Polymorphisms in the gene encoding lipoprotein lipase in men with low HDL-C and coronary heart disease: The Veterans Affairs HDL Intervention Trial[1]

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Abstract **Our goal was to further define the role of *LPL* gene polymorphisms in coronary heart disease (CHD) risk. We determined the frequencies of three *LPL* polymorphisms (D9N, N291S, and S447X) in 899 men from the Veterans Affairs HDL Intervention Trial (VA-HIT), a study that examined the potential benefits of increasing HDL with gemfibrozil in men with established CHD and low high density lipoprotein cholesterol (HDL-C; ≤40 mg/dl), and compared them with those of men without CHD from the Framingham Offspring Study (FOS). In VA-HIT, genotype frequencies for *LPL* D9N, N291S, and S447X were 5.3, 4.5, and 13.0%, respectively. These values differed from those for men in FOS having an HDL-C of >40, who had corresponding values of 3.2% ($P = 0.06$), 1.5% ($P < 0.01$), and 18.2% ($P < 0.01$). On gemfibrozil, carriers of the *LPL* N9 allele in VA-HIT had lower levels of large LDL (−32%; $P < 0.01$) but higher levels of small, dense LDL (+59%; $P < 0.003$) than did noncarriers. Consequently, mean LDL particle diameter was smaller in *LPL* N9 carriers than in noncarriers (20.14 ± 0.87 vs. 20.63 ± 0.80 nm; $P < 0.003$). In men with low HDL-C and CHD: *1*) the *LPL* N9 and S291 alleles are more frequent than in CHD-free men with normal HDL-C, whereas the X447 allele is less frequent, and *2*) the *LPL* N9 allele is associated with the LDL subclass response to gemfibrozil.**—Brousseau, M. E., A. L. Goldkamp, D. Collins, S. Demissie, A. C. Connolly, L. A. Cupples, J. M. Ordovas, H. E. Bloomfield, S. J. Robins, and E. J. Schaefer. **Polymorphisms in the gene encoding lipoprotein lipase in men with low HDL-C and coronary heart disease: The Veterans Affairs HDL Intervention Trial. *J. Lipid Res.* 2004.** 45: **1885–1891.**

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Coronary heart disease (CHD) remains the leading cause of death in the United States (1). A number of studies have shown that plasma lipoprotein concentrations are significant predictors of CHD risk (2–5). Thus, genes with key roles in lipoprotein metabolism are excellent candidates for interindividual variation in susceptibility to CHD (6). Among these candidate genes is *LPL*, which hydrolyzes triglyceride-rich lipoproteins, such as chylomicrons and VLDLs, generating free fatty acids and glycerol for energy utilization and storage (7, 8). By acting as a ligand in lipoprotein-cell surface interactions, LPL also mediates the cellular uptake of lipoproteins (9).

Several common variants, or polymorphisms, have been identified in the *LPL* gene, as reviewed by Murthy, Julien, and Gagne (10). The majority of these are synonymous polymorphisms (11) that do not affect the protein sequence of LPL. However, three nonsynonymous variants that alter the protein sequence of LPL, namely D9N, N291S, and S447X, are among the most widely studied in terms of their relationships with plasma lipoproteins and CHD risk (12, 13). The D9N and N291S variants are associated with decreased LPL activity (14), whereas the

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S447X variant, which causes a deletion of the last two amino acids in the LPL protein, is associated with increased LPL activity (15).

The present study represents an extension of our earlier work with the Framingham Offspring Study (FOS) population. In FOS, the D9N and N291S alleles were associated with lower levels of high density lipoprotein cholesterol (HDL-C) and a trend toward increased triglycerides (16). In contrast, the S447X allele was associated with higher levels of HDL-C, decreased triglycerides, and reduced CHD risk in FOS males (17). To further explore the role of these *LPL* variants in CHD risk, we examined their associations with plasma lipid and lipoprotein levels, response to gemfibrozil therapy, and CHD end points in men participating in the Veterans Affairs HDL-C Intervention Trial (VA-HIT), a study designed to examine the potential benefits of increasing HDL-C in men with established CHD, who had low HDL-C as their primary lipid abnormality. Additionally, we have compared the genotypic data generated in VA-HIT with that generated in men without CHD from FOS, stratified according to HDL-C level.

TABLE 1. Demographic and biochemical characteristics of VA-HIT subjects

Parameter	Units[a]	Value
Age	years	64 ± 7
Body mass index	kg/m^2	29 ± 5
Total cholesterol	mg/dl	177 ± 25
LDL-C	mg/dl	114 ± 22
HDL-C	mg/dl	32 ± 5
HDL_2-C	mg/dl	5 ± 3
HDL_3-C	mg/dl	27 ± 5
TC:HDL-C		5.7 ± 1.1
Triglycerides	mg/dl	162 ± 67
apoA-I	mg/dl	107 ± 17
apoB	mg/dl	97 ± 21
Race		
White	%	93
Nonwhite[b]	%	7
Smokers	%	18

apoA-I, apolipoprotein A-I; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; VA-HIT, Veterans Affairs HDL-C Intervention Trial. Data shown are means ± SD for 899 males from VA-HIT.

[a] To convert values for cholesterol and triglycerides to millimoles per liter, multiply by 0.02586 and 0.01129, respectively.

[b] Nonwhite includes the categories of black and other.

METHODS

Subjects

The rationale, design, and methods for VA-HIT have been described elsewhere in detail (18). Briefly, men were recruited at 20 Department of Veterans Affairs medical centers throughout the United States. Eligibility for the trial required a documented history of CHD, an age of <74 years, an absence of coexisting conditions, an HDL-C level of ≤40 mg/dl (1.0 mmol/l), an LDL-C of ≤140 mg/dl (3.6 mmol/l), and a plasma triglyceride concentration of ≤300 mg/dl (3.4 mmol/l). Information on age, alcohol consumption, smoking status, blood pressure, body mass index (BMI), and diabetes were available for all subjects enrolled in VA-HIT. However, informed consent for DNA analysis was only obtained from some of the subjects; thus, only these samples could be used in our genotyping analysis. Ninety-three percent of these subjects were white (**Table 1**), with no differences noted in the race distribution among subjects within each genotype. Data used in our statistical analyses were obtained at baseline, with the exception of those used to examine the relationships between genotype and the plasma lipid response to gemfibrozil (1,200 mg/day), which were obtained at month 7.

The subjects included in the comparisons presented in Table 3 were men from FOS who were free of CHD, had an age of >39 years, were not taking any medications known to influence plasma lipid levels, and had genotype information for at least two of the three *LPL* polymorphisms. FOS men were further stratified according to those having an HDL-C level of <40 mg/dl (n = 367) and those having an HDL-C level of ≥40 mg/dl (n = 577). Characteristics of the FOS subjects are as follows: *1*) HDL-C ≥ 40: age, 58 ± 9 years; BMI, 27.8 ± 4.1 kg/m^2; HDL-C, 52 ± 11 mg/dl; LDL-C, 131 ± 31 mg/dl; triglyceride, 110 ± 61 mg/dl; *2*) HDL-C < 40: age, 58 ± 9 years; BMI, 29.4 ± 4.5 kg/m^2; HDL-C, 33 ± 5 mg/dl; LDL-C, 126 ± 30 mg/dl; triglyceride, 195 ± 297 mg/dl. The contrast between VA-HIT and FOS men with HDL-C ≥ 40 provides a comparison of those with low HDL-C and CHD and those who have neither, whereas the contrast between VA-HIT and FOS men with HDL-C < 40 provides a comparison of those who have CHD versus those without CHD among men with low HDL-C.

Measurement of plasma lipid, lipoprotein, and apolipoprotein levels

Blood samples were collected from subjects after a 12–14 h fast into tubes containing 0.1% EDTA. Plasma was isolated and frozen for subsequent analysis of plasma lipid, lipoprotein, and apolipoprotein concentrations. Plasma total cholesterol (TC) and triglyceride concentrations were determined using enzymatic assays (19). Plasma HDL-C concentrations were measured after dextran sulfate-magnesium precipitation of apolipoprotein B (apoB)-containing lipoproteins (20), and HDL subfractions were separated by differential polyanion precipitation (21). LDL-C levels were calculated with the equation of Friedewald, Levy, and Fredrickson (22). ApoA-I and apoB levels in the plasma were measured with an immunoturbidimetric assay using reagents and calibrators from Incstar Corp. (Stillwater, MN) (23, 24).

Analysis of lipoprotein subclass concentrations and particle size by NMR

The distribution of lipoprotein subclasses and particle size were determined by proton NMR spectroscopy, as previously described (25, 26). The concentrations of six VLDL (V6–V1), three LDL (L3–L1), and five HDL (H5–H1) subclasses, listed from largest to smallest with respect to particle size (nm), are provided by this methodology. VLDL levels are expressed in units of triglyceride (mg/dl), whereas LDL and HDL subclass levels are expressed in units of cholesterol (mg/dl).

DNA analysis

Genomic DNA was extracted from whole blood samples using either QIAamp mini kits (Qiagen) or Generation Capture Column kits (Gentra Systems). Genotyping of the LPL N291S and S447X variants was carried out on an Applied Biosystems 7700 sequence detection system, using TaqMan probes for allelic discrimination. A detailed description of this methodology has previously been reported (27, 28). For the N291S and S447X variants, PCR amplification was performed with the following primer pairs: 5′-AGGGCTCTGCTTGAGTTGTAGAAA-3′ and 5′-CTGAGAACGAGTCTTCAGGTACATTTT-3′ (N291S) and 5′-AGGAAAGGC-

ACCTGCGGTAT-3′ and 5′-CAGGATGCCCAGTCAGCTTTA-3′ (S447X). For each genotype, PCR amplification was carried out in the presence of two probes, each of which had a different reporter dye attached to its 5′ end (FAM™ or VIC™) and a fluorescent quencher (TAMRA™) attached to its 3′ end. One probe was complementary to the wild-type DNA strand and the other to the DNA strand with the mutation. PCR was performed in a 10 μl reaction volume, as previously described (27, 28), using two steps of 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Genotyping of the D9N variant was performed manually, as previously described (29).

Statistical analyses

To evaluate the relationships between *LPL* genotype and plasma lipids and lipoproteins, we used analysis of covariance techniques, adjusting for the potential confounders of age, BMI, smoking status, hypertension, and diabetes. Chi-square tests were used for categorical measures, and either two-sample *t*-tests or Wilcoxon rank sums tests were used for continuous measures. SAS version 8 was used for all analyses. Analyses of time-to-event data were performed, and relative risks were calculated, using Cox proportional hazards models, with *LPL* genotype as the explanatory variable and adjustments made for the aforementioned confounders as well as treatment group.

RESULTS

Subject characteristics

To further investigate the frequency distribution and phenotypic effects of three common *LPL* variants, we analyzed 899 men in VA-HIT who had consented to DNA analysis. The demographic and biochemical characteristics of these subjects are presented in Table 1. This group of men had a mean age of 64 ± 7 years and a mean BMI of 29 ± 5 kg/m^2, with normal levels of TC, LDL-C, and triglycerides. Because of the selection criteria of VA-HIT, the mean level of HDL-C in these men was low (32 ± 5 mg/dl). This level resulted in a mean ratio of TC to HDL-C of 5.7, a value that is significantly higher than that defined as favorable (30).

Genotype distribution of LPL variants in VA-HIT

DNA analysis revealed that, among men in VA-HIT, 5.3% were carriers of the *LPL* N9 allele, 4.5% were carriers of the S291 allele, and 13.0% were carriers of the X447 allele (**Table 2**). Comparatively, the meta-analysis of Wittrup, Tybjaerg-Hansen, and Nordestgaard (13) identified heterozygote frequencies of 2–4% for *LPL* D9N, 1–7% for *LPL* N291S, and 17–22% for *LPL* S447X. Based on these data, it appears that the *LPL* N9 allele occurs with increased frequency in the VA-HIT population, whereas the *LPL* X447 allele occurs with decreased frequency.

Comparison of LPL genotype distribution in VA-HIT versus FOS

To evaluate whether the *LPL* D9N, N291S, and S447X polymorphisms influence CHD risk via HDL, or possibly through other mechanisms, we compared the genotype distribution of each *LPL* variant in the VA-HIT population with age-matched men without CHD from FOS. In these analyses, men in FOS were classified as having an HDL-C level of <40 or ⩾40 mg/dl. The contrast between VA-HIT and FOS men with HDL-C ⩾ 40 provided a comparison of those with low HDL-C and CHD and those who have neither, whereas the contrast between VA-HIT and FOS men with HDL-C of <40 provided a comparison of those who have CHD versus those without CHD among men with low HDL-C. As shown in **Table 3**, significant differences were observed between the VA-HIT and FOS populations in terms of *LPL* genotype distribution. The only statistically significant difference observed for the comparison of men in VA-HIT with men in FOS having low HDL-C was the increased frequency of the *LPL* D9N allele in VA-HIT (5.3% vs. 1.7%; $P < 0.01$). When men in VA-HIT were compared with men in FOS having an HDL-C level of ⩾40, significant differences were seen in the percentage of carriers of the *LPL* N291S and S447X variants. Significantly more men in VA-HIT were carriers of the S291 allele (4.1% vs. 1.5%; $P < 0.01$), whereas significantly fewer were carriers of the X447 allele (13.0% vs. 18.2%; $P < 0.01$). Nearly identical differences were observed for the comparison of men in FOS with low HDL-C levels and men in FOS with normal HDL-C levels for both the *LPL* S291 (4.1% vs. 1.5%; $P < 0.01$) and X447 (11.8% vs. 18.2%; $P < 0.01$) alleles.

TABLE 2. Genotype distribution of *LPL* polymorphisms in VA-HIT subjects

LPL Variant	n	Frequency	Rare Allele Frequency
		%	
D9N			
Carrier	47	5.3	
Noncarrier	834	94.7	0.026
N291S			
Carrier	40	4.5	
Noncarrier	846	95.5	0.024
S447X			
Carrier	117	13.0	
Noncarrier	782	87.0	0.066

TABLE 3. Comparison of the genotype distribution of *LPL* polymorphisms in VA-HIT with those of men in FOS stratified according to HDL-C level

LPL Variant	VA-HIT	FOS HDL-C < 40	FOS HDL-C ⩾ 40
D9N			
Carrier[a]	47 (5.3)[b,c]	5 (1.7)	15 (3.2)
Noncarrier	834 (94.7)	291 (98.3)	459 (96.8)
N291S			
Carrier	40 (4.5)[d]	12 (4.1)[e]	7 (1.5)
Noncarrier	846 (95.5)	283 (95.9)	467 (98.5)
S447X			
Carrier	117 (13.0)[d]	43 (11.8)[d]	105 (18.2)
Noncarrier	782 (87.0)	322 (88.2)	472 (81.8)

FOS, Framingham Offspring Study. Mean HDL-C (mg/dl) for each group was 32 ± 5 for VA-HIT, 33 ± 5 for FOS < 40, and 52 ± 11 for FOS ⩾ 40.

[a] Heterozygous or homozygous carriers of the rare allele.

[b] Values in parentheses indicate percentage of subjects within each group.

[c] $P < 0.01$ for comparison with FOS HDL-C < 40.

[d] $P < 0.01$ for comparison with FOS HDL-C ⩾ 40

[e] $P = 0.02$ for comparison with FOS HDL-C ⩾ 40.

TABLE 4. Associations between *LPL* genotype and concentrations of plasma lipids in VA-HIT subjects at baseline adjusted for age, diabetes, hypertension, and smoking status

	LPL D9N			*LPL* N291S			*LPL* S447X		
Lipid	Noncarrier (n = 834)	Carrier (n = 47)	*P*	Noncarrier (n = 846)	Carrier (n = 40)	*P*	Noncarrier (n = 782)	Carrier (n = 117)	*P*
TC	177 ± 26	174 ± 24	0.56	177 ± 26	178 ± 15	0.87	177 ± 25	180 ± 27	0.34
HDL-C	31.9 ± 5.3	30.7 ± 4.2	0.30	31.9 ± 5.4	30.7 ± 3.3	0.30	31.7 ± 5.1	32.7 ± 6.3	0.21
LDL-C	113 ± 23	112 ± 21	0.78	113 ± 23	111 ± 11	0.91	112 ± 23	117 ± 23	0.16
Triglyceride	162 ± 69	158 ± 49	0.80	162 ± 70	169 ± 47	0.64	164 ± 69	153 ± 66	0.29

All data shown are means ± SD (mg/dl).

Association of LPL variants with plasma lipid and lipoprotein subclass concentrations at baseline

We have previously reported that male carriers of either the *LPL* N9 or S291 alleles in FOS had significantly lower levels of plasma HDL-C (−7.1%; $P = 0.03$) than did noncarriers (16), whereas carriers of the *LPL* X447 allele had significantly higher levels of HDL-C (+5.3%; $P = 0.008$) and lower triglyceride levels (−14%; $P = 0.01$) (17). In VA-HIT, we did not observe significant associations between baseline plasma lipid levels and *LPL* genotype (**Table 4**). Although HDL-C concentrations were lower in carriers of the D9N and N291S variants relative to noncarriers, neither of these differences was statistically significant ($P = 0.30$ for both comparisons). As was the case in FOS, carriers of the *LPL* X447 allele in VA-HIT had relatively lower levels of triglycerides (153 ± 66 vs. 164 ± 69 mg/dl) and higher levels of HDL-C (32.7 ± 6.3 vs. 31.7 ± 5.1 mg/dl) than did noncarriers, but these differences were not statistically significant. Because VA-HIT consisted of a high number of men with diabetes and/or insulin resistance, we also examined our data for interactions between diabetes and insulin resistance and each *LPL* genotype in predicting plasma levels of HDL-C and triglycerides. Although no statistically significant ($P < 0.05$) interactions were observed, a few were close to statistical significance. Specifically, the *P* value for the interaction between *LPL* S447X and insulin resistance in predicting plasma HDL-C was 0.05, whereas that between *LPL* D9N and diabetes was 0.06.

To examine whether or not *LPL* genotypes were associated with lipoprotein subclass levels, we determined VLDL, LDL, and HDL subclass concentrations, as well as particle size, in a subgroup of men from VA-HIT using automated NMR spectroscopy. No statistically significant associations ($P < 0.01$) were observed between any of the *LPL* variants and concentrations of lipoprotein subclasses at baseline (data not shown).

Association of LPL variants with the plasma lipid and lipoprotein response to gemfibrozil

When on-trial concentrations of plasma lipids were compared in noncarriers versus carriers of either the *LPL* N9 or S291 allele, no statistically significant differences were observed (data not shown). This was likewise the case when data were expressed in terms of percentage change from baseline. In carriers of the *LPL* S447X variant, a relatively greater percentage reduction in plasma triglycerides versus placebo was observed in noncarriers compared with carriers (−27% vs. −20%; $P < 0.04$). However, this finding was most likely attributable to the higher plasma triglycerides seen in noncarriers at baseline, with on-trial concentrations of triglycerides being nearly identical in carriers (111 ± 51 mg/dl) versus noncarriers (114 ± 49 mg/dl) of the *LPL* S447X variant.

On-trial concentrations of plasma lipoprotein subclasses according to *LPL* genotype are provided in **Table 5**. The most striking differences in lipoprotein subclass concentrations in carriers versus noncarriers were seen among the LDL subclasses according to *LPL* D9N genotype. On gemfibrozil, carriers of the *LPL* N9 allele had significantly lower levels of large LDL (−32%) but significantly higher levels of small, dense LDL (+59%) than did noncarriers. This is in contrast to baseline values, in which differences in LDL subclass levels were not observed among the *LPL* D9N genotypes. The substantial differences in concentrations of large and small LDL observed in N9 carriers versus noncarriers translated into a mean LDL particle diameter of 20.14 ± 0.87 nm for carriers, which was significantly smaller than that of 20.63 ± 0.80 nm for noncarriers ($P < 0.003$). In contrast, no statistically significant differences were observed in on-trial concentrations of lipoprotein subclasses for the *LPL* N291S and S447X genotypes.

Association of LPL variants with CHD end points

Because VA-HIT consisted solely of men with established CHD, we also evaluated our data for associations between each *LPL* genotype and CHD end points, defined as death attributable to CHD or nonfatal myocardial infarction. After adjustment for treatment group, age, hypertension, smoking status, diabetes, and BMI, no statistically significant relationships were observed between risk for CHD end points and *LPL* genotype among carriers of the LPL N9 [hazard ratio (HR) = 1.27, 95% confidence interval (CI) = 0.59–2.75; $P = 0.54$], S291 (HR = 1.21, 95% CI = 0.53–2.75; $P = 0.66$), or X447 (HR = 1.23, 95% CI = 0.73–2.07; $P = 0.43$) alleles.

DISCUSSION

HDL deficiency is the most common lipid abnormality observed among patients with premature CHD (31). It has been reported that more than 50% of the variation in

TABLE 5. Associations between *LPL* genotype and concentrations of plasma lipoprotein subclasses in VA-HIT subjects on gemfibrozil adjusted for age, diabetes, hypertension, and smoking status

	LPL D9N		*LPL* N291S		*LPL* S447X	
Lipoprotein Subclass	Carrier (n = 24)	Noncarrier (n = 379)	Carrier (n = 13)	Noncarrier (n = 393)	Carrier (n = 54)	Noncarrier (n = 356)
VLDL						
V6[a]	3.4 ± 7.6	3.7 ± 9.8	1.4 ± 2.8	3.7 ± 9.7	2.1 ± 4.3	3.8 ± 10.1
V5	24.5 ± 22.5	21.2 ± 24.2	18.1 ± 27.5	21.5 ± 23.9	19.3 ± 19.8	21.6 ± 24.6
V4	40.2 ± 22.3	30.8 ± 17.6	32.4 ± 19.6	31.3 ± 18.1	30.1 ± 18.1	31.7 ± 18.1
V3	1.0 ± 1.4	2.4 ± 4.0	3.0 ± 3.9	2.2 ± 3.9	2.1 ± 2.9	2.3 ± 4.0
V2	9.2 ± 5.9	8.9 ± 6.4	10.6 ± 6.7	8.8 ± 6.3	8.8 ± 6.1	8.9 ± 6.3
V1	5.2 ± 2.5	5.6 ± 2.5	6.7 ± 1.7	5.6 ± 2.5	5.9 ± 2.5	5.6 ± 2.4
VLDL size[b]	47.85 ± 9.44	46.52 ± 10.20	42.69 ± 9.06	46.7 ± 10.1	45.69 ± 8.90	46.70 ± 10.25
LDL						
L3	35.4 ± 26.0[c]	52.0 ± 29.9	49.5 ± 32.0	51.0 ± 29.7	56.1 ± 29.6	50.2 ± 29.7
L2	45.1 ± 14.1	42.6 ± 17.4	38.1 ± 12.7	42.8 ± 17.5	44.5 ± 17.9	42.3 ± 17.2
L1	39.2 ± 28.0[d]	24.6 ± 22.6	23.6 ± 29.4	25.7 ± 22.9	21.6 ± 22.3	26.2 ± 23.2
LDL size	20.14 ± 0.87[d]	20.63 ± 0.80	20.61 ± 1.04	20.60 ± 0.80	20.74 ± 0.79	20.58 ± 0.81
HDL						
H5	1.0 ± 1.8	1.6 ± 2.1	1.4 ± 1.7	1.6 ± 2.1	2.1 ± 3.2	1.5 ± 1.9
H4	4.1 ± 2.4	4.7 ± 3.1	4.8 ± 3.7	4.7 ± 3.1	4.4 ± 3.3	4.7 ± 3.1
H3	2.1 ± 3.1	1.9 ± 3.3	2.2 ± 4.1	1.9 ± 3.2	1.9 ± 3.2	1.9 ± 3.3
H2	21.0 ± 3.9	21.7 ± 4.6	21.6 ± 4.3	21.7 ± 4.6	22.2 ± 3.6	21.7 ± 4.7
H1	1.3 ± 1.9	1.9 ± 3.1	1.5 ± 1.9	1.9 ± 3.1	2.1 ± 2.9	1.9 ± 3.1
HDL size	8.33 ± 0.27	8.38 ± 0.27	8.38 ± 0.25	8.37 ± 0.28	8.39 ± 0.35	8.37 ± 0.26

All data shown are means ± SD.

[a] Subclasses are listed from largest to smallest with respect to particle size. VLDL subclass concentrations are expressed in units of triglycerides (mg/dl), whereas LDL and HDL subclass concentrations are expressed in units of cholesterol (mg/dl).

[b] Particle size is given in nanometers.

[c] $P < 0.01$ for comparison with noncarriers.

[d] $P < 0.003$ for comparison with noncarriers.

HDL-C levels in humans is genetically determined (32), with gene products that influence the amount and nature of lipid contained within HDL particles having important effects on the metabolism of HDL and apoA-I. Included among these gene products is LPL. We have previously reported that genetic variation at the *LPL* locus was associated with plasma lipoprotein levels and CHD risk in men from the FOS (16, 17). To further explore the role of common *LPL* variants in the modulation of CHD risk at the population level, we examined the relationships between three *LPL* polymorphisms, plasma lipoprotein levels, response to gemfibrozil therapy, and CHD end points in men from the VA-HIT. VA-HIT is distinct from other populations used previously to examine such associations in that it consists solely of men with preexisting CHD, low HDL-C, and normal LDL-C.

In VA-HIT, 5.3% of men were carriers of the *LPL* N9 allele, 4.5% were carriers of the *LPL* S291 allele, and 13.0% were carriers of the *LPL* X447 allele. Comparatively, the meta-analysis of Wittrup, Tybjaerg-Hansen, and Nordestgaard (13) identified heterozygote frequencies of 2–4% for *LPL* D9N, 1–7% for *LPL* N291S, and 17–22% for *LPL* S447X for white subjects. To further investigate the associations between these *LPL* genotypes and CHD risk, we compared men in VA-HIT with men without CHD from FOS, who were stratified according to HDL-C level (≥40 or <40 mg/dl). The contrast between VA-HIT and FOS men with HDL-C ≥ 40 provided a comparison of those with low HDL-C and CHD with those who have neither, whereas the contrast between VA-HIT and FOS men with HDL-C < 40 provided a comparison of those who have CHD versus those without CHD among men with low HDL-C. In contrast to what was seen in VA-HIT, these analyses revealed that the frequency of the *LPL* N9 allele was not significantly increased in the group of men from FOS having an HDL-C of <40 mg/dl compared with men from FOS having an HDL-C of ≥40. However, similar to what was seen in VA-HIT, the *LPL* S291 and X447 alleles were significantly increased and decreased, respectively, in men from FOS with low HDL-C relative to men in FOS with normal HDL-C. Taken together with the meta-analysis of Wittrup, Tybjaerg-Hansen, and Nordestgaard (13), our data suggest that the *LPL* N9 allele is associated with increased CHD risk, whereas the *LPL* S291 and X447 alleles may be more strongly associated with HDL-C phenotype than with CHD risk. In support of this concept, a number of studies have reported increased frequency of the mutant *LPL* N9 allele in patients with CHD (13, 33–38).

The *LPL* N9, S291, and X447 alleles have been reasonably consistent in terms of their associations with plasma lipid concentrations (13). The N9 and S291 alleles are associated with lower levels of HDL-C and higher levels of triglycerides, whereas the converse is true for the X447 allele. The differential effects of these variants on plasma lipid levels may be related to their location within the LPL protein, with the D9N and N291S variants located in the N terminus and the S447X variant located in the C terminus (10). In the present study, we did not observe any statistically significant relationships between these common

LPL variants and plasma lipid concentrations at baseline. The lack of significant association is likely the result of the homogenous nature of VA-HIT with respect to plasma lipid levels. Alternatively, the lack of association may be attributable to the fact that the effects of these variants on HDL-C levels is masked by other, more potent genetic factors in this population of men with low HDL-C.

A novel aspect of this study concerns our examination of the relationships between these *LPL* variants and the lipoprotein response to a fibric acid derivative (gemfibrozil). To our knowledge, only one other study has addressed the influence of *LPL* genotype on response to a lipid-lowering agent (fluvastatin) (39). Because gemfibrozil has been shown to modulate triglyceride levels, in part, via its effects on *LPL* gene expression (40), we believe that our study is particularly relevant. As discussed above, we did not detect significant relationships between any of the *LPL* genotypes and plasma lipid concentrations at baseline or on trial. However, when we examined the distribution of cholesterol among lipoprotein particles, significant associations were observed between the *LPL* D9N variant and on-trial concentrations of LDL subclasses. Specifically, carriers of the *LPL* N9 allele had significantly higher plasma concentrations of small, dense LDL during gemfibrozil therapy than did noncarriers. This is in contrast to baseline values, in which significant differences in LDL subclass levels were not observed among the genotypes. Thus, carriers of the *LPL* N9 allele experienced an increase in small, dense LDL in response to gemfibrozil, whereas noncarriers experienced a decrease. The differential response may relate to genotype-specific differences in LPL activity (14) and, in turn, their effects on triglyceride levels, which are known to be an important determinant of LDL composition and particle size (41). On the other hand, it is also known that LPL can bind to LDL (42), suggesting that the less common N9 allele may be associated with altered uptake of LDL during gemfibrozil therapy. Compatible with this concept, Fisher et al. (43) have recently reported that the *LPL* N9 allele was associated with the bridging of LDL to monocytes in vitro. It is further possible that the *LPL* D9N variant is in linkage disequilibrium with a polymorphism located within, or near, the peroxisome proliferator response element of the *LPL* promoter, whereby the N9 allele could mediate the functional responsiveness of LPL to fibrate therapy. Finally, the differences in response seen in carriers versus noncarriers may be attributable to distinct structural changes in LPL as a result of the aspartic acid-to-asparagine amino acid substitution, a concept supported by the molecular modeling work of Razzaghi et al. (44).

We also evaluated our data for associations between *LPL* genotype and CHD outcomes. In VA-HIT, no statistically significant relationships were observed between the *LPL* D9N and N291S genotypes and CHD end points, with only a trend toward increased risk for CHD end points seen in carriers of the *LPL* N9 (HR = 1.27) and S291 (HR = 1.21) alleles. These results are compatible with the meta-analysis of Wittrup, Tybjaerg-Hansen, and Nordestgaard (13), in which odds ratios for ischemic heart disease in carriers of the N9 and S291 alleles ranged from 0.8 to 2.4 (mean of 1.4) and from 0.9 to 1.5 (mean of 1.2), respectively, with neither value being statistically significant ($P = 0.2$). The *LPL* S447X variant was not associated with risk for CHD end points in our study. Although this finding is in contrast to studies that have shown an inverse association between the X447 allele and CHD risk (13, 17), other studies have yielded results similar to ours (33, 37–39). Included among the latter is the study of Spence, Ban, and Hegele (38), in which no association was found between the S447X variant and either carotid artery plaque at baseline or carotid artery plaque progression. Overall, the literature is not consistent with respect to associations between LPL polymorphisms and cardiovascular disease end points. As noted by Spence, Ban, and Hegele (38), these disparities are not surprising, considering the multiple roles of LPL in vivo. Thus, a particular association in one specific population may not translate to others.

In conclusion, our data show that, in men with low HDL-C and CHD, *1*) the *LPL* N9 and S291 alleles are more frequent than in CHD-free men with normal HDL-C levels, whereas the X447 allele is less frequent, and *2*) the *LPL* N9 allele is associated with the LDL subclass response to fibrate therapy. These results provide new insight into the mechanisms responsible for the wide range of response to lipid-lowering therapy that is often observed at the population level.

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