

# Association of the *HindIII* and S447X polymorphisms in *LPL* gene with hypertension and type 2 diabetes in Mexican families

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**Abstract.** Lipoprotein lipase (LPL) is a key enzyme in lipid metabolism and is associated with obesity, dyslipidemias, hypertension (HTN) and type 2 diabetes mellitus (T2DM). *LPL* gene polymorphisms can be related with the development of cardiovascular risk factors. The present study was conducted to analyze the relationship of the *HindIII* and S447X polymorphisms in *LPL* gene with cardiovascular risk factors in Mexican families. The study population comprised ninety members of 30 Mexican families, in which an index case had obesity, were included in the study. We evaluated the body composition by bioelectrical impedance. Peripheral blood samples were collected to determine biochemical parameters. Screening for both polymorphisms was made by PCR-RFLPs. In the parents, both polymorphisms were in Hardy-Weinberg's equilibrium. We found that the genotype T/T of *HindIII* was associated with diastolic blood pressure  $\geq 85$  mmHg (OR = 1.1;  $p = 0.011$ ), whereas the genotype C/C of S447X was associated with systolic blood pressure  $\geq 130$  mmHg (OR = 1.2;  $p < 0.001$ ), diastolic blood pressure  $\geq 85$  mmHg (OR = 1.3;  $p < 0.001$ ), T2DM (OR = 1.3;  $p < 0.001$ ) and with increase of total cholesterol ( $\beta = 23.6$  mg/mL;  $p = 0.03$ ). These data suggest that the *HindIII* and S447X *LPL* gene polymorphisms can confer susceptibility for the development of hypertension and T2DM in Mexican families.

Keywords: Lipoprotein lipase, polymorphisms, obesity, hypertension, type 2 diabetes

## 1. Introduction

A sedentary lifestyle and high calorie diet, together with a specific genetic susceptibility, in populations of Amerindian origin, are key determinants for the increased prevalence of overweight and obesity in the

Mexican population. These changes have been accompanied by an increase in the prevalence of chronic diseases and obesity-associated comorbidities, such as type 2 diabetes mellitus (T2DM), hypertension (HTN), atherogenic dyslipidemia and thereby the development of cardiovascular disease (CVD) [1]. In México, the estimated prevalence HTN is 30.8%, and T2DM is 7% [1]. The prevalence in the State of Guerrero is 14.3% for HTN and 13.4% for T2DM [2].

Hypertension and prehypertension is closely associated with obesity [3,4]. Accumulated evidence indicates the existence of several pathways through which

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free fatty acids (FFA) could promote blood pressure (BP) elevation. These include  $\alpha$ -1-adrenergic stimulation, endothelial dysfunction, increase in oxidative stress, growth of the vascular smooth muscle, an increase in vascular sympathetic tone and an increase in blood pressure [5,6]. Collectively, these data support a central role of non-esterified fatty acid (NEFA) in the development of hypertension in patients with obesity.

Insulin resistance (IR) is present in the majority of hypertensive subjects, and constitutes a common pathophysiological feature linking to obesity, glucose intolerance and hypertension. IR may contribute to hypertension by mechanisms involving inflammation, oxidative stress overlaid to abnormalities in both glucose and lipid metabolism, activation of the sympathetic nervous system (SNS), renal sodium retention, altered membrane cation transport, vascular smooth muscle growth and remodeling, and vasoconstriction [7]. Hypertension and T2DM are also related to the rise of triglycerides (TG), very low density lipoprotein-cholesterol (VLDL-c) levels, high concentrations of low density lipoprotein-cholesterol (LDL-c) and decrease of high density lipoprotein-cholesterol (HDL-c) levels [8]. Furthermore, long term elevated plasma FFA leads to  $\beta$ -cell dysfunction in T2DM and consequently to changes in insulin secretion in pancreatic islets [9]. Thus, the disorder of lipid metabolism plays a crucial role in the occurrence and development of hypertension and T2DM.

Lipoprotein lipase (LPL) is a key enzyme in the processing of triglycerides and plays a key role in lipid metabolism [10]. The LPL mutations influence LPL function in different ways. Catalytic activity, dimerization, secretion, and heparin binding are affected differentially and in certain combinations may be change the three-dimensional structure [11]. Several polymorphisms have been identified in the coding and noncoding regions of the gene, including, *HindIII* and *S447X*. The *HindIII* polymorphism (rs320) has been shown that is in linkage disequilibrium with the polymorphism *S447X*, where the T allele has been associated with increased levels of triglycerides and decreased HDL levels, although the G allele also has been associated with decrease levels of triglycerides [11–14]. The *S447X* polymorphism (rs328) has been shown that the stability and catalytic activity in the truncated form is normal, but may be in high concentrations in circulation, resulting a high activity of LPL [15] and has been considered protective, because has been associated with decreased levels of triglycerides and increase HDL levels, decreasing the risk of CVD [11,16].

Is very important to avoid the problem of ethnic confounding in the identification of genes that increase susceptibility to cardiovascular risk factors in Mexican subjects, though this problem may resolve with the family-based association studies.

However, few is yet known about the prevalence of those polymorphisms in Mexican populations and their putative association with cardiovascular risk factors. Therefore, the aim of this study was to analyze the relationship of the *HindIII* and *S447X* polymorphisms in *LPL* gene with cardiovascular risk factors in Mexican families.

## 2. Subjects, materials and methods

Ninety members of 30 Mexican families, with an index case with obesity ( $\text{BMI} \geq 30 \text{ kg/m}^2$ ) and  $\geq 18$  years old were included in the study. The families were integrated by case-parents trios and grouped in fathers ( $n = 30$ ), mothers ( $n = 30$ ), sons ( $n = 14$ ) and daughters ( $n = 16$ ). The participants were all born in the State of Guerrero, located in southern Mexico, with a family history of guerrerenses ancestors, at least back to the third generation. The study was reviewed and approved by the Research Ethics Committee of the University of Guerrero. All of the participants provided written informed consent for the project and subsequent medical research.

Blood pressure was measured twice with an automatic sphygmomanometer (HEM-712C, Omron Corporation, Japan), with subjects in a sitting posture, after resting for  $\geq 5$  minutes with the cuff placed on the arm. Two consecutive measures were obtained at 5-minute intervals. Hypertension was diagnosed according to the criteria of JNC 7 [17]. Height and weight were measured with the subjects in standing position and barefoot. Height was recorded to the nearest 0.5 cm and weight to the nearest 0.1 kg. Height was determined with a stadiometer (BM-214, Seca, Germany). Weight and body mass index were evaluated by bioelectrical impedance analysis with a body composition analyzer (TFB-300 GS, Tanita Corporation of America Inc, USA). Waist circumference (WC) was measured at the level midway between the lower rib margin and the iliac crest with an anthropometric tape (203, Seca, Germany), rounded to the nearest 0.1 cm. Abdominal obesity was considered in women with a  $\text{WC} \geq 88$  cm and in men with a  $\text{WC} \geq 102$  cm, according to WHO criteria [18]. Hip circumference was measured at the level of the greater trochanters, with the subject should

stand with relaxed buttocks and feet together, with an anthropometric tape (203, Seca, Germany), and rounded to the nearest 0.1 cm. Waist/hip ratio (WHR) was calculated by dividing the mean waist circumference by the mean hip circumference. Normal values were considered if WHR were  $< 0.9$  in men and  $< 0.85$  in woman, according to WHO criteria [18].

Blood samples were drawn after an overnight fast to the families who accepted to participate in the study, for biochemical analyses using standard protocols. Serum total cholesterol (TC), triglycerides (TG), HDL-c and glucose concentrations were measured with an automated analyzer (Cobas Mira, F. Hoffmann-La Roche Ltd, Germany) according to the manufacturer instructions. The concentration of LDL-c was calculated with the Friedewald equation [19]. The normal values for TG was  $< 150$  mg/dL,  $< 200$  mg/dL to TC, for HDL-c was  $> 60$  mg/dL, and  $< 100$  mg/dL to LDL-c, according with NCEP/ATPIII [20]. T2DM was defined if fasting serum glucose was  $\geq 126$  mg/dL in accord with the American Diabetes Association (ADA) [21].

Genomic DNA was extracted from white blood cells by the modified Miller's technique and the screening for *HindIII* and *S447X LPL* gene polymorphisms were performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Genotyping for *HindIII LPL* gene polymorphism was used the following primers: 5'-AGATGCTA CCTGGATAATCAAAG-3' (forward) and 5'-AATTTGTCAATCCTAACTTAGAG-3' (reverse), obtaining a product of 229 bp. The PCR product was digested with *HindIII* enzyme (New England Biolabs Inc, USA) using 10 U of the enzyme at 37°C by 3 hours, obtaining fragments of 139 and 90 bp for T/T genotype; 229, 139 and 90 bp for the T/G; and the only fragment of 229 bp for the G/G. Genotyping for *S447X LPL* gene polymorphism was used the following primers: 5'-AGGAAAGGCACCTGCGGTAT-3' (forward) and 5'-CAGGATGCCCAGTCAGCTTTA-3' (reverse), obtaining a product of 99 bp. The PCR product was digested with *MnII* enzyme (Vivantis Technologies, USA) using 8 U of the enzyme at 37°C by 5 hours, obtaining the only fragment of 99 bp for the CC; 99, 50 and 49 bp fragments for the CG; and fragments of 50 and 49 bp for G/G genotype.

Statistical analyses were performed using STATA software (version 9.2, StataCorp LP.), with significance set at  $p < 0.05$ . The absolute frequencies for the qualitative variables; average and standard deviation for symmetrical quantitative; median and percentile 5 and 95 for the non-symmetrical quantitative variables were

determined. The differences between the values of the quantitative variables were made using ANOVA of one factor and Kruskal-Wallis test, the differences of the quantitative variables between the genotypes were made using *t*-Student and Mann-Whitney test. The genotypic and allelic frequencies for the *HindIII* and *S447X LPL* gene polymorphisms were determined in all members of the families and the Hardy-Weinberg equilibrium was determined in the parents. Correlations between TG and CT with other characteristics studied were assessed with the Pearson's correlation coefficient after logarithmic transformation. The association between the genotypes for both polymorphisms with the anthropometric, clinical and biochemical measurements was realized using generalized linear regression models for correlated data based on generalized estimating equations (GEE) model.

### 3. Results

The anthropometric and biochemical measurements between all family members are shown in the Table 1. We found that waist circumference is increased in sons, daughter and mothers ( $p = 0.003$ ), according with the criteria to "risk waist" (WC  $\geq 102$  cm in men and WC  $\geq 88$  cm in woman). Concerning the WHR, we found that in all members is increased, according with the normal index reported by WHO. Also, there is a significant difference in SBP ( $p = 0.018$ ), being highest in the sons, mothers and fathers, indicated a prehypertensive state, according to JNC 7 criteria. Likewise, we found in the groups of parents and sons, a significant increase in TG levels ( $p = 0.004$ ), according with the normal values reported by NCEP/ATPIII.

The association between serum lipids and other characteristics studied were assessed with Pearson's correlation coefficient, we found a positive correlation between the TC levels with SBP ( $r = 0.25$ ,  $p = 0.020$ ), DBP ( $r = 0.25$ ,  $p = 0.019$ ), LDL-c ( $r = 0.85$ ,  $p < 0.001$ ) and glucose ( $r = 0.22$ ,  $p = 0.037$ ). The TG levels were logarithmic transformed and was associated with waist circumference ( $r = 0.28$ ,  $p = 0.007$ ), SBP ( $r = 0.21$ ,  $p = 0.043$ ), DBP ( $r = 0.27$ ,  $p < 0.001$ ), HDL-c ( $r = -0.28$ ;  $p = 0.009$ ) and glucose ( $r = 0.26$ ,  $p = 0.013$ ).

Concerning the distribution of genotypic and allelic frequencies for the *HindIII* polymorphism of *LPL* gene in all subjects studied, there was highest frequency of T/T genotype (52%), followed by T/G genotype (36%) and G/G genotype (2%). The T allele frequency was

Table 1  
Anthropometric and biochemical measurements of the families

Characteristic	Fathers <i>n</i> = 30	Mothers <i>n</i> = 30	Sons <i>n</i> = 14	Daughters <i>n</i> = 16	<i>p</i> */ <sup>a</sup>
Age, years	53(46–77)	51(40–71)	25.5(21–51)	26(18–47)	< 0.001 <sup>a</sup>
Weight, kg	79.4 ± 11.5	80 ± 12.5	98.0 ± 11.9	81.8 ± 10.4	0.886*
BMI, kg/m <sup>2</sup>	29.7 ± 4.2	30.7 ± 4.5	33.0 ± 2.8	33.4 ± 2.9	0.117*
WC, cm	100.5(87–116)	96(83–110)	103.5(98–132)	98(90–115)	0.003 <sup>a</sup>
HIP, cm	103.2 ± 6.5	106.2 ± 8.0	113.6 ± 6.3	113.8 ± 9.4	0.884
WHR	0.99(0.9–1.09)	0.9(0.8–1.05)	0.95(0.8–1.05)	0.89(0.74–0.99)	< 0.001 <sup>a</sup>
SBP, mmHg	128.2 ± 18.9	126.1 ± 22.1	124.8 ± 9.1	110.8 ± 14.8	0.018*
DBP, mmHg	80.2 ± 10.9	75.3 ± 11.6	79.2 ± 9.7	71 ± 14.1	0.531*
Glucose, mg/dL	100(69–267)	97.5(71–287)	91.5(69–148)	85(70–193)	0.097 <sup>a</sup>
TC, mg/dL	217 ± 48.5	208.6 ± 44.6	222.6 ± 31.7	186.1 ± 32.9	0.198*
TG, mg/dL	200(87–600)	165.5(83–382)	189(125–444)	133.5(67–307)	0.004 <sup>a</sup>
HDL-c, mg/dL	40.5(24–54.9)	41(30–58)	42.2(25–63.7)	37(29.5–69)	0.861 <sup>a</sup>
LDL-c, mg/dL	129.3 ± 44.1	129.3 ± 41	136.6 ± 38.3	117 ± 30.5	0.486*

Values are mean ± SD and median (percentile 5–percentile 95). BMI indicates body mass index; WC, waist circumference; WHR, waist/hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL-c, high density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol. The *p* values were obtained with ANOVA of one factor test\* and Kruskal-Wallis test<sup>a</sup>.

77.8% (140) and allele G of 22.2% (40). In the case of the *S447X* polymorphism was found more frequently the *C/C* genotype (72%), followed by *C/G* genotype (18%) and *G/G* genotype (0%). The *C* allele frequency was 90% (162) and 10% (18) allele *G*. The genotypic frequencies for both polymorphisms were in Hardy-Weinberg's equilibrium in the parents ( $x^2 = 0.59$ ,  $p = 0.44$  for *HindIII* polymorphism and  $x^2 = 1.11$ ,  $p = 0.29$  for the *S447X* polymorphism) (data not shown).

When the anthropometric and biochemical variables were analyzed for each genotype of the *HindIII* polymorphism, the *T/G* and *G/G* genotypes were grouped in a dominant genetic model, we found that *T/T* carriers had highest SBP compared to the *T/G+G/G* carriers (127.4 vs. 119.1 mmHg,  $p = 0.038$ ). While for *S447X* polymorphism, we found significant differences in the *C/C* carriers compared to *C/G* carriers in DBP (78.1 vs. 71.3 mmHg,  $p = 0.029$ ) and glucose (96 vs. 84 mg/dl;  $p = 0.017$ ) (Table 2).

To estimate the relative contribution of both polymorphisms to BMI, blood pressure, glucose and serum lipids, we used generalized linear regression models adjusted by age, sex and WC. We found that the *T/T* genotype of *HindIII* polymorphism contributed to increase in SBP ( $\beta = 10.1$  mmHg,  $p = 0.002$ ) and DBP ( $\beta = 4.5$  mmHg,  $p = 0.053$ ). The *C/C* genotype of *S447X* polymorphism contributed to rise in DBP ( $\beta = 7.6$  mmHg,  $p = 0.008$ ), glucose ( $\beta = 35.6$  mg/mL,  $p = 0.008$ ) and TC ( $\beta = 23.6$  mg/mL;  $p = 0.03$ ) (Table 3).

In the studied families, the genotype *T/T* of *HindIII* was associated with a DBP  $\geq 90$  mmHg (OR = 1.2; IC95% 1, 1.4;  $p = 0.011$ ), in a model adjusted by age, sex and abdominal obesity. Also, we found a

significant association between the *C/C* genotype of *S447X* polymorphism with SBP  $\geq 140$  mmHg (OR = 1.2; IC95% 1.1, 1.4;  $p < 0.001$ ), DBP  $\geq 90$  mmHg (OR = 1.3; IC95% 1.1, 1.4;  $p < 0.001$ ) and T2DM (OR = 1.3; IC95% 1.1, 1.4;  $p < 0.001$ ) (Table 4).

#### 4. Discussion

To our knowledge, this is the first study in Mexican families to investigate the association between *LPL* gene polymorphisms with HTN and T2DM. In this study, the prevalence of abdominal obesity, it is higher in sons and daughters than fathers and mothers, respectively. Previous studies have shown that increased WC, independently of BMI, is associated with higher concentration levels of TC, TG and lipoproteins, making it a better marker of risk for dyslipidemia and CVD [22]. In this study, both TC and TG levels were higher in fathers and sons than mothers and daughters. This suggests an association of male gender with dyslipidemias in these families. Also, the SBP is increased in the group of fathers, mothers and sons, which is very important, because is known that increased in SBP, regardless of the DBP, has been associated with a similar CVD risk [23]. Additionally, a highest frequency of hypertriglyceridemia in men was observed, this is of great significance because has been associated with abdominal obesity and IR as a result of increased hepatic VLDL production in the presence of increased FFA mobilization from adipose tissue, so it is a major risk factor for the development of T2DM and CVD [24]. And all members of the families present increase lev-

Table 2  
Relationship of anthropometric and biochemical measurements with polymorphisms of *LPL* gene

Characteristic	<i>HindIII</i>		$p^{*/\text{a}}$	<i>S447X</i>		$p^{*/\text{a}}$
	T/G+G/G <i>n</i> = 38	T/T <i>n</i> = 52		C/G <i>n</i> = 20	C/C <i>n</i> = 70	
BMI, kg/m <sup>2</sup>	31.7 ± 4.1	30.9 ± 4.3	0.316*	30.5 ± 3.8	31.4 ± 4.3	0.484*
WHR	0.94(0.8–1.05)	0.92(0.8–1.05)	0.211 <sup>a</sup>	0.92(0.86–1.09)	0.93(0.8–0.93)	0.787 <sup>a</sup>
SBP, mmHg	119.1 ± 16.1	127.4 ± 20.4	0.038*	122.6 ± 14.6	124.2 ± 20	0.940*
DBP, mmHg	75.1 ± 13	78 ± 11	0.267*	71.3 ± 6.9	78.1 ± 12.5	0.029*
Glucose, mg/dL	95(70–303)	93(74–267)	0.925 <sup>a</sup>	84(71–173)	96(70–270)	0.017 <sup>a</sup>
TC, mg/dL	211 ± 43.7	209 ± 43.8	0.833*	193.9 ± 39.9	213.8 ± 43.7	0.082*
TG, mg/dL	184.5(79–557)	173.5(95–444)	0.784 <sup>a</sup>	164.5(79–358)	178.5(87–487)	0.217 <sup>a</sup>
HDL-c, mg/dL	40(25–54)	41(28.4–61.8)	0.673 <sup>a</sup>	41(24–65.5)	41(30–57)	0.815 <sup>a</sup>
LDL-c, mg/dL	130.4 ± 41.2	126.5 ± 39	0.663	117 ± 38.6	131.1 ± 39.8	0.182*

Values are mean ± SD and median (percentile 5–percentile 95). BMI indicates body mass index; WHR, waist/hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL-c, high density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol. The *p* values were obtained with *t*-Student test\* and Mann-Whitney test<sup>a</sup>.

Table 3

Effect on anthropometric and biochemical measurements of the T/T genotype of *HindIII* polymorphism and C/C genotype of *S447X* polymorphism of *LPL* gene

Characteristic	Without adjusted		Multiple models <sup>&amp;</sup>	
	$\beta$ (IC95%)	<i>p</i>	$\beta$ (IC95%)	<i>p</i>
<i>HindIII</i> <sup>a</sup>				
BMI (kg/m <sup>2</sup> )	−0.9(−2.6, 0.8)	0.307	−0.2(−1.22, 0.76)	0.650
WHR	−0.02(−2.3, 0.5)	0.209	−0.01(−0.03–0.02)*	0.478
SBP (mmHg)	8.3(0.6, 16.1)	<b>0.035</b>	10.1(3.62, 16.66)	<b>0.002</b>
DBP (mmHg)	2.8(−2.1, 7.7)	0.258	4.5(0.06, 9.09)	0.053
Glucose (mg/dL)	−0.9(−24.6, 22.8)	0.941	−0.9(−24.25, 22.49)	0.941
TC (mg/dL)	−2(−20.1, 16.1)	0.830	1.5(−16.33, 19.23)	0.873
TG (mg/dL)	−1.6(−49.4, 46.1)	0.947	12.9(−31.66, 57.54)	0.570
HDL-c (mg/dL)	1.4(−2.8–5.5)	0.515	1.1(−3.1–5.3)	0.612
LDL-c (mg/dL)	−3.8(−20.8–13.1)	0.658	−3.4(−20.5–13.8)	0.700
<i>S447X</i> <sup>b</sup>				
BMI (kg/m <sup>2</sup> )	0.8(−1.4, 2.9)	0.477	−0.1(−1.3, 1.1)	0.850
WHR	−0.001(−0.04–0.04)	0.964	0.006(−0.03–0.04)	0.707
SBP (mmHg)	1.6(−8.2, 11.4)	0.753	5.5(−2.9, 13.9)	0.199
DBP (mmHg)	6.8(0.9, 12.7)	<b>0.025</b>	7.6(2, 13.1)	<b>0.008</b>
Glucose (mg/dL)	29.2(0.5, 57.8)	<b>0.046</b>	37.6(9.7, 65.4)	<b>0.008</b>
TC (mg/dL)	20(−2, 41.9)	0.075	23.6(2.1, 45)	<b>0.031</b>
TG (mg/dL)	40(−18.3, 98.4)	0.179	50.5(−3.7, 104.8)	0.068
HDL-c (mg/dL)	0.7(−5.7–4.4)	0.795	−0.7(−5.8–4.4)	0.789
LDL-c (mg/dL)	14.1(−6.2–34.4)	0.174	15.8(−4.8–36.4)	0.134

BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL-c, high density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol. <sup>&</sup>Adjusted by age, sex and waist circumference. \*Adjusted by age and sex.  $\beta$  = Regression coefficient of generalized estimation equation (GEE); CI 95%, confidence interval of 95%. <sup>a</sup>Reference category (T/G+G/G genotypes). <sup>b</sup>Reference category (C/G genotype).

els of LDL-c and low levels of HDL, these are a very important features because concentration increment of LDL-c and concentration decrement of HDL-c are independent risk factors for the development of CVD [25–27]. Parents may have a significant influence on the heritability of different risk factors contributing to their offspring at increased risk for developing dyslipidemia, regardless of environmental factors, because it suggests that the estimated heritability is high, including values of 40–60% for HDL, 40–50% for LDL and 35–48% for TG [28].

Although the two polymorphisms have been studied in different populations around the world, this is the first report of genotypic and allelic frequencies for both polymorphisms in a Mexican population. The frequencies obtained for *HindIII* and *S447X* polymorphisms are very similar with the reported in other populations [11,29–32].

Yang et al. [33] associated *HindIII* and *S447X* polymorphisms with DBP in Chinese families for first time. Interestingly, our study shows that the T/T carriers of *HindIII* polymorphism had an increase of both SBP and

Table 4  
Association of *HindIII* and *S447X* polymorphisms with cardiovascular risk factors

			Without adjusted		Multiple models <sup>bc</sup>	
			OR (IC95%)	<i>p</i>	OR (IC95%)	<i>p</i>
<i>HindIII</i>	*T/G+G/G n(%)	T/T n(%)				
SBP						
<140 mm de Hg	28(73.7)	35(67.3)	1.1 (0.9, 1.4)	0.387	1.1 (1, 1.3)	<b>0.083</b>
≥140 mm de Hg	10(26.3)	17(32.7)				
DBP						
<90 mm de Hg	32(84.2)	38(73.2)	1.2 (0.9, 1.3)	0.192	1.2 (1, 1.4)	<b>0.011</b>
≥90 mm de Hg	6(15.8)	14(26.8)				
Diabetes						
No	29(76.3)	37(71.1)	1.05 (0.88, 1.26)	0.580	1 (0.9, 1.2)	0.496
Yes	9(23.9)	15(28.9)				
<i>S447X</i>	*C/G n(%)	C/C n(%)				
SBP						
<140 mm de Hg	14(77.8)	49(68.1)	1.1 (0.9, 1.4)	0.387	1.2 (1.1, 1.4)	< <b>0.001</b>
≥140 mm de Hg	4(22.2)	23(31.9)				
DBP						
<90 mm de Hg	17(84.2)	53(73.6)	1.2 (1.1, 1.4)	0.005	1.3 (1.1, 1.4)	< <b>0.001</b>
≥90 mm de Hg	1(15.8)	19(26.4)				
Diabetes						
No	16(88.9)	50(69.4)	1.2 (1, 1.5)	0.034	1.3 (1.2, 1.4)	< <b>0.001</b>
Yes	2(11.1)	22(30.6)				

SBP indicates systolic blood pressure; DBP, diastolic blood pressure. <sup>bc</sup>Adjusted by age (men ≥ 55 years; women ≥ 65 years), sex and abdominal obesity (waist circumference: men ≥ 102 cm; women ≥ 88 cm). OR= Odds ratio of generalized estimation equation (GEE); CI 95%, confidence interval of 95%. \*Reference category.

DBP measurements compared with TG+GG carriers. Tu et al. [34] previously reported similar finding, they also showed that T allele is related with an increased hypertension risk, but no statistically significant association was found. These findings have not been replicated in Caucasian populations and suggest that *LPL* might have a race-specific role in the development of HTN.

The main finding was that we found a significant association of C/C genotype of *S447X* polymorphism with T2DM, SBP and DBP. Clee et al. [35] showed for first time that C/C carriers have an increase DBP compared to C/G and G/G carriers, suggesting that G allele may influence vascular tone, independent of serum lipid profile, because *LPL* is anchored to vascular endothelium by heparansulfate proteoglycan and may be alter the cell signaling by increased nitric oxide production. Afterward, Talmud et al. [36] found a highest risk for hypertension for C allele and they found that the G allele is associated with a decrease 50% for CVD in normotensive patients. The same group suggests that the C allele could be involved in the pathophysiology of cardiovascular disease, due to that the availability of FFA may affect cardiac function, this leading to a heart growth as a compensatory effect, increasing the risk for CVD. In the case of the association of C/C

genotype with T2DM, Komurcu-Bayrak et al. [37] reported that the C/C genotype is associated with an increase in fasting glucose levels compared to C/G and G/G genotypes, also reported that the G allele is protective for CVD. Goodarzi et al. [38] showed evidence of that *LPL* gene play a significant role in the development of IR in Mexican-American population. These results suggest that overexpression of *LPL* might improve insulin resistance, although the underlying mechanism is not clear. These results imply that the association between the *LPL* gene and T2DM may be tissue specific. Cruz et al. [39] indicate that increased FFA levels could be delivered to pancreatic  $\beta$ -cells by rising *LPL* activity, consequently impairing  $\beta$ -cell function and promote apoptosis in the patients with hypertriglyceridemia, hyperinsulinemia and T2DM. Hypertriglyceridemia rise the utilization of TG as fuels inhibits the intake and oxidization of glucose [40]; intracellular fatty acid metabolites interfere with propagation of insulin signaling cascade [41] and delivering more FFA to pancreatic  $\beta$ -cells, impairs  $\beta$ -cell function and promote apoptosis [39].

The above findings may be partly responsible for the association between *LPL* and T2DM. As evidence of the relationship of the C/C genotype with TC levels, AshokKumar et al. [32] found increased TC levels in

C/C carriers compared to other genotypes. The underlying mechanisms might include the effect of high TG and CT on hemodynamics, sodium re-absorption, retention and vascular hypertrophy [42], and the role of LPL in the regulation of arterial stiffness [43]. However, the causal mechanism is complicated and tissue specific. Overexpression of LPL in monocyte-derived macrophages (MDM) induces unregulated uptake of NEFA and 2-monoacylglycerol and re-esterified into TG [44]. The lipid saturated macrophages evolve into foam cells and then penetrate the endothelium cells into the middle layer of vascular wall [45]. In addition, the LPL could trigger other pro-atherogenic events, such as the proliferation of smooth muscle cells [46]. In the adipose and muscle tissues, however, LPL acts protectively because it aids in the clearance of circulating lipoprotein particles through storage [47].

One limitation of our study is a low number of families; therefore the findings should be further confirmed in a larger population of Mexican families.

In summary, the *HindIII* and S447X polymorphisms of the *LPL* gene were associated with hypertension and Type 2 diabetes and could be genetically involved in the susceptibility for these diseases in Mexican families.

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