CORONARY ARTERY CALCIUM IS ASSOCIATED WITH *LPA* GENE VARIANT RS7765803-C IN MEXICAN MESTIZO POPULATION. THE GEA PROJECT

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ABSTRACT

Background: Lipoprotein(a) [Lp(a)] levels are genetically determined; high levels are a risk factor for coronary disease, although their association with coronary artery calcium (CAC) is controversial. **Objective:** The objective of the study was to assess the association of *LPA* gene polymorphisms with CAC in a Mexican Mestizo population. **Methods:** We included 1594 subjects 35-70 years old. Six polymorphisms of the *LPA* gene were analyzed. CAC score was determined by tomography and Lp(a) serum levels by immunonephelometry. The association of *LPA* polymorphism with CAC and Lp(a) was evaluated by logistic regression. **Results:** The prevalence of Lp(a) ≥ 30 mg/dL was 10%, and of CAC > 0 was 26.9%. Three polymorphisms were associated with high Lp(a) levels: rs10455872-G ($p = 0.013$), rs6907156-T ($p = 0.021$), and rs7765803-C ($p = 0.001$). Homozygotes (CC) for the rs7765803 variant compared with the G allele (CG + GG) carriers had higher Lp(a) levels (8.9 [3.3-23.9] vs. 4.9 [2.3-11.2] mg/dL; $p = 0.015$) and higher prevalence of CAC > 0 (36.5% vs. 26.3%, $p = 0.045$) and were associated with CAC > 0 (odds ratio = 1.7, 95% confidence interval: 1.06-2.7; $p < 0.026$). The other polymorphisms were not associated with CAC. **Conclusions:** This is the first study to demonstrate in a Mexican Mestizo population that carriers of the rs7765803-C allele of *LPA* gene have 2.6 times greater risk for high Lp(a) values and 1.7 times higher risk for coronary artery disease. (REV INVEST CLIN. 2020;72(2):61-8)

Key words: *LPA* gene. Calcium score. Mexican Mestizo Population.

INTRODUCTION

Several retrospective, prospective, and meta-analyses studies have shown that a high serum level of lipoprotein(a) [Lp(a)] is an independent risk factor for atherosclerotic disease¹⁻³. For more than 25 years,

the presence of Lp(a) has been described in atheroma and venous coronary grafts⁴⁻⁶. However, there are controversial data regarding the relationship between Lp(a) levels and coronary artery calcium (CAC) score: some authors found evidence of a positive relationship^{7,8}, while others reported no association^{9,10}. The

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usefulness of CAC as an atherosclerosis marker lies in that it can predict future cardiovascular events¹¹⁻¹³. Some studies show significant heterogeneity in the relationship between Lp(a) levels and coronary heart disease (CHD), especially with regard to (1) gender and race, (2) prevalence of coronary risk factors between populations, and (3) the prevalence of *LPA* gene variant. It is thus possible that all these differences could explain the controversial data^{12,14-16}.

In adult Mexican population, we have previously reported a prevalence of 14% for Lp(a) ≥ 30 mg/dL and high Lp(a) levels associated with myocardial infarction in 33% of atherosclerotic subjects¹⁷. Moreover, subclinical atherosclerosis (evaluated by CAC score) was recently found in 27% of a Mexican Mestizo population without personal or familial history of coronary heart disease (CHD)¹⁸. There is no information on the genetic variants of *LPA* in Mexican Mestizo population; however, some single-nucleotide polymorphisms (SNPs) in Mexican-American population¹⁹ and in non-Hispanic Whites²⁰, have been found associated with Lp(a) levels, although the association of *LPA* gene variants with coronary artery calcification (CAC) was not evaluated. Therefore, the purpose of this study was to investigate the role of *LPA* gene variants in both plasma Lp(a) levels and subclinical atherosclerosis (assessed by CAC score) in a genetically well-characterized Mexican Mestizo population.

METHODS

Participants

The study included 1594 Mexican Mestizos, who participated in the Genetics of Atherosclerotic Disease (Genética de la Enfermedad Ateroesclerosa [GEA]) Mexican study²¹. In summary, all GEA participants were Mexican Mestizos, 35-70 years of age, without a family history of premature CHD and free of clinically apparent cardiovascular disease. Subjects were recruited from donors at the blood bank of the National Institute of Cardiology in Mexico City or by advertisements in social service centers. Participants were free of any acute illness and had normal performance status.

The study protocol was approved by the Institutional Ethics Committee and conducted according to the

Helsinki Declaration guidelines. All participants signed an informed consent letter.

Demographic data and laboratory studies

Validated questionnaires were applied to the participants to obtain demographic information, family and personal history of cardiovascular risk, physical activity, and use of medications. Weight was determined in kilograms (kg) and height in meters using a calibrated scale and a wall stadiometer. Body mass index (BMI) was calculated with the formula weight/height² [kg/m²]. Waist was measured with a fiberglass metric tape at the midpoint of the distance between the lower part of the last rib and the iliac crest (with a 0.5 cm approximation). Systolic and diastolic arterial pressures were measured 3 times in the sitting position after at least 5 min rest; the average of the last two consecutive measurements was considered for the analysis.

Blood samples were obtained from an antecubital vein after a 10 h fasting period and 20 min in the sitting position. Glucose, total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) concentrations were determined in plasma by enzymatic methods (Roche/Hitachi, Germany) in a Hitachi 902 auto-analyzer (Hitachi LTD, Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) was calculated with the Friedewald formula modified by DeLong et al.²²; non-HDL-C was calculated subtracting the HDL-C from total cholesterol. Reproducibility and accuracy of lipid and lipoprotein determinations were periodically evaluated by the Lipid Standardization Program of the Centers for Disease Control and Prevention (Atlanta, GA, USA). Intra- and inter-assay variation coefficients were below 3%. Lp(a) concentrations were measured by kinetic immunonephelometry with the N Latex Lp(a) reagent (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany)²³ in automated BN ProSpec® equipment following manufacturer's instructions. Intra- and inter-assay variation coefficients were below 6%.

Tomographic measurements

Computed tomography is a validated method for measuring visceral adipose tissue²⁴. In the present study, coronary artery calcification (CAC) expressed

in Hounsfield units (HU) was assessed using 64-detector helical tomography (Somatom Sensation, Siemens, Malvern, PA, USA) with cardiac synchronization by a prospective protocol with the following parameters: 120 kV, 120 mA, and 3 mm slices according to the Agatston method²⁵, interpreted by experienced radiologists. Twenty different scans were randomly selected to evaluate the consistency of interpretation. Intraobserver coefficient correlation was 0.99 ($p < 0.001$).

Determination of *LPA* genotypes

DNA was extracted from peripheral blood according to the Lahiri and Nurnberger method²⁶. Lp(a) is encoded by the *LPA* gene which is located on the chromosome 6q26-q27 region. Recent studies have associated six relevant SNPs in the *LPA* gene with increased Lp(a) levels, myocardial infarction, coronary artery disease (CAD), and major adverse cardiovascular events^{19,20}: one located in exon 15 C4192G Leu1358Val (rs7765803); four located in the introns 3, 15, 18, and 38 (positions T132049C [rs6919346], A82290G [rs10455872], T76399C [rs6907156], and C8252T [rs1321195], respectively); and one upstream of the transcription site (position G12460658A [rs12212807]). The SNPs were genotyped using 5' exonuclease TaqMan genotyping assays on an ABI Prism 7900HT Fast Real-Time PCR System, according to the manufacturer's instructions (Applied Biosystems, Foster City, USA). For each polymorphism, we identified the CAD-risk allele or the minor allele frequency; subjects carrying the risk allele were compared to those carrying the wild-type allele.

Because the Mexican Mestizo population is admixed, a panel of 265 ancestry informative markers distinguishing mainly Amerindian, European, and African ancestries was selected²⁷ and genotyped on Illumina Bead Station using the GoldenGate assay. Duplicate control samples were genotyped on each chip, which served as internal controls for the quality of clustering and reproducibility. Genotyping accuracy was also assessed by genotype clustering using the Illumina gene train score, which is a measure of the clustering confidence of individual SNP alleles. Global ancestry (Caucasian, Amerindian, and African) was determined in all individuals using the ADMIXTURE software.

Statistical analysis

Data are expressed as mean and standard deviation or median and interquartile range for continuous variables, and as simple frequencies and percentages for categorical variables. Comparisons for continuous variables were performed with Student's t-test or Mann–Whitney U-test, according to the variable distribution. We used the Chi-square test for categorical variables. CAC was analyzed as a categorical variable (CAC >0 HU). Allelic and genotypic polymorphism frequencies were obtained through direct counting; the Hardy–Weinberg equilibrium (HWE) was assessed by a Chi-square test. To investigate the independence of the association of *LPA* gene polymorphisms with Lp(a) ≥ 30 mg/dL or the presence of CAC >0 HU, multivariate logistic regression analysis adjusted by gender and age in model 2, plus BMI, triglycerides, LDL-C, diabetes mellitus, hypertension, and smoking in model 3 was used. $p < 0.05$ was considered statistically significant. We used the statistics software SPSS V.16 to perform the analyses.

RESULTS

Characteristics of the study sample

Table 1 summarizes the general characteristics of the 1594 study subjects. The mean age of the population was 53.1 ± 9.3 years; 50.75% were women and 49.25% men. Of the six polymorphisms assessed in this study, rs10455872-G, rs6907156-T, and rs7765803-C alleles were associated with high median Lp(a) levels when compared with the major allele frequency (14.7 vs. 4.66 mg/dL, $p < 0.001$; 22.4 vs. 5.04 mg/dL, $p < 0.021$; and 8.6 vs. 4.8 mg/dL, $p < 0.001$, respectively). In addition, the rs7765803-C variant was simultaneously associated with a high prevalence of subclinical atherosclerosis (CAC >0 HU, 36.4% vs. 26.3 %, $p = 0.022$).

The sample was then stratified according to rs7765803 *LPA* genotype into two groups: (1) CC genotype carriers (6.8%) and (2) GC + GG genotype carriers (93.2%) (Table 1). Subjects carrying the rs7765803-C allele had higher systolic and diastolic blood pressures ($p < 0.01$) and higher levels of Lp(a) (8.6 mg/dL vs. 4.8 mg/dL, $p = 0.001$). The prevalence

Table 1. Characteristics of Mexican Mestizo participants according to rs7765803 genotype (CC and CG+GG) of *LPA* gene

	rs 7765803		p
	CC	CG + GG	
n (%)	108 (6.8)	1486 (93.2)	
Sex, F/M	45/63	764/722	
Age, years	53.3 ± 9.4	53.2 ± 9.3	0.95
Weight, kg	76.3 ± 15.0	74.2 ± 13.8	0.13
BMI, kg/m ²	28.4 ± 4.4	28.4 ± 4.4	0.90
SBP, mmHg	121.6 ± 21.9	116.9 ± 17.1	0.007
DBP, mmHg	74.7 ± 10.5	72.0 ± 9.3	0.004
TC, mg/dL	192.04 ± 36.3	192.9 ± 37.5	0.81
TG, mg/dL*	144.6 (100.5-198.7)	148.7 (112.0-204.0)	0.32
LDL-C, mg/dL	118.4 ± 31.5	118.0 ± 31.6	0.88
HDL-C, mg/dL	46.4 ± 13.4	46.0 ± 13.4	0.76
Apo-B, mg/dL	96.5 ± 27.9	96.5 ± 28.0	0.99
Apo-AI, mg/dL	136.3 ± 33.1	138.0 ± 36.1	0.63
Glucose, mg/dL	99.2 ± 29.2	99.0 ± 33.6	0.30
Insulin IU/mL*	17.8 (12.3-26.4)	17.3 (12.5-23.9)	0.49
HOMA-IR*	4.1 (2.8-6.2)	3.9 (2.7-5.8)	0.51
hsCRP mg/dL*	1.73 (0.7-7.8)	1.5 (0.8-3.2)	0.96
Lp(a), mg/dL*	8.6 (3.7-20.1)	4.8 (2.3-11.0)	0.001

Mean ± SD, *median (interquartile range). BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C and LDL-C, high- and low-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; Lp(a), lipoprotein(a).

of Lp(a) ≥30 mg/dL and of CAC >0 HU were significantly higher in rs7765803-C genotype carriers (9.4% vs. 20.5%, 20.5%, and 36.4%, respectively; Fig. 1).

Global ancestry proportions were estimated as 54% Native American, 35.8% Caucasian, and 10% African. Observed and expected frequencies were in HWE ($p > 0.05$). Age, BMI, and the concentration of lipids, HDL and LDL, apo-A, apo-B, and plasma glucose were similar to those observed in the rs7765803-G allele carriers ($p = \text{ns}$) (Table 1). No significant differences were found in the prevalence of traditional risk factors, such as smoking habit, hypertension, and diabetes mellitus. A simple Spearman correlation analysis between clinical and metabolic variables with Lp(a) levels ≥ 30 mg/dL showed that only BMI in rs7765803-C allele carriers correlated negatively with Lp(a) levels ($p < 0.001$) (Table 2). In the GG + CG genotype carrier group, the systolic and diastolic blood pressure, glucose, insulin, and HOMA-IR correlated negatively ($p < 0.001$) with Lp(a) levels (Table 2).

The independence of the association between the rs7765803-C allele and the risk of Lp(a) ≥30 mg/dL or CAC >0 HU was investigated by multivariate logistic regression analysis (Fig. 2). The rs7765803-C allele carriers had a higher risk of Lp(a) ≥30 mg/dL: (a) odds ratio (OR) = 2.4 (95% confidence interval (CI) = 1.07-5.6, $p < 0.034$) under an unadjusted model; (b) OR = 2.5 (95% CI = 1.09-5.8, $p = 0.030$) under model 2 adjusted for gender and age; and (c) OR = 2.7 (95% CI = 1.1-6.3, $p = 0.02$) under model 3 after complete adjustment. Allele rs7765803-C was also associated with a risk of presenting CAC >0 HU: (a) unadjusted model, OR = 1.6 (95% CI = 1.06-2.4, $p < 0.023$); (b) gender and age-adjusted model, OR = 1.6 (95% CI = 1.01-2.5, $p < 0.045$); and (c) OR = 1.6 (95% CI = 1.01-2.6, $p = 0.047$) after adjusting for several traditional coronary risk factors. In both cases, the rs7765803-C allele was significantly associated with increased risk of developing CAC >0 HU, as well as with higher Lp(a) levels in our population (Fig. 2).

Figure 1. Lipoprotein(a) ≥ 30 mg/dL and CAC > 0 HU prevalence in Mexican Mestizo population according to rs7765803 genotype. * $p = 0.029$, ** $p = 0.022$. CC versus CG + GG. Chi-square test.

Figure 2. Association of rs7765803-C allele with high lipoprotein(a) levels or coronary artery calcification in a Mexican Mestizo population. Model 1: not adjusted. Model 2: adjusted by gender and age. Model 3: adjusted by Model 2 plus body mass index, triglycerides, low-density lipoprotein cholesterol, diabetes mellitus, hypertension, and smoking.

DISCUSSION

Lp(a) serum levels have long been recognized as an independent risk factor for CAD¹⁻³. However, Lp(a) concentrations and their relationship with cardiovascular disease vary across races/ethnicities. The distinctive feature of Lp(a) is the presence of Apo(a), a glycoprotein similar to plasminogen. Apo(a) contains a single copy of plasminogen-like kringle V and multiple copies of kringle IV (KIV) type 2 that have been classified into 10 different types based on their sequence homology²⁸. The *LPA* gene is a major determinant of the KIV type 2 repetitions in Apo(a) and accounts for more than 90% of Lp(a) concentration in plasma²⁹. However, Lp(a) concentrations and their

relationship with cardiovascular disease vary across races/ethnicities³⁰.

This is the first study conducted in a large genetically well-characterized Mexican Mestizo population without CHD, with the purpose of investigating the relationship between CAC and Lp(a) depending on *LPA* gene polymorphisms. The main findings were that (a) 6.8% of the subjects were genotyped as homozygous for the CC allele of the rs7765803 *LPA* polymorphism; (b) the levels of Lp(a) were ≥ 30 mg/dL in 20.5% of the subjects; and (c) 36.4% had subclinical atherosclerosis defined by CAC score > 0 HU. Both percentages (50% and 10%) were significantly higher than those observed in rs7765803-G allele carriers

Table 2. Spearman correlation of clinical and metabolic variables with plasma lipoprotein(a) levels categorized by rs7765803 genotype

	rs7765803	
	CC	CG +GG
n	108	1486
Age, years	-0.09	-0.04
BMI	-0.33**	-0.06
SBP, mmHg	-0.42	-0.137**
DBP, mmHg	-0.13	-0.114**
TC, mg/dL	-0.043	0.15
TG, mg/dL	0.17	-0.080*
HDL-C, mg/dL	0.04	0.065*
LDL-C, mg/dL	-0.12	0.07*
Glucose, mg/dL	-0.068	-0.114**
Insulin, UI/mL	-0.10	-0.124**
HOMA-IR	-0.12	-0.139**
hsCRP, mg/dL	-0.15	-0.040

*p < 0.05, **p < 0.001. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C and LDL-C, high- and low-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; cholesterol; TG, triglyceride; HDL-C and LDL-C, high- and low-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; Lp(a), lipoprotein(a).

(p < 0.02). Compared with subjects with the rs7765803-G variant, homozygous rs7765803-C allele carriers had 2.7 times greater risk of Lp(a) levels ≥ 30 mg/dL and 1.6 times greater risk of CAC >0 HU; this comparison is independent of traditional risk factors (sex, BMI, blood pressure, lipids, lipoproteins, and HOMA-IR) for CHD.

Lp(a) has been found in atheroma plaques for more than 25 years and has already been identified as a predictor for major cardiovascular events⁵. Despite this evidence, the relationship between Lp(a) and subclinical atherosclerosis is still controversial. A positive relationship has been observed in some studies^{6,20,28,29}, while no association has been reported in others^{10,12,31}. These inconsistencies may in part be due to (1) small sample sizes; (2) inclusion of patients with diabetes¹⁵, hypertension³², or dyslipidemia^{4,33} in clinical studies; (3) studies with exclusively Caucasian, Afro-American, or multiethnic subjects despite the well-known

fact that Lp(a) levels are clearly different between racial groups³⁴ and widely diverse across ethnic groups; and (4) mediated by *LPA* gene polymorphism distribution³⁵. Some studies have shown that *LPA* SNPs could be associated with elevated Lp(a) levels in certain populations and with low levels in others²⁰. Differences in both Lp(a) levels between ethnic groups and the distribution of *LPA* gene polymorphisms could explain the inconsistencies indicated above. In addition, these differences prompted us to investigate in a Mexican Mestizo population, the relationship of *LPA* gene polymorphism with plasma Lp(a) levels and with subclinical atherosclerosis evaluated by CAC score.

In this study, three out of six *LPA* SNPs were significantly associated with elevated Lp(a) levels (rs10455872-G, rs6907156-T, and rs7765803-C). Of note, only one allele (rs7765803-C) was additionally associated with increased Lp(a) levels and the presence of CAC. These results are consistent with the previous reports that included 20 SNPs for *LPA* in a cohort of 3145 Europeans and 1749 Mexican-Americans; the rs7765803 SNP was found significantly associated with high Lp(a) levels, p < 1×10^{-6} and p < 8.54×10^{-5} , respectively^{19,20}.

The rs10455872-G allele of *LPA* has a prevalence of 7%³⁶-15%¹⁹ in the European population and has been associated with high Lp(a) levels, CHD, and aortic valve stenosis. In contrast, in a previous study, we found in a Mexican Mestizo population that this *LPA* allele was associated with higher levels of Lp(a) but not with subclinical atherosclerosis³⁷. Our results are consistent with the previous reports in Latin American and East Asian populations. In those studies, a significant association with myocardial infarction was found, suggesting a causal relationship²⁰. Interestingly, in this Mexican Mestizo population, we found an association of rs10455872-G and rs6907156-T alleles with high Lp(a) levels, which had already been reported for Mexican-American subjects¹⁹. However, these two alleles present in a Mexican Mestizo population without a history of CHD were not associated to the presence of subclinical atherosclerosis. These results underscore the importance of familial history of CHD, which frequently is not taken into account in study designs.

This study has several strengths. First, this is a large genetically well-characterized Mexican Mestizo

population without a personal or familial history of CHD. Second, to the best of our knowledge, this is the first study to include, in a Mexican Mestizo population, Lp(a) levels, CAC score, and *LPA* SNPs, simultaneously. Third, our study provides evidence for an association between the *LPA* variants and CAC independently of clinical, biochemical, or metabolic coronary risk factors. Of note, these relationships have not been reported before in this population. Finally, assessment of CAC score has many potential clinical applications: supplementing prognostic information for CHD, identifying subjects who may benefit from a more aggressive treatment and/or further diagnostic workup, and evaluating the efficacy of risk factor modification or medical treatments on plaque burden³⁸.

Being a cross-sectional study, one cannot establish a causal relationship but can only make inferences. Since studies reporting associations with SNPs need replication in an independent sample before stating significant associations, and considering that this is the first report of an association of the rs7765803 polymorphisms with CAC, our results should be replicated in other Mexican Mestizo samples. Another limitation of the present work is that since the sample consisted of volunteers, participants may not have represented the general population. Nevertheless, the prevalence of CHD risk factors observed in this study is similar to that found in the ENSANUT, a survey with national representation³⁹.

Our study, performed in a genetically well-characterized Mexican Mestizo population, demonstrated that rs7765803-C allele of *LPA* is significantly and independently associated with Lp(a) concentrations ≥ 30 mg/dL as well as with the presence of subclinical atherosclerosis, CAC > 0 HU. The Lp(a) prevalence of ≥ 30 mg/dL was 10%, and the presence of CAC was 26.9%. Compared to subjects carrying the G allele, the subjects homozygous for the rs7765803-C allele of the *LPA* gene had a 2.6-fold greater risk of high Lp(a) serum levels and a 1.7 times higher risk of subclinical atherosclerosis. Our results highlight the importance of replicating these results in another sample of the Mestizo population without premature atherosclerosis to advance the understanding of the genetic basis of subclinical atherosclerosis. Hence, longitudinal studies would be useful to demonstrate a causal relationship of rs7765803-C allele of *LPA* as

a genetic marker of subclinical atherosclerosis in the Mexican population.

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