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Lipoprotein lipase (LPL) gene variation and progression of carotid artery plaque

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Lipoprotein Lipase (*LPL*) Gene Variation and Progression of Carotid Artery Plaque

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Background and Purpose—Coding single nucleotide polymorphisms (cSNPs) in the lipoprotein lipase (*LPL*) gene have been associated with lipoprotein phenotypes and vascular disease risk. We studied the association between *LPL* cSNPs and a novel noninvasive measure of disease, namely, cross-sectional carotid plaque area (CPA) on B-mode ultrasound.

Methods—Four hundred fifty-two patients from an atherosclerosis prevention clinic had determinations of baseline and total CPA. Traditional atherosclerosis risk factors were recorded, and the *LPL* D9N, N291S, and S447X cSNPs were genotyped. Multiple regression analysis was used to identify determinants of CPA.

Results—Minor allele frequencies for *LPL* D9N, N291S, and S447X were 2.8%, 0.9%, and 4.4%, respectively. There were no significant between-genotype differences in treated fasting lipids. The *LPL* D9N genotype was a significant predictor of both baseline CPA ($P=0.008$) and plaque progression from baseline to 1 year later ($P=0.001$). Heterozygotes for the N9 allele had higher mean baseline CPA and plaque progression than did *LPL* D9/D9 homozygotes.

Conclusions—*LPL* D9N genotype may be a determinant of atherosclerosis as estimated by static baseline CPA and by progression of CPA. **(*Stroke*. 2003;34:1176-1180.)**

Key Words: atherosclerosis ■ carotid artery plaque ■ genetics ■ lipoprotein lipase

Lipoprotein lipase (LPL) plays a key role in the hydrolysis of circulating triglyceride-rich lipoproteins, such as chylomicrons and VLDL, which appear to be determinants of vascular disease risk.[1] LPL is the predominant plasma triglyceride lipase and is bound to vascular endothelium through interaction with membrane-anchored proteoglycans.[1] While the main catalytic activity of LPL is within the capillary beds of skeletal muscle and adipose tissue, it also has a nonenzymatic molecular bridging function, which mediates the cellular uptake of lipoproteins.[1] Thus, the catalytic function of LPL is probably antiatherogenic, while the noncatalytic bridging function may be proatherogenic.

Over the last decade, several DNA polymorphisms in the *LPL* gene have been evaluated for their association with clinical traits.[2–7] *LPL* coding sequence single nucleotide polymorphisms (cSNPs) that alter the protein sequence have been associated with variation in fasting lipoproteins,[2–7] postprandial lipoproteins,[8,9] coronary artery disease,[10–19] and cerebrovascular disease,[20,21] although there are some important disparities.[13] The relationship between *LPL* variation and the progression of vascular disease has not yet been examined. We previously demonstrated that the total cross-sectional area of all plaques determined by B-mode ultrasonography of common, internal, and external carotid arteries (carotid plaque area [CPA]) was associated with traditional and nontraditional atherosclerosis risk factors.[22–25] In addition, both baseline CPA and the rate of progression of CPA were strong independent predictors of vascular disease risk.[26] Patients in the top quartile of baseline plaque have a 3.5-fold increase in risk of stroke, death, or myocardial infarction over 5 years compared with the lowest quartile, and patients with progression have a 2-fold increase in risk compared with those with regression or stable plaque.[26] Thus, CPA may represent a useful adjunctive noninvasive measure for assessment and prediction of vascular disease risk. In the present report we tested for association between genetic variation in *LPL* marked by nonsynonymous cSNPs and interindividual variation in both baseline CPA and the progression of CPA.

Subjects and Methods

Study Sample

Study participants each attended the Atherosclerosis Prevention Clinic of the London Health Sciences Centre, London, Canada. Each subject had measurement of CPA at baseline and at 1 year, together with baseline medical history, physical examination, and fasting plasma lipoprotein profile. Genotypes were based on the nonsynonymous cSNPs at *LPL* codons 9, 291, and 447, namely, D9N, N291S, and S447X. Briefly, from genomic DNA that was extracted from peripheral blood leukocytes, a thermostable DNA polymerase was used to amplify the coding region surrounding the polymorphic sites at codons 9, 291, and 447 in 3 different amplification reactions for each DNA sample. The amplified fragments were then exposed to

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the appropriate restriction endonucleases.[17] Electrophoresis in 2% agarose gels was used to identify size polymorphism of the digested fragments.[17] Polymorphism in the length of the restriction fragment was used to score the genotype for the specific SNP, as described.[17] Sequence-proven control samples were used for each genotyping reaction.

Measurement of Atherosclerosis

CPA was measured as described previously[22–26] with the use of a high-resolution duplex ultrasound scanner (initially an ATL Mark 9, more recently an ATL 5000 HDI; Advanced Technology Laboratories). Plaque was defined as a local thickening of the intima >1 mm. Measurements were made in magnified longitudinal views of each plaque seen in the right and left common, internal, and external carotid artery. The plane for measurement of each plaque was chosen by scanning to find the largest extent of plaque. The image was then frozen and magnified, and the plaque was measured by tracing around the perimeter with a cursor on the screen. The microprocessor in the scanner then displayed the cross-sectional area of the plaque. The operator then moved on to the next plaque and repeated the process until all observed plaques were measured. The sum of cross-sectional areas of all plaques between the clavicle and the angle of the jaw was taken as total plaque area. Intraobserver reliability was 0.94 for repeated measurements; interobserver reliability was 0.85.[22,26] All measurements used in this study were made by the same certified vascular ultrasound technologist.

Statistical Methods

Data were entered and analyzed with the use of SPSS PC+ 10.0 (SPSS). Deviation from Hardy-Weinberg equilibrium was determined by χ^2 analysis. Multiple regression analysis was performed with a cube root transformation of CPA, which resulted in a variable with a distribution not significantly different from normal, and a linear probability plot. Independent variables included in the regression model were those traditional risk factors that we previously identified as being significant predictors of CPA, including age, sex, systolic blood pressure, plasma total cholesterol, pack-years of smoking, treatment of hyperlipidemia, and treatment of hypertension.[23–25] We used χ^2 analysis to test for differences among genotypes with respect to baseline history of myocardial infarction, stroke, or transient ischemic attack, assuming a dominant model for the minor allele in each instance.

Results

Clinical Attributes

Baseline clinical features of the study population are shown in Table 1. The patients were typical of a sample drawn from an atherosclerosis prevention clinic: middle-aged, overweight as indicated by body mass index (BMI), and having several risk factors.

LPL Genotypes

Allele and genotype frequencies among the study subjects of the 3 *LPL* cSNPs are shown in Table 2. Genotype frequencies did not deviate from Hardy-Weinberg expectations.

Genotype-Phenotype Associations

There were no significant associations between any *LPL* genotype and any clinical or biochemical trait (data not shown). The multiple regression analysis of determinants of baseline CPA is shown in Table 3. Variables entered were based on our previous work, in which the proportion of explained plaque area (r^2) was 0.513; all variables were significant predictors of baseline plaque with a probability value <0.007, in a sample of >1600 patients.[26] For the present study of 452 cases, in the overall regression model before *LPL* genotypes were entered, the proportion of baseline CPA that was explained by the risk factors (r^2) was 0.394.

TABLE 1. Clinical Attributes of Study Sample

Age, y	50.8±11.5
Male, %	53.8
Body mass index, kg/m^2	26.3±4.1
Previous myocardial infarction, %	5.8
Previous stroke, %	2.5
Previous transient ischemic attack, %	3.9
History of diabetes, %	3.8
Systolic blood pressure, mm Hg	128±18
Diastolic blood pressure, mm Hg	79±11
Total cholesterol, mmol/L	5.39±0.97
Triglycerides, mmol/L	1.80±1.09
LDL cholesterol, mmol/L	1.15±0.41
HDL cholesterol, mmol/L	3.89±1.03
Smoking, pack-years	8.74±13.3
Baseline CPA, cm^2	0.46±0.71
CPA after 1 year, cm^2	0.54±0.75

Variances are ±SD.

When BMI and the genotypes were entered singly into the complete regression model (Table 4), only D9N was a significant predictor of baseline plaque (β=0.144, P=0.003). Among the subjects who had been genotyped for *LPL* D9N, the baseline CPA (mean±SD) was 0.94±0.32 cm^2 in the N9/D9 heterozygotes compared with 0.53±0.06 cm^2 in the D9/D9 homozygotes (P<0.05). The effect of N291S genotype approached significance (β=−0.087, P=0.06) but that of S447X genotype did not (β=−0.028, P=0.47). The effect of BMI was not significant (β=−0.028, P=0.13). With pairwise entry of BMI and each *LPL* genotype, the standardized β for D9N increased to 0.157 (P=0.006), whereas the β for BMI decreased to −0.023 (P=0.68), suggesting an interaction between genotype and BMI. This interaction was specifically tested with the use of an interaction term in the multivariate analysis (general linear model) and was found to be significant (P=0.007). There was no interaction of BMI with the other *LPL* cSNPs.

In the multiple regression model for prediction of progression of plaque area from baseline to 1 year later (Table 5), only *LPL* D9N genotype predicted progression of plaque from baseline to 1 year later (β=0.241, P=0.001). None of

TABLE 2. Genetic Attributes of Sample

LPL SNP	Genotype	Frequency	Allele	Frequency
D9N	D9/D9	0.944	N9	0.028
	N9/N9	0.056		
N291S	N291/N291	0.983	S291	0.008
	S291/N291	0.017		
S447X	S447/S447	0.917	X447	0.044
	X447/S447	0.079		
	X447/X447	0.005		

TABLE 3. Multiple Regression Model for Baseline CPA

	Standardized Coefficients β	t	P Value
(Constant)		−5.31	0.000
Age (at baseline)	0.436	11.1	0.000
Total cholesterol	0.060	1.60	0.110
On antihypertensive therapy	0.133	3.37	0.001
On lipid therapy	0.211	5.60	0.000
Pack-years of smoking	0.185	4.95	0.000
Sex (M=0; F=1)	−0.078	−2.04	0.042
Systolic blood pressure	0.126	3.09	0.002

Dependent variable: cube root transform of baseline carotid plaque area.

the traditional risk factors was a significant predictor of progression. Among the subjects who had been genotyped for *LPL* D9N, the progression in CPA was 0.53 ± 0.41 cm^2/y in the N9/D9 heterozygotes compared with 0.077 ± 0.023 cm^2/y in the D9/D9 homozygotes ($P<0.001$).

Discussion

There is still no uniform consensus regarding the relationship between candidate gene variation and plasma triglyceride concentrations. Common variants, in particular *LPL* D9N and N291S, appear to be fairly consistently associated with variation in plasma lipoprotein concentrations.[13] Common variants of some other candidate genes, such as hepatic lipase, have not been shown to have consistent associations with plasma triglycerides. Studies of newer candidates such as the mitochondrial genome, nuclear lamin A/C, and interleukin-6 indicate that many different genes may contribute importantly to plasma triglyceride in different populations.[3]

The mechanistic basis for the observed association between *LPL* D9N and both CPA at baseline and plaque progression is not clear. The product of the D9N allele has been associated with low enzyme activity,[5] although LPL activity as an intermediate trait was not assessed in this study sample. Furthermore, the relationship between the *LPL* D9N marker and carotid plaque was independent of such established risk factors as plasma lipids. Talmud and Humphries[5] recently speculated that *LPL* D9N polymorphism may be associated with an increased bridging function, resulting in increased uptake of lipoprotein particles into cells of the vascular wall. While this has yet to be tested in vitro, if such a mechanism were correct, then *LPL* D9N could be associated with variation in atherosclerosis end points but not have any relationship with variation in lipase activity and/or plasma lipoprotein concentrations. The reason why the *LPL* genotype was related to plaque progression while traditional risk factors were not could have been due to the fact that the traditional risk factors were being treated over the period between the baseline and follow-up measurements.

TABLE 4. Multiple Regression Model of Baseline CPA With *LPL* Genotype

	Standardized Coefficients β	t	P Value
(Constant)		−4.04	0.000
Age (at baseline)	0.493	9.33	0.000
Total cholesterol baseline	0.047	0.93	0.353
On antihypertensive therapy	0.130	2.40	0.017
On lipid therapy	0.209	4.16	0.000
Pack-years of smoking	0.219	4.40	0.000
Sex (M=0; F=1)	−0.063	−1.22	0.224
Systolic blood pressure	0.110	2.01	0.045
LPL D9N genotype	0.144	2.97	0.003

Dependent variable: cube root transform of baseline carotid plaque area.

TABLE 5. Stepwise Multiple Regression for CPA Progression Over 1 Year

	Standardized Coefficients β	t	P Value
(Constant)		2.45	0.015
LPL D9N genotype	0.241	3.34	0.001
	Standardized Coefficients β	**t**	**Significance**
Excluded variables			
Age (at baseline)	0.052	0.714	0.476
Total cholesterol baseline	0.134	1.87	0.064
On antihypertensive therapy	−0.016	−0.219	0.827
On lipid therapy	−0.064	−0.883	0.378
Pack-years of smoking	0.065	0.906	0.366
Sex (M=0; F=1)	−0.064	−0.872	0.384
Systolic blood pressure	0.051	0.709	0.479

The literature is inconsistent with respect to associations between *LPL* SNP genotypes and vascular disease end points.[3–21] For instance, in the Framingham Offspring Study,[7] both D9N and N291S were associated with lipoprotein changes compatible with increased atherosclerosis risk, while in the Atherosclerosis Risk in Communities Study,[21] S447X was associated with MRI-detected strokes but not with changes in plasma lipoproteins. A more extensive review of all *LPL* gene association studies performed to the present is beyond the scope of this article. However, disparities may not be that surprising when it is considered that LPL has several functions in vivo, some of which are mechanistically opposed. Thus, any particular associations found in one specific population may not translate to others. Another point that may contribute to discrepancies may be intersample differences with respect to linkage disequilibrium between a measured marker and an unmeasured functional allele at or near the *LPL* locus.

Like the D9N allele, the product of the less common *LPL* N291S allele has been associated with low enzyme activity. In contrast, the product of the less common S447X allele has been associated with increased enzyme activity.[27] LPL activity has been associated with angina severity in a study of statin drugs and angiographic progression of coronary disease.[28] In that study 47% of patients in the lowest quartile of

LPL activity had severe angina compared with only 29% of subjects in the highest quartile of LPL activity.[28] There has been some indication that the *LPL* S447X variant may protect against elevated triglycerides, depressed HDL cholesterol, and coronary heart disease in men.[14] We found no association of this variant with reduced or increased CPA at baseline or with CPA progression.

There is increasing evidence that postprandial lipids are as important as fasting lipids as determinants of disease states.[29–31] Even dietary cholesterol, which has traditionally not been thought to be important, is emerging as a significant contributor to fasting lipids and oxidized LDL and as a risk factor for vascular disease.[32–34] A high-fat meal impairs endothelial function for approximately 4 hours, an effect that can be reduced by antioxidant vitamins.[35,36] A Mediterranean diet has been shown to improve endothelial function.[37] These observations suggest that oxidative stress may be an important mechanism underlying the adverse effect of diets high in animal fat.

Postprandial lipemia appears to be related both to fat intake and to variations in postprandial lipid metabolism.[38] Polymorphisms of some genes, including *LPL*, have been reported to alter the relationship between visceral obesity and plasma lipoproteins.[39] *LPL* polymorphism has also been shown to be associated with greater response of lipid levels to caloric restriction in obese patients.[40] There are several genetic factors that can affect responsiveness of plasma lipoproteins to dietary intervention.[41] Some studies have shown that *LPL* genotypes are important determinants of postprandial lipids[8,9] and response of plasma lipids to diet.[42] Our observation that the interaction between *LPL* variation and BMI was associated with CPA mirrors a similar interaction reported in association with plasma triglycerides.[43] LPL activity has been shown to be upregulated in response to caloric restriction,[44] and therefore it is possible that an interaction between *LPL* genotype and BMI could also affect clinical phenotypes, such as plasma lipoproteins or vascular disease.

In summary, we found that the *LPL* D9N genotype was a significant predictor of baseline CPA and that this association might have been modulated by BMI. In addition, the *LPL* D9N genotype was strongly associated with plaque progression over a 1-year period. The findings suggest that *LPL* D9N genotype may be an important determinant of atherosclerosis as estimated by progression of CPA.

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