The *D9N*, *N291S*, and *T495G* Polymorphisms of the Lipoprotein Lipase Gene Are Not Associated with Cerebral Infarction

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Background: Lipoprotein lipase (LPL) plays an important role in plasma lipoprotein metabolism and its polymorphisms are possibly implicated in the etiology of ischemic cerebrovascular disease (CVD). The aim of this work was to determine the association of the of *D9N*, *N291S*, and *T495G* polymorphisms of the *LPL* gene as a risk factor for the development of CVD. *Methods:* A case–control study was conducted that included 100 patients with CVD and 120 healthy controls. All the subjects were genotyped for the *D9N*, *N291S*, and *T495G* polymorphisms of the *LPL* gene through polymerase chain reaction–restriction fragment length polymorphism, and the results were analyzed for their association with CVD. *Results:* The *D9N* genotype was not significantly correlated with CVD; the odds ratio (OR) between the control subjects and CVD patients was .29 (95% confidence interval [CI], .03-2.66; $P = .27$). The *N291S* polymorphism was not significantly correlated with CVD either; the OR between the control subjects and CVD patients was 1.2 (95% CI, .07-19.46; $P = .89$). And the *T495G* mutation was not significantly correlated with CVD; the OR between the control subjects and the CVD patients was 1.21 (95% CI, .7-2.08; $P = .48$). *Conclusions:* In the present study, the *D9N*, *N291S*, and *T495G* polymorphisms of the *LPL* gene were not risk factors for the development of CVD. **Key Words:** Protein lipase—ischemic cerebrovascular—polymorphisms—association—risk.

Introduction

In 2012, cerebrovascular disease (CVD) was responsible for 6.7 million deaths worldwide, placing second place following ischemic heart disease.[1] CVD is defined as a disease of the vessels that irrigate the brain, or diseases of the brain resulting from an abnormality of the blood vessels or normal blood supply to the brain. Its onset is not always sudden and on occasion it presents as progressive clinical deterioration.[2] In 2013, in Mexico and in the State of Colima, the incidence rate of CVD was 38.8 and 32.51 per 100,000 inhabitants, respectively.[3] CVD etiology is multifactorial.[4] Studies have posited the lipoprotein lipase (*LPL*) gene as the most viable candidate for study due to its contribution to the interindividual variability in lipid levels and their consequent role in atherosclerosis, as well as to the possible correlation between

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Received November 30, 2015; accepted January 6, 2016.
Grant support: This work was supported by the Medical Emergency Center of the UMF No. 1 of the IMSS, Colima, and the School of Medicine of the Universidad de Colima.
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1052-3057/$ - see front matter

http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2016.01.017

hyperlipidemia and CVD.[5,6] The *LPL* gene is approximately 32 kb in length and is located on the 8p22 chromosome, containing 10 exons.[7,8] Nearly 100 naturally occurring mutations in the *LPL* gene have been described in humans.[9] In different studies, the *D9N* and *N291S* mutations of the *LPL* gene have been associated with hyperlipidemia,[10] hypercholesterolemia,[11] higher diastolic arterial pressure, and hypertriglyceridemia,[11-13] whereas the *T495G* mutation has been associated with hyperlipidemia and diabetic nephropathy.[14] All these are risk factors for developing CVD. Currently, there are no studies that determine the association between the *D9N*, *N291S*, and *T495G* polymorphisms of the *LPL* gene and CVD in a Latin population.

Methods

Study Population

In the city of Colima, Mexico, a case–control study included 100 patients with a clinical and computed axial tomography diagnosis of cerebral infarction who arrived at the emergency services of the Instituto Mexicano de Seguro Social of Colima and 120 controls. Patients who were referred to a tertiary care center before any diagnostic studies were done because of the severity of their status were excluded. The patients voluntarily participated in the study and signed statements of informed consent. Their medical history and population data were collected in a personal interview. The study was approved by the local research ethics committee and was carried out according to the guidelines of the 2004 Declaration of Helsinki.

Polymorphism Analysis

DNA was obtained from 7 mL of peripheral blood. Two washouts were done with 25 mL of red blood cell lysis buffer (NH_4Cl .131 M, NH_4HCO_3 9.1×10^{-4} M); 3 mL of white blood cell lysis buffer (Tris 10 mM, NaCl 400 mM, EDTA .002 M, pH 8.2) was added to the leukocyte pellet, with 100 µL of sodium dodecyl sulfate at 10% and 45 µL of proteinase K (10 mg/mL). The mixture was incubated all night at 37°C. A solution of phenol : chloroform was used for DNA extraction and was precipitated with absolute ethanol. Finally, the samples were resuspended in 200 µL of 1× Tris–EDTA buffer solution, pH 7.5, and were stored at −20°C until analysis.[15] The quantity and purity of the extracted DNA were determined through spectrophotometry. The polymerase chain reactions (PCRs) were developed in a total volume of 10 µL that contained 1× PCR buffer, 1.5 mM $MgCl_2$, dNTP mix (.2 mM each), primers (10 µM each) (Table 1), .25 U of Taq DNA polymerase, and 100 ng of genomic DNA. The PCRs were developed in a programmable thermocycler (RoboCycler Gradient 40; Stratagene Corp., San Diego, CA) under the following conditions: a 94°C cycle for 5 minutes and 35 cycles at 94°C for 30 seconds. The alignment temperature changed according to the polymorphism to 57°C (*T495G*), 52°C (*N291S*), and 62°C (*D9N*) for 30 seconds, 72°C for 30 seconds, and a final extension cycle at 72°C for 5 minutes.

Individually, 5 µL of PCR product was mixed with the reaction buffer and 1 U of restriction enzyme, *Hind III* (New England Biolabs, Inc., Ipswich, MA), for *T495*, *Taq I* (New England Biolabs, Inc.) for *D9N*, and *Rsa I* (New England Biolabs, Inc.) for *N291S*, respectively, following manufacturer instructions. The digestion product (Table 2) was loaded in a polyacrylamide gel at 6% for electrophoresis and stained with $AgNO_3$ solution at .02%.

Statistical Analysis

A Hardy–Weinberg equilibrium evaluation was carried out using the chi-square test, counting method, and percentage calculation for allelic frequency; the chi-square test was used for the distribution. Odds ratio (OR) and confidence intervals (CIs) for the polymorphisms were calculated. When the groups were different, the OR was adjusted through poststratification with the Mantel–Haenszel test, using the SPSS V21.0 statistics program (SPSS Inc., Chicago, IL).

Results

The mean age for the cases and controls was 67.9 years (standard deviation 12.5) and 61.6 years (standard

Table 1. *Primer sequence used in the PCR*

Polymorphism	Sequence (5′-3′)	Length of the amplified DNA (bp)
T495G	GATGTCTACCTGGATAATCAAAG CTTCAGCTAGACATTGCTAGTGT	355
D9N	AAAATCAAGCAACCCTCAAG TAGGGCAAATTTACTTGCGA	234
N291S	TCTGCCGAGATACAATCTTGG TAATATAAAATATAATACTGCTTCTTTTGGCTCTGACTGTA	243

Abbreviation: PCR, polymerase chain reaction.