Genetic variants of the lipoprotein lipase gene and myocardial infarction in the Central Valley of Costa Rica

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Abstract To assess common variants of the LPL gene that could influence susceptibility to myocardial infarction (MI), we assessed three functional single-nucleotide polymorphisms (SNPs), D9N, N291S, and S447X, in 1,321 survivors of a first acute MI and 1,321 population-based controls, matched for age, gender, and area of residence, all living in the Central Valley of Costa Rica. Conditional logistic regression was used to estimate odds ratio (OR) and 95% confidence interval (CI). The frequency of the X447 mutant allele was significantly lower in cases than in controls (6.2% vs. 7.6%; $P < 0.01$), whereas no association with MI was found for D9N or N291S. The OR (95% CI) for carriers vs. noncarriers of the X447 allele was 0.80 (0.63–1.01); when considering the haplotype that contained X447 and normal alleles of D9N and N291S, the OR (95% CI) was 0.66 (0.48–0.91). Twelve other SNPs were assessed in a subgroup of the population, of which the four functional SNPs were found to be monomorphic, and no correlation with MI was observed for the other eight neutral SNPs. The X447 mutant allele of the LPL gene may protect from MI risk, although this effect is small.— Yang, Y., E. Ruiz-Narvaez, T. Niu, X. Xu, and H. Campos. **Genetic variants of the lipoprotein lipase gene and myocardial infarction in the Central Valley of Costa Rica. *J. Lipid Res.* 2004.** 45: **2106–2109.**

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Lipoprotein lipase is a major enzyme responsible for the hydrolysis of circulating triglyceride (1). Independent of its lipolytic activity, LPL is capable of anchoring lipoproteins to the vessel wall (2); it can act as a ligand for the LDL receptor family (3), and it can mediate the selective uptake of lipoprotein-associated lipids and lipophilic vitamins (2, 4). The LPL gene is located on chromosome 8p22, spans ~30 kb containing 10 exons, and ~100 naturally occurring mutations have been described in this gene (5). A few of these mutations are relatively common in the general population, although their individual frequency differs widely across populations (6). Two common mutations, D9N and N291S, reduce LPL activity (7). Other highly frequent LPL mutations are population-specific, such as P207L and G188E in French Canadians (8). Overall, these mutations are associated with increased triglycerides and decreased HDL cholesterol in plasma (9). Two common LPL mutations, the S447X that causes a C-terminal truncation by two amino acids and −93 T/G, are associated with low triglyceride concentrations (9, 10).

Despite the relatively consistent LPL gene effects on plasma lipids and enzyme activity, results from studies addressing the effect of LPL genetic mutations on clinical end points in the general population are sparse. The N9 allele has been associated with increased coronary heart disease risk (11), but most studies do not show an association (9). A strong association between the S291 allele and ischemic heart disease was found in a large study (12) but not in others (9). On the other hand, the X447 allele is likely to be protective, although not conclusively (9, 13–16). In the present study, we examined 15 single-nucleotide polymorphisms (SNPs) in the LPL gene to test whether these genetic variants are associated with the risk of nonfatal myocardial infarction (MI) in the Central Valley of Costa Rica.

METHODS

Studied population and data collection

The catchment area consisted of the 34 counties that constitute the Central Valley of Costa Rica. All MI cases and population-based controls were ascertained between 1994 and 2000. Eligible case subjects were men and women who were diagnosed as survivors of a first acute MI by two independent cardiologists at

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any of the six recruiting hospitals in the catchment area, according to the World Health Organization criteria for MI, which require typical symptoms plus either increases in cardiac enzyme levels or diagnostic changes in the electrocardiogram (17). One free-living control subject for each case, matched for age (±5 years), sex, and area of residence (county), was randomly selected using the information available at the National Census and Statistics Center of Costa Rica. Participation was 97% for cases and 89% for controls. All subjects gave informed consent on documents approved by the Human Subjects Committee of the Harvard School of Public Health and the University of Costa Rica.

Selection of LPL gene polymorphisms and genotyping

Seven functional and eight neutral SNPs were selected for assessment. After the initial genotyping of 300 subjects, the −39 T/C, G188E, and P207L polymorphisms were found to be monomorphic, and the −93 T/G had very low frequency; therefore, they were not genotyped further. The neutral SNPs (rs252, rs258, rs270, rs285, rs301, rs314, rs320, and rs326) (http://www.ncbi.nlm.nih.gov/SNP/) were genotyped in 492 cases and 520 controls. Genotyping was carried out using a variation of the allele-specific assay (ASA). DNA fragments were obtained using PCR primers designed according to each SNP's vicinity sequence. The reverse primers contained an artificially introduced sequence (derived from the bacteriophage M13) at the 5′ end, which was identical across all SNPs. SNPs were genotyped in three different multiplex reactions, with allele-specific forward primers and a reverse primer whose sequence was universal for all SNPs. Universal primers were labeled at the 5′ end with one of three fluorescent dyes (FAM, HEX, and TET). ASA products were separated by capillary electrophoresis with the ABI Prism 310 genetic analyzer (Applied Biosystems, Perkin-Elmer) and analyzed using Genotyper software. The two alleles of each polymorphism were distinguished by size and fluorescent label. Eight randomly selected QA/QC samples were genotyped on each plate throughout the study, and reproducibility was >99%. Overall, less than 1% of genotypes had missing values.

Statistical and genetic analysis

All data were analyzed with the Statistical Analysis Systems software version 8 (SAS Institute, Inc., Cary, NC). Of the 2,886 subjects recruited, 1,321 cases and 1,321 controls with no missing general information or genotyping data were used in this analysis. Differences in health characteristics and potential confounders between cases and controls were assessed by Wilcoxon rank-sum tests for continuous variables and with Chi-square tests for categorical variables. Haplotype frequencies were estimated with the Arlequin software (Arlequin version 2.000) (18). Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using conditional logistic regression for single loci of D9N, N291S, and S447X and for haplotypes defined by these loci. The effect estimation of haplotypes was carried out in 1,255 case-control pairs with no phase ambiguity. Models were adjusted for waist-to-hip ratio, physical activity, household income, smoking, history of diabetes, and history of hypertension.

RESULTS

Table 1 shows the general characteristics of the study subjects, mutant allele frequencies of functional polymorphisms, and frequencies of haplotypes defined by the functional polymorphisms. The frequencies of the mutant alleles were all <10%. The X447 allele frequency was significantly lower in cases than in controls (6.2% vs. 7.6%; $P = 0.007$). No significant case-control differences were observed for D9N or N291S. Among the haplotypes defined by the functional polymorphisms, the H0 haplotype was the most prevalent, and it was lower in controls, 77.8%, than in cases, 80.3% ($P = 0.002$). All haplotypes containing mutant alleles were more prevalent in controls than in cases, but significant differences were detected only for the H3 haplotype, which contained the X447 allele and normal alleles at the other two loci (6.7% vs. 5.0%, respectively; $P = 0.0003$). All other haplotypes were rare (∼1% combined).

TABLE 1. General characteristics and allele and haplotype frequencies of D9N, N291S, and S447X in myocardial infarction cases and population-based controls from the Central Valley of Costa Rica

Characteristics	Controls (n = 1,321)	Cases (n = 1,321)
Age (years)[a]	58 (11)	58 (11)
Sex (% female)	26	26
Area (% rural)	33	33
Waist-to-hip ratio[a]	0.95 (0.08)	0.97 (0.07)[b]
Physical activity (METS)[a,c]	1.55 (0.75)	1.50 (0.76)[b]
Monthly household income (U.S $)[a]	565 (449)	475 (410)[b]
Current smokers (%)[d]	23	41[b]
History of diabetes (%)	13	23[b]
History of hypertension (%)	27	38[b]
Single-locus allele		
D9N (%)	8.4	7.9
N291S (%)	7.5	7.0
S447X (%)	7.6	6.2[b]
Three-loci haplotype[e]		
+++ (H0)	77.8	80.3[b]
− ++ (H1)	7.5	6.9
+ − + (H2)	6.9	6.6
++ − (H3)	6.7	5.0[b]
Others	1.1	1.2

[a] Mean (SD).
[b] $P < 0.01$ compared with controls.
[c] METS, metabolic equivalent tasks.
[d] At least one cigarette per day.
[e] Defined by alleles in the order D9N, N291S, and S447X. +, normal allele; −, mutant allele.

Table 2 shows the frequencies of neutral SNP variants. The frequencies ranged between 24.7% and 49.5% in the control group. There were no significant case-control dif-

TABLE 2. Allele frequencies of LPL gene polymorphisms assessed in a subgroup of myocardial infarction cases and population-based controls from the Central Valley of Costa Rica

Locus[a]	Controls (n = 520)	Cases (n = 492)
rs252	49.5	50.4
rs258	48.0	47.8
rs270	24.7	24.1
rs285	49.0	50.4
rs301	30.1	28.7
rs314	33.2	32.7
rs320[b]	27.9	27.2
rs326	46.8	47.7

[a] Four other loci were genotyped in 300 subjects: three (G188E, P207L, and −39 T/C) were monomorphic, and the allele frequency of −93 T/G was the same (2.3%) in both cases and controls.
[b] Recognized as *Hin*dIII restriction fragment length polymorphism in previous literature.

ferences in allele frequency of these nonfunctional SNPs. Hardy-Weinberg proportions were observed for all SNPs studied.

Carriers of the X447 allele had a lower risk of MI compared with noncarriers (OR = 0.80, 95% CI = 0.63–1.01). After adjusting for potential confounders and the putative effect of D9N and N291S, the point estimate was not substantially changed. When the SNP effect was estimated for the haplotype H3, which contained the X447 allele and normal alleles of loci D9N and N291S, the OR for MI was 0.66 (95% CI = 0.48–0.91). All models were adjusted for waist-to-hip ratio, physical activity, household income, smoking, history of diabetes, and history of hypertension.

We repeated the analysis after deleting carriers of the APOE gene variants APOE2 and APOE4, and the point estimates remained the same.

DISCUSSION

We studied the effect of genetic variation in the LPL gene and risk of MI in a large population-based study in the Central Valley of Costa Rica. Three functional SNPs, D9N, N291S, and S447X, were frequently found in this population, but promoter variants were rare. The carriers of the X447 allele, known to decrease triglyceride levels in plasma, had a decreased risk of MI. The validity of our finding is demonstrated by the population-based case-control design with high participation rate, the standardized study procedures for inclusion of cases of a first MI and randomly selected controls, and the large number of case subjects.

Our finding of a protective effect of the mutant X447 allele, although small, is supported by previous studies (9). Surprisingly, this effect has been observed despite of the relatively modest effects of this SNP on decreasing triglyceride and increasing HDL levels, compared with the larger effects of the D9N and N291S polymorphisms on these lipids (9). Thus, it is possible that the X447 allele affects MI through other mechanisms that cannot be measured using biochemical markers in blood. It is known that LPL is expressed in lesion macrophages and smooth muscle cells (19), where it can retain lipoproteins by direct bridging between lipoproteins and subendothelial matrix or cellular proteoglycans in the arterial wall (20). During this process, the X447 LPL mutant may have a decreased capacity to anchor lipoproteins and directly mediate cholesteryl ester influx into macrophages and smooth muscle cells, thus reducing foam cell formation.

Consistent with several studies (21–26) except one (12), we found no association between the D9N and N291S polymorphisms and MI. This finding is in contrast to the expected results based on the effect of their mutant alleles on plasma lipoproteins. The N9 and S291 alleles are associated with 20% and 31% higher triglyceride and 0.08 and 0.12 mmol/l lower HDL cholesterol (9), respectively, which are well-known established risk factors for coronary disease (27, 28). Although it is an attractive explanation that D9N and N291S may be in linkage disequilibrium with other functional SNPs of the LPL gene that have opposite effects on MI, our data suggest that this is unlikely (data not shown). Among the 15 SNPs we examined across the LPL gene, we found no other associations between MI and these SNPs or haplotypes, except the locus of S447X. Other relatively common SNPs in other populations that may have large effects on MI, such as G188E and P207L, were monomorphic in this population, and the −93 T/G in the promoter had very low frequency. When carriers of the G-93 variants were deleted from the analysis, the results remained unchanged.

In summary, the X447 mutant allele is associated with a modest decrease of MI risk among residents of the Central Valley of Costa Rica. The observed effect is consistent with the role of the X447 allele on plasma lipoproteins.

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