOD = −4.52) to the SOD2 locus with phenotype A/B (75). No evidence of linkage was demonstrated for the other candidate genes tested with LDL particle phenotypes, including APOAII (71, 75), APOE-CII-CI gene cluster (71, 72, 75), high density lipoprotein binding protein (71), hormone-sensitive lipase (72), insulin receptor (72, 75), apo[a] (71), and microsomal triglyceride transfer protein (MTP) (72, 75).

Taken together, linkage studies based on the candidate gene approach have provided positive but mainly inconsistent results. Based on these observations, Austin et al. (75) emphasized the necessity of finding new genetic loci,

other than those harboring known candidate genes, to identify genes potentially involved in determining the small, dense LDL phenotype. Genome-wide scans are particularly suited for this purpose. To date, three genome-wide linkage scans have been reported in the literature for LDL-PPD. Results of these genome-wide searches are indicated in boldface in Table 3 and are illustrated in **Fig. 1**. The first whole-genome scan on LDL-PPD was performed on 240 individuals ascertained through 18 unrelated FCHL probands (78). The results suggested a locus located ~12 Mb from the HL gene on chromosome 15 with a LOD score of 2.2. Suggestive linkage (LOD = 1.6) was also observed for a marker located on chromosome 19q13, which contains the APOE-CII-CI gene cluster. The second genome scan was carried out in 681 subjects from 236 nuclear families participating in the QFS (80). The strongest evidence of linkage was found on chromosome 17q23, with a LOD score of 6.76 for the phenotype adjusted for age, BMI, and triglyceride levels. The APOH gene is a particularly interesting candidate gene in this area. Other chromosomal regions provided LOD greater than 2.0, including 1p33-p31, 2q33-q36, 4p15-q13, 5q13-q14, and 14q23-q32. Although this genome scan gives strong evidence for the presence of a major quantitative trait locus (QTL) located on 17q, it also demonstrated the multilocus nature of LDL size. The third genome scan on LDL-PPD was based on 140 subjects from 26 hypertriglyceridemia families participating in the GET Study (81). For the whole-genome scan, only one chromosomal region provided possible evidence of linkage on chromosome 6q (LOD = 2.1). When the LDL-PPD was adjusted for other lipoprotein covariates, the LOD score decreased slightly but the location of the peak remained unchanged, suggesting that the signal is independent of other lipoprotein levels. The SOD2 and apo[a] genes are located within the one LOD score support interval. An additional genome scan of cholesterol concentrations within LDL size subfractions is also worth mentioning. Rainwater et al. (82) found two QTLs on chromosomes 3 and 4 with LOD scores greater than 3 for LDL size 3 (LDL-3), a subfraction that contains small LDL particles. Suggestive linkage was also observed on 3p26-p25 and 6q24-q27 for LDL-3, 19p13-q12 for LDL-1 (a subfraction that contains large particles), and 19q13 for LDL-2 (a subfraction that contains particles of intermediate diameter). This study evaluated LDL size-related phenotypes, but QTLs identified are those affecting the cholesterol concentration within a particular subpopulation of LDL and do not correspond to QTLs affecting the size of the particles.

This genome scan and the three others on LDL-PPD have generated new leads in finding genes involved in LDL particle heterogeneity. Interestingly, these QTLs harbor a good number of candidate genes that have not been tested previously in linkage and association studies. Among these genome scans, only the two suggestive loci observed for LDL-PPD (81) and LDL-3 (82) on chromosome 6q show replication (Fig. 1). This locus contains the SOD2 gene, which has been linked to the phenotype before (71, 74). However, the number of loci identified by genome-wide scans clearly reveals the complex genetic architecture underlying LDL particle characteristics.

Association studies

A considerable number of association studies have been conducted to identify the genes influencing LDL particle characteristics. **Table 4** presents a summary of these studies organized by genes and ordered by chromosome number.

APOE. The gene that encodes apoE lies on chromosome 19, and its three common alleles, ε2, ε3, and ε4, code for the isoforms apoE2, apoE3, and apoE4, respectively. To the best of our knowledge, a total of nine studies have investigated the association between LDL size and apoE genotypes (Table 4). The largest among them by far was conducted by Schaefer et al. (83) with 2,258 men and women participating in the Framingham Offspring Study. In this study, the age-, BMI-, and plasma triglyceride-adjusted LDL particle type (a larger LDL type reflects smaller LDL particles) was significantly different in men with different apoE genotypes. However, the same trend was observed in men and women for higher LDL type from the ε2 to ε4 subjects. The lowering effect of the ε4 allele was confirmed in Japanese subjects (84) and in men of North European descent (85), showing that carriers of this allele had smaller LDL particle size than those without the ε4 allele. Consistent with these observations, Haffner et al. (86) demonstrated a progressive decrease in LDL size in both men and women from apoE2/3, apoE3/3, and apoE3/4 genotypes. The same study also showed that the risk of having LDL subclass pattern B was higher for subjects carrying the apoE3/4 genotype compared with both the apoE2/3 and apoE3/3 genotypes. Consistent with these observations, Nikkilä et al. (87) reported that LDL size was lowest in E4/4 and increased in the order E3/4, E3/3, and E2/3. In contrast, an earlier study conducted in healthy middle aged men (88) and a second one performed in children (89) reported no difference in LDL particle size among the different apoE genotypes. To complicate the interpretation even more, two additional studies, one conducted with 132 subjects from a small 800 individual island (90) and the other performed in 212 subjects with or without recent onset of angina (91), reported lower particle size among carriers of the ε2 allele compared with noncarriers. In the former study, the difference disappeared when data were adjusted for plasma triglyceride levels, whereas the effect of the ε2 allele in the latter study was still evident after such adjustment.

HL. The human HL gene is located on chromosome 15q and encodes a protein that plays an important role in lipoprotein metabolism. Two polymorphisms, −250G>A and −514C>T (also referred to as −480C>T), have been tested with LDL particle size/density. These two polymorphisms are in linkage disequilibrium (92), and the rare allele is associated with lower HL activity (93, 94). Zambon et al. (93) were the first to describe an association between the −250G>A polymorphism and LDL particle flotation rate (LDL-Rf) measured by DGU. They have shown that the less common A allele was associated with more buoyant LDL particles among normolipidemic subjects

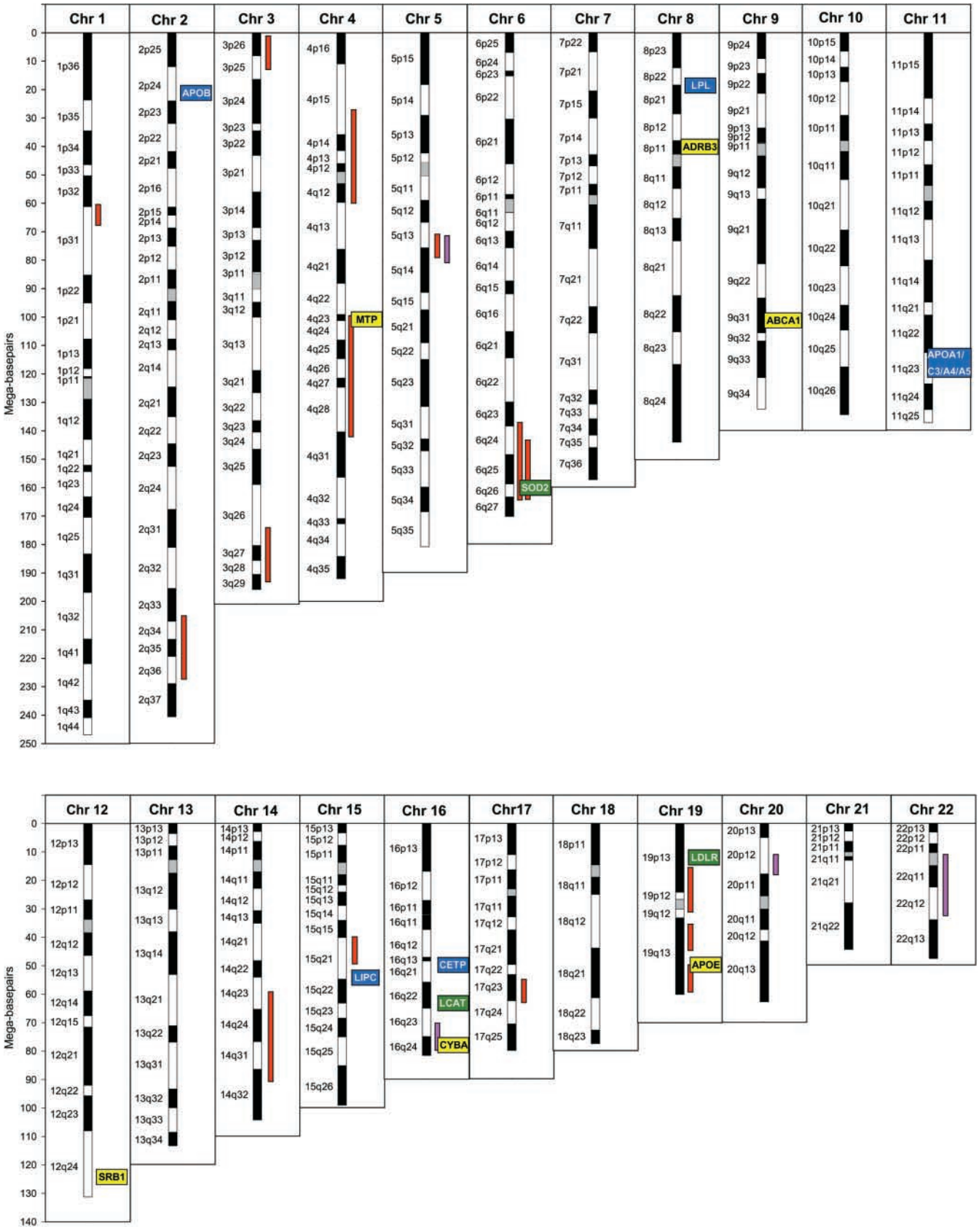

Fig. 1. Ideogram of human karyotype showing chromosomal locations of genes and quantitative trait loci (QTLs) potentially involved in LDL size/density identified from various lines of evidence reviewed in this article. Only positive findings are included (see text for the complete results). Red and purple lines indicate QTLs identified by genome-wide linkage scans in humans and animals, respectively. Genes linked (green rectangles), associated (yellow rectangles), or both linked and associated (blue rectangles) to LDL particle characteristics are shown. Genes and QTLs are placed on the hybrid map showing the sequence and the cytogenetic locations. Information to construct the

and men with CAD. This finding was then replicated in a group of premenopausal women showing more buoyant LDL particles among carriers of the T-514 allele (94). However, a larger study, conducted in 2,667 subjects participating in the Framingham Offspring Study, found no relationship between HL polymorphism at position −514 and the LDL particle size measured by GGE (95). This lack of association between this variant and LDL size measured by GGE was also observed in a subgroup of unrelated subjects from FCHL Dutch families (78) and in a cohort of healthy, middle-aged men (85). The different methods used to characterize LDL particles might explain the inconsistency. However, an additional study rejected the hypothesis of association between the −514C>T polymorphism and LDL particle size measured by NMR (96).

CETP. The CETP gene lies on chromosome 16q and encodes a protein that facilitates the exchange of triglyceride and cholesterol between lipoproteins. The LDL particles of CETP-deficient patients constitute a group of abnormal heterogeneous particles that show polydispersity on GGE with a smaller mean particle size (97). The most studied restriction fragment length polymorphism (RFLP) in this gene, Taq1B in intron 1, was associated with CETP activity and mass. In fact, the B2 allele (absence of the Taq1 restriction site) was associated with decreased CETP activity and mass, which mimics a mild form of CETP deficiency (94, 98). The Framingham Offspring Study again provided the largest population-based cohort (n = 2,916) investigating this polymorphic site with LDL size (98). This study reported that the B2 allele in men was associated with increased levels of the large LDL subfraction, whereas B1B1 homozygous subjects had increased levels of the small LDL subfraction. Therefore, the B2 allele was associated with increased LDL particle size, an effect seen only in men. The effect of the B2 allele was also observed in the Columbia University BioMarker Study, but this time only in women (96). In this study, women homozygous for the B2 allele had larger particles compared with carriers of the B1 allele. This difference was also observed in men, but the small number of men tested might have lacked the power to detect a significant effect. In contrast, the absence of association between the Taq1B polymorphism and LDL-Rf was reported in a cohort of normolipidemic premenopausal women (94). A trend toward greater LDL size with increasing numbers of B2 alleles was observed in the Veterans Affairs HDL Cholesterol Intervention Trial group, but the effect did not reach statistical significance (99). This lack of association was also observed in a cohort of patients with type 2 diabetes (100) and in Japanese subjects (101). However, a second polymorphism in the CETP gene, namely I405V, showed a significant association in this Japanese study. The authors demonstrated lower LDL size in patients with the VV genotype compared with carriers of the I allele. A significant effect of this polymorphism was also observed in families ascertained for exceptional longevity (53). However, in this cohort, subjects carrying the VV genotype had larger LDL particles. The latter study also reported no association between LDL size and a third gene variant in linkage disequilibrium with the Taq1B polymorphism named −629C>A. In contrast, carriers of the −629C>A polymorphism had increased LDL-PPD compared with CETP-629C homozygotes in a cohort of healthy, middle-aged men (85). Finally, a less frequent missense mutation, D442G in exon 15, in the CETP gene was investigated in patients with CAD. The presence of this mutation was associated with lower CETP concentrations and larger LDL size (102).

MTP. The MTP gene, located on chromosome 4q, encodes a protein essential in the assembly and secretion of apoB-containing lipoproteins in hepatocytes and enterocytes. A common functional polymorphism in the promotor of the MTP gene, −493G>T, was investigated in relation to LDL particle size. Couture et al. (103) showed no association between the −493G>T genotype and LDL size in 2,510 subjects participating in the Framingham Offspring Study. This absence of association was also observed in a group of viscerally obese men (104). The only positive association between the −493G>T variant and LDL size was observed in a small sample of Chinese subjects with type 2 diabetes (105). The authors of this study reported smaller LDL particle size among TT homozygotes compared with the other genotypes.

LPL. The LPL gene is located on chromosome 8p and encodes a protein that is responsible for the hydrolysis of triglyceride within apoB-containing lipoproteins. Several mutations have been identified in the LPL gene, and some of them have been associated with LDL particle size. First, a missense mutation at codon 188 causes a clear reduction in LPL activity. Carriers of this defective mutation had smaller LDL size compared with noncarrier family members (106). Similarly, lower LDL size was observed among carriers of the LPL Tyr302-Ter mutation in an Italian family (107). Hokanson et al. (77) confirmed the LDL size-reducing effect of LPL deficiency in five families with structural mutations in the LPL gene. Subsequently, the Ser447-Ter mutation in exon 9 was associated with larger LDL size (85, 108). This mutation causes a premature termination codon that, surprisingly, increases the enzymatic activity of LPL (85, 109). These observations suggested that the mutations that decrease LPL activity cause a reduction in LDL size and that the mutations that increase LPL activity increase LDL size. This hypothesis was confirmed in a cohort of 206 heterozygote subjects carrying either the null P207L or the defective D9N mutation

Fig. 1. *continued* ideogram was obtained from the University of California, Santa Cruz Genome Browser (http://genome.ucsc.edu). The alternating black and white colors on the chromosomes have been used to distinguish a cytogenic band from the adjacent ones and do not correspond to the band colors observed on Giemsa-stained chromosomes. ADRB3, β3-adrenergic receptor; APOB, apolipoprotein B; Chr, chromosome; CETP, cholesteryl ester transfer protein; CYBA, p22 phox; LDLR, low density lipoprotein receptor; LIPC, hepatic lipase; MTP, microsomal triglyceride transfer protein; SOD2, manganese superoxide dismutase; SRB1, scavenger receptor class B type I.

TABLE 4. Association studies between candidate genes and LDL particle characteristics

Genes	Chromosome Band	Mutation	Phenotype	n	Study Population	Effect	Reference
APOB	2p24.1	*Eco*RI	LDL-PPD and LDL score	65	Caucasian men	Carriers = noncarriers	Vohl et al. (115)
		*Xba*I	Relative charge	104	Hypercholesterolemic men	+/+ < −/+ < −/−	Védie et al. (113)
		*Eco*RI				−/− and −/+ = +/+	
		*Msp*I				−/− and +/− > +/+	
		*Bsr*DI				−/− and +/− > +/+	
		I/D				II < ID/DD	
		3′VNTR				SS > SB/BB	
MTP	4q23	−493G>T	LDL size (NMR)	2,510	Framingham Study	GG = GT = TT	Couture et al. (103)
		−493G>T	LDL-PPD	227	Viscerally obese men	GG = GT/TT	St-Pierre et al. (104)
		−493G>T	LDL-PPD	76	Chinese subjects	TT < GT/GG[a]	Chen et al. (105)
ADRB3	8p12–p11.2	W64R	LDL-PPD	136	Japanese subjects	RR/RW < WW	Okumura et al. (117)
LPL	8p21.3	G188E	LDL-PPD	16	LPL deficiency families	GE < GG	Miesenböck et al. (106)
		Y302-Ter	LDL-PPD	22	LPL deficiency family	Carriers < noncarriers	Bertolini et al. (107)
		LPL-HTZ	LDL-PPD	120	LPL deficiency families	LPL-HTZ < LPL-HMZ normal	Hokanson et al. (77)
		S447-Ter	LDL-PPD	189	Japanese subjects	SS < SX/XX	Sawano et al. (108)
		S447-Ter	LDL-PPD	377	Men of North European descent	SS < SX/XX	Skoglund-Andersson et al. (85)
		S447-Ter	LDL size (NMR)	358	Hispanic and non-Hispanic white	SS = SX/XX	Humphries et al. (96)
		D9N and P207L	LDL-PPD	206	LPL deficiency subjects	P207L HTZ < D9N HTZ	Ruel et al. (110)
CYP7	8q12.1	−204A>C	LDL size (NMR)	2,330	Framingham Study	AA = AC = CC	Couture et al. (116)
ABCA1	9q31.1	Compound HTZ	LDL size (NMR)	71	One patient with TD	TD patient < controls	Schaefer et al. (123)
		ABCA1-HTZ	LDL size (NMR)	54	ABCA1 deficiency families	ABCA1-HTZ = controls	Kuivenhoven et al. (124)
APOA5	11q23.3	−3A>G	LDL-PPD	558	Japanese American families	Transmission of allele G ↓ LDL-PPD	Austin et al. (114)
APOC3	11q23.3	*Sst*I	LDL size (NMR)	2,485	Framingham Study	Carriers < noncarriers[b]	Russo et al. (111)
		−455T>C	LDL size (NMR)	358	Hispanic and non-Hispanic white	TT = TC + CC	Humphries et al. (96)
		−455T>C and −625T>del	LDL-PPD	320	Residents from Costa Rica	−455TT−625TT = −455CX−625delX	Brown, Ordovas, and Campos (112)
		C3238G				CC = CG/GG	
		*Sac*I	Relative charge	104	Hypercholesterolemic men	−/− and +/− < +/+	Védie et al. (113)
		−625T>del				−/+ and +/+ < −/−	
		−482C>T				−/− and −/+ = +/+	
APOA1	11q23.3	*Pst*I	Relative charge	104	Hypercholesterolemic men	−/− and −/+ = +/+	Védie et al. (113)
		*Msp*I				−/− and −/+ = +/+	
SR-BI	12q24.31	Exon 1 G>A	LDL size (NMR)	2,650	Framingham Study	GG > GA/AA[a]	Osgood et al. (120)
		Intron 5 C>T				CC = CT/TT	
		Exon 8 C>T				CC = CT = TT	
LIPC	15q21.3	−250G>A	LDL-Rf	128	Normolipidemic and CAD subjects	GG < GA/AA	Zambon et al. (93)
		−514C>T	LDL-Rf	120	Premenopausal women	CC < CT/TT	Carr et al. (94)
		−514C>T	LDL type	2,667	Framingham Study	CC = CT = TT	Couture et al. (95)
		−514C>T	LDL-PPD	225	Dutch subjects	CC = CT/TT	Allayee et al. (78)
		−514C>T	LDL-PPD	377	Men of North European descent	CC = CT/TT	Skoglund-Andersson et al. (85)
		−514C>T	LDL size (NMR)	358	Hispanic and non-Hispanic white	CC = CT/TT	Humphries et al. (96)

(110). In this study, LDL particle size was smaller in the P207L carriers than in the D9N subjects, suggesting that a greater reduction in LPL activity results in smaller LDL particle size. However, this relation between LPL genetic variants, LPL activity, and LDL size is not without controversy, given that the greater LDL particle size observed among carriers of the Ser447-Ter mutation was not confirmed in the Columbia University BioMarkers Study (96).

APOA1/CIII/A4/A5 cluster. The APOA1/CIII/A4/A5 cluster lies on chromosome 11q and encodes four proteins involved in lipoprotein metabolism. Genetic variations within individual genes have been associated with LDL particle characteristics. Russo et al. (111) tested the *Sst*I polymorphism on the 3′ untranslated region of the APOC3 gene and showed that male carriers of the S2 allele had significantly lower concentrations of large

TABLE 4. (*Continued*)

Genes	Chromosome Band	Mutation	Phenotype	n	Study Population	Effect	Reference
CETP	16q13	CETP deficiency	LDL-PPD	6	Two CETP-deficient patients	CETP-deficient < normal	Sakai et al. (97)
		Taq1B	LDL size (NMR)	2,916	Framingham Study	B1B1 < B1B2 < B2B2[b]	Ordovas et al. (98)
		Taq1B	LDL size (NMR)	358	Hispanic and non-Hispanic white	B1B1 + B1B2 < B2B2[c]	Humphries et al. (96)
		Taq1B	LDL-Rf	120	Premenopausal women	B1B1 = B1B2 = B2B2	Carr et al. (94)
		Taq1B	LDL size (NMR)	852	VA-HIT Study	B1B1 = B1B2 = B2B2	Brousseau et al. (99)
		Taq1B	LDL-PPD	60	Subjects with type 2 diabetes	B1B1 = B2B2	Bernard et al. (100)
		Taq1B I405V	LDL-PPD	136	Japanese subjects	B1B1 = B1B2 = B2B2 VV < VI/II	Okumura et al. (101)
		I405V	LDL size (NMR)	1,276	Families with high longevity	II < VV	Barzilai et al. (53)
		−629C>A				CC = CA = AA	
		−629C>A	LDL-PPD	377	Men of North European descent	CC < CA/AA	Skoglund-Andersson et al. (85)
		D442G	LDL-PPD	47	CAD Chinese patients	DD < DG/GG	Wang et al. (102)
CYBA	16q24.2	C242T	LDL-PPD	260	Japanese subjects	CC = CT/TT	Hayaishi-Okano et al. (118)
ACE	17q23.3	I/D	LDL-PPD	136	Japanese subjects	II = ID/DD and DD = DI/II	Okumura et al. (84)
FATP1	19p13.11	Intron 8 G>A	LDL-PPD	373	Swedish men	GG = GA = AA	Gertow et al. (119)
APOE	19q13.32	ε2/3/4	LDL type	2,258	Framingham Study	E4 < E3 < E2[b]	Schaefer et al. (83)
		ε2/3/4	LDL-PPD	136	Japanese subjects	E4 < no E4	Okumura et al. (84)
		ε2/3/4	LDL-PPD	361	Men of North European descent	E4 < E3 = E2	Skoglund-Andersson et al. (85)
		ε2/3/4	LDL-PPD	337	San Antonio Heart Study	E4 < E3 < E2	Haffner et al. (86)
		ε2/3/4	LDL-PPD	321	Men and women of 40 and 70 years of age	E4 < E3 < E2	Nikkilä et al. (87)
		ε2/3/4	LDL-PPD	196	Healthy men	E2 = E3 = E4	Zhao et al. (88)
		ε2/3/4	LDL size (NMR)	505	Children	E2 = E3 = E4	Isasi et al. (89)
		ε2/3/4	LDL-PPD	132	Subjects from Ustica Island	E2 < E3/E4	Barbagallo et al. (90)
		ε2/3/4	LDL-PPD	212	Subjects with or without angina	E2 < no E2	Dart et al. (91)

ADRB3, β3-adrenergic receptor; CAD, coronary artery disease; CYBA, p22 phox; CYP7, cholesterol 7α-hydroxylase; FATP1, fatty acid transport protein-1; HMZ, homozygotes; HTZ, heterozygotes; LDL-Rf, low density lipoprotein flotation rate; MTP, microsomal triglyceride transfer protein; SR-BI, scavenger receptor class B type I; TD, Tangier disease; VA-HIT, Veterans Affairs HDL Cholesterol Intervention Trial; VNTR, variable number of tandem repeats.

[a] Effect seen only in diabetic subjects.

[b] Effect seen only in men.

[c] Effect seen only in women.

LDL and a significant reduction in LDL size. In women, there was no significant effect on LDL size. The other polymorphisms tested in the APOC3 gene (−455T>C, −625T>del, and C3238G) showed no association with LDL particle size (96, 112). However, the *Sac*I and the −625T>del polymorphisms, located in the 3′ untranslated and promotor regions of the APOC3 gene, respectively, were significantly associated with LDL charge (113). In contrast, the same study reported no association between APOA1 restriction sites (*Pst*I and *Msp*I) and LDL charge. Recently, Austin et al. (114) demonstrated, with several analytic approaches, that common single nucleotide polymorphism (SNP) variants in the APOA5 gene were associated with LDL particle size in a community-based sample of Japanese American families. This study particularly pinpointed the −3A>G variant to decrease LDL size. However, considering the close proximity of the four genes in the cluster, it is difficult to determine with certainty that the effect observed with one of them is mediated by the gene tested. Indeed, a positive finding in one gene might be attributable to linkage disequilibrium of the tested marker with a polymorphism in a second gene within the cluster. Accordingly, further studies in the APOA1/CIII/A4/A5 gene cluster will be required to identify the functional site.

Other candidate genes: APOB, cholesterol 7α-hydroxylase, angiotensin-converting enzyme, β3-adrenergic receptor, phox 22, fatty acid transport protein-1, scavenger receptor class B type I, LDLR, and ABCA1. One study verified the effect of the APOB *Eco*RI polymorphism in a group of Caucasian men and showed no effect on either LDL-PPD or LDL score (115). However, five polymorphisms in the APOB gene were found to influence LDL charge heterogeneity evaluated by relative electrophoretic mobility (113). A common A-to-C substitution at position −204 in the promoter of the cholesterol 7α-hydrolase gene showed no association with LDL particle size (116). The hypothesis that the angiotensin-converting enzyme gene insertion/deletion polymorphism

was associated with LDL size was also rejected in a small Japanese cohort (84). However, a recent paper suggested a positive association between the Trp64Arg variant in the β_3-adrenergic receptor and LDL-PPD (117). The authors reported that the LDL particle size was smaller in subjects with the Arg64 allele than those without the Arg64 allele. The effect remained significant after triglyceride adjustment but disappeared after adjustment for BMI or parameters of insulin resistance. The phox 22 gene, which is a small subunit of vascular NAD(P)H oxidase that plays an important role in superoxide production, was also investigated in a group of healthy Japanese subjects (118). A trend ($P = 0.08$) toward larger LDL-PPD was observed among carriers of the C242T polymorphism compared with noncarriers. In addition, the proportion of subjects with pattern B was significantly larger in the CC group than in the CT/TT group. No association was observed between a functional intronic variation in the fatty acid transport protein-1 (FATP1) gene and LDL-PPD in a cohort of healthy Swedish men (119). However, the cholesterol concentration ratio of the largest and smallest LDL subfractions (LDL-I/LDL-III ratio) was found to be different between FATP1 intron 8 genotypes. No clear association was observed between three SNPs located within the scavenger receptor class B type I (SR-BI) gene and LDL size in the Framingham Study (120). However, this study showed reduced LDL particle size in carriers of the A allele at the SR-BI exon 1 gene in the subgroup of diabetic subjects. Finally, no study investigated the effect of common polymorphisms in the LDLR gene on LDL particle characteristics. However, earlier studies showed that the LDL particles of patients with familial hypercholesterolemia are characterized by higher peak flotation rates and lower density (121, 122). Similarly, one patient with Tangier disease was shown to have smaller particle size compared with control subjects, highlighting the possible implication of the ABCA1 gene (123). However, the reduced LDL size effect of a defective mutation in the ABCA1 gene was not reported in a group of heterozygous subjects (124).

COMPLEMENTARY GENETIC APPROACHES

LDL size: a component of the metabolic syndrome and a trait representing a common atherogenic lipoprotein profile

The metabolic syndrome is characterized by a cluster of CAD risk factors, including hypertension, upper body obesity, glucose intolerance, and the atherogenic lipoprotein phenotype, which consists of increased plasma triglyceride levels, low plasma levels of HDL cholesterol (HDL-C), and a predominance of small, dense LDLs (125). The strong association between the small, dense LDL phenotype and the atherogenic lipoprotein profile raises the question of whether the gene proposed by complex segregation analyses is also responsible for the associated lipid and lipoprotein levels. Using factor analysis, Edwards et al. (126, 127) investigated the clustering of risk factors in the Kaiser Permanente Women Twins Study by examining the correlation structure among the components of the metabolic syndrome. Factor analysis reduced 10 correlated risk factors to 3 uncorrelated factors, each reflecting a different aspect of the metabolic syndrome. One of the factors was considered the lipid factor because of the strong factor loading for the lipid variables, including triglyceride, HDL-C, and LDL-PPD. Heritability estimates for the lipid factor were calculated using various approaches and ranged from 0.25 to 0.32. Thus, the authors suggested that approximately one-fourth to one-third of the variance in this composite lipid factor may be attributable to genetic influences. Using a candidate gene strategy, the same research group subsequently found strong evidence of linkage between the lipid factor and the CETP gene (128). The authors proposed that the CETP gene variations influence the covariation in LDL size and triglyceride and HDL-C levels and may account for a portion of the phenotypic correlation between these risk factors.

To investigate the interrelationship between LDL particle size and triglyceride and HDL-C levels, Edwards et al. (52) reported genetic correlations between pairs of traits. The genetic correlation between LDL-PPD and triglyceride was -0.87, suggesting that 76% [$\rho_G^2 = (-0.87)^2 = 0.76$] of the additive genetic variance in LDL size is shared with triglyceride. The genetic correlation between LDL-PPD and HDL-C was more modest (0.65) but suggested that nearly 50% of the additive genetic variance in each of these traits is attributable to shared genes. However, based on the likelihood-ratio test, the hypothesis of complete pleiotropy was rejected for the two genetic correlations, suggesting the existence of unique genes for each trait. These results demonstrated that the observed phenotypic associations between these three traits are largely under genetic control and indicated that searching for genes implicated in LDL size may actually mean searching for genes also involved in triglyceride and HDL-C. A similar study conducted by Rainwater, Martin, and Comuzzie (54) reported a genetic correlation between lipoprotein size traits (ΔLDL and ΔHDL) and triglyceride. Triglyceride and ΔHDL were strongly correlated with ΔLDL, with genetic correlations of -0.76 and 0.56, respectively. Thus, shared genes accounted for 58% and 31% of the genetic variance in each pair of traits.

Small, dense LDL is also metabolically associated with increased plasma apoB levels, and both features are found in patients with FCHL (37). Thus, some investigators searched for a common genetic mechanism between these two traits in families characterized by FCHL. Using bivariate segregation analysis, Juo et al. (129) reported evidence of a common genetic mechanism controlling both apoB levels and the distribution of LDL subfraction (parameter K) in FCHL families. The best-fitting model proposed a common gene with a codominant allele for both traits, plus distinct polygenic components for each trait. This major gene explained 37% and 23% of the variance in parameter K and apoB levels, respectively. On the other hand, Jarvik et al. (130) have shown that LDL subclass phenotype B and apoB levels are two traits influenced by

two Mendelian loci independent of each other and modulating the risk of FCHL. This conclusion was drawn by showing *1*) that the major gene effect seen in segregation analysis for apoB levels remained after adjustment for LDL subclass phenotypes and *2*) that a lack of association between LDL subclass phenotype and the apoB level predicted genotypes in contingency analysis. Finally, using commingling analysis, Austin et al. (131) reported bimodality of apoB levels in individuals with LDL subclass phenotype B. This finding suggested distinct genetic mechanisms for LDL subclass phenotype and apoB levels in FCHL families. The conflicting results between these studies may be attributable to the different statistical strategies used or may simply reflect the complexity of the genetic mechanisms for these traits.

Taken all together, it appears that distinct sets of genes influence LDL size: those that influence LDL size independent of triglyceride and other lipid parameters and those that affect several components of the lipid profile. Thus, in addition to the genes uniquely influencing LDL size, there appear to be genetic factors that are responsible for covariation in lipoprotein/lipid traits, which demonstrates the complexity of characterizing genetic influences on LDL size.

Animal model

Few, but relevant, studies on animal models have confirmed the presence of genetic factors influencing LDL size. First, LDL size varies substantially between different strains of mice, showing the effect of the genetic background. Jiao et al. (132) characterized LDL size by liquid chromatography in 10 inbred strains and observed a LDL size range from 24.16 nm in BALB/c strain to 29.39 nm in SWR strain, with the whole spectrum of sizes within this interval for the other strains. In an attempt to test whether LDL size was an inherited trait in mice, three sets of recombinant inbred strains were produced by crossing strains with different LDL sizes. By this means, authors have shown that the LDL size of recombinant inbred strains segregated to one or another progenitor 88% of the time, implying that LDL size may be controlled by the product of a major gene. Attempts to identify the major LDL size-determining gene yielded only marginal significant results for a RFLP analysis in the APOB gene.

An attempt was also made to establish whether genes control variation in LDL size in baboons (133). A total of 150 baboon members of 19 sire groups were investigated. Baboons were fed three diets contrasting in levels of fat and cholesterol. A multifactor ANOVA revealed that 18.3% of the variation in LDL size was explained by the sire groups. In addition, there was a significant sire-diet interaction on the phenotype, indicating that members of different sire groups responded differently to various dietary compositions. Taken together, these results suggested that genes influence LDL size and the patterns of LDL response to different diets in baboons. Recently, a genome-wide linkage scan was performed among an enlarged group of these baboons to localize the genes that control LDL size fractions (134). Using GGE, four LDL size-related phenotypes were constructed based on fractional absorbance in four intervals of LDL (LDL-4, 24–26 nm; LDL-3, 26–27 nm; LDL-2, 27–28 nm; and LDL-1, 28–30 nm). The LDL median diameter was also estimated, which is the diameter at which half of the LDL absorbance is on larger and half is on smaller particles. Genome scans were performed on LDL size-related phenotypes taken from blood samples collected at the end of each experimental diet. On a high-cholesterol, high-fat diet, significant evidence of linkage (LOD = 4.22) for LDL-2 was observed on the baboon homologs of human chromosomes 20 and 22 (Fig. 1). Two additional QTLs were suggested, one on the baboon homolog of human chromosome 16 for LDL-3 when exposed to a low-cholesterol, low-fat diet (LOD = 2.15) and one on the baboon homolog of human chromosome 5 for LDL-3 when exposed to a low-cholesterol, high-fat diet (LOD = 2.67). The latter QTL is particularly relevant because the signal was also observed for the LDL median diameter (LOD = 2.21). In addition, this locus overlapped with a QTL for LDL-PPD observed in the QFS (Fig. 1), which contains the HMG-CoA reductase gene.

These results have clearly shown the usefulness of animal studies to identify the LDL size genes. Because of our ability to tightly control the animals' environment, these studies might prove to be even more relevant in the future for testing gene-environment interactions.

Gene-gene and gene-environment interactions

Relatively little is known about gene-gene and gene-environment interactions in LDL particle characteristics, but it would be surprising if they were not important. A preliminary study has shown that the LDL bands of MZ twins were more concordant than those of DZ twins before but not after a 22 week exercise program, suggesting that the genetic contribution of LDL subfractions decreases with exercise (135). It was also demonstrated that the LDL size response to a low-fat diet in children was predicted by the parental LDL subclass pattern (136). Tentative evidence of interactions with LDL size phenotypes was also reported for specific loci. A significant interaction was observed between SR-BI exon 1 genotypes and type 2 diabetes on LDL size, indicating that diabetes status modifies the effect of this polymorphism on LDL particle size (120). St-Pierre et al. (104), for example, have shown an inverse effect of the MTP −493G>T genotypes according to visceral adipose tissue and fasting insulin. It is also apparent from association studies (Table 4) that the effects of some loci are sex specific or confined to subgroups of the population (e.g., diabetic). Zambon et al. (137) also reported an interesting pharmacogenetic interaction on LDL density. They showed that the −514C>T polymorphism in the HL gene promoter strongly influences the LDL-Rf response in middle-aged men undergoing intensive lipid-lowering therapy. Although these studies are interesting examples, they demonstrate the high number of interactions that could be tested and the difficulty of doing so in humans. Clearly, when the loci that control small LDLs

are mapped, there will be a greater potential for determining the gene-gene and gene-environment interaction effects.

CONCLUSION

Here, we synthesized the accumulating evidence of the complex genetic etiology underlying LDL particle heterogeneity. Genetic epidemiology studies have clearly shown a genetic contribution to the LDL subclass phenotypes. Heritability studies have shown that at least 30% to 60% of the variations are attributable to genetic factors. In addition, complex segregation analyses have consistently demonstrated the existence of a single gene with major effect. On the other hand, searching the DNA-based variations responsible has proven to be a difficult task owing to inconsistency and lack of replication among studies. Indeed, linkage and association studies with candidate genes have produced some of the expected results, but in general the effect of positive hits does not seem to be uniform in all populations and environmental backgrounds. Genome-wide linkage scans have been undertaken to fill the gap and have produced interesting leads that need to be followed up.

It is becoming obvious that several different genetic loci contribute to the expression of small, dense LDL. This observation suggests that different genetically determined metabolic mechanisms may give rise to the phenotype. For most of the loci identified to date, it is unclear whether the effect is direct or mediated through the interrelationship with other metabolic parameters, such as glucose/insulin homeostasis and triglyceride metabolism. The false positives reported are difficult to assess but may be important as a result of publication bias toward positive findings. Accordingly, this summary should be interpreted with caution and awareness because some of the positive loci may eventually prove to be false positives.

Understanding the genetic cause of small, dense LDL will help us elucidate the complex multifactorial network involved in the progression of atherosclerosis and its ultimate consequence, CHD. Although searching the genes has been and continues to be a demanding undertaking, the challenge may still be ahead to identify the combination of genes and environmental circumstances that predispose to small, dense LDL. It should be emphasized, however, that the nongenetic factors influencing the expression of small, dense LDL can be used to our advantage by treating genetically susceptible individuals with appropriate lifestyle modifications.

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