Relationship of lipoprotein lipase gene variants and fasting triglyceride levels in a pediatric population: The CASPIAN-III study

Roya Kelishadi^{1, A–F}, Mahin Hashemipour^{2, A, B, D, F}, Behnoosh Esteki^{1, A, B, D, F}, Mohammad Hasan Tajadini^{3, A–F}, Laleh Rafiei^{3, A–F}, Mohammad Esmail Motlagh^{4, A, B, D, F}, Golnaz Vaseghi^{3, A–F}, Gelayol Ardalan^{1, A, B, D, F}, Shaghayegh Haghjooy Javanmard^{3, A, B, D, F}

¹ Pediatrics Department, Child Growth and Development Research Center, Research Institute for Primordial Prevention of Non-Communicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran

² Alzahra Hospital, Pediatrics Department, School of Medicine, Isfahan University of Medical Sciences, Iran

³ Physiology Department, Applied Physiology Research Center, Isfahan University of Medical Sciences, Iran

⁴ Pediatrics Department, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

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Address for correspondence

Shaghayegh Haghjooy Javanmard
E-mail: shaghayegh.haghjoo@gmail.com

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Abstract

Background. Lipoprotein lipase (LPL) is one of the major enzymes responsible for the hydrolysis of triglyceride (TG)-rich lipoprotein. The effects of LPL polymorphisms on serum TG are inconsistent among different populations.

Objectives. This study aims to assess the TG serum concentration and distributions of three LPL single nucleotide polymorphisms (SNPs), namely D9N, HINDIII and S447X, in a nationally representative sample of Iranian adolescents.

Material and methods. We studied the associations between SNP genotypes and TG levels in a nationally representative sample of Iranian adolescents. Genotyping was performed in 750 randomly selected participants. We compared the genotypes according to different TG levels.

Results. This study comprised 746 participants, with mean $\pm$ SD age of 14.6 ± 2.5 years. The distribution of genotypes of D9N and S447X were not significantly different according to TG levels. Regarding the HINDIII polymorphism, the distribution of GG, GT, and TT genotypes were significantly different in participants with low, borderline-high, and elevated TG ($p = 0.02, 0.03$, and 0.01 , respectively). The mean TG was not significantly different according to the genotype distribution.

Conclusions. In this study, most of the LPL gene variants were not significantly different in adolescents with normal and elevated TG, and the mean TG was not different in participants with various genotypes. As the first evidence from the pediatric population of the region of the Middle East and North Africa (MENA), these results might be used in international comparisons. Our findings might suggest that the high prevalence of hypertriglyceridemia in Iranian adolescents is more likely to be a result of lifestyle rather than genetic factors.

Key words: single nucleotide polymorphisms, adolescence, triglycerides, lipoprotein lipase

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Lipid disorders are major risk factors for cardiovascular diseases (CVDs). Epidemiological evidence indicates that plasma triglyceride (TG) concentration is a robust and independent CVD risk factor.^{1,2}

Plasma TG levels are complex traits with inter-individual variations; they are mainly influenced by genetic variants.³ The genetic determinants of severe hypertriglyceridemia have yet to be determined.⁴ Lipoprotein lipase (LPL) is the major enzyme responsible for the hydrolysis of the TG-rich lipoprotein of circulating lipoproteins.⁵ LPL has an important function in TG metabolism; LPL deficiency would result in hypertriglyceridemia.^{6–8}

Several genome-wide association studies (GWAS) have investigated plasma TG levels.^{9–11} They have confirmed a number of genes with inter-individual variations in TG which were found in previous functional and/or candidate gene association studies. However, the association of these variants with hypertriglyceridemia in other populations has yet to be determined. Few post-GWAS replication studies have been conducted in various ethnic groups. Therefore, more studies investigating GWAS-identified variants are needed in diverse racial/ethnic groups.

Hypertriglyceridemia is quite prevalent in the Middle East and North Africa (MENA) region.^{12,13} National studies have documented that elevated TG is highly prevalent in Iranian adult and pediatric populations.^{14,15} As the first national survey in the pediatric population of MENA, the present investigation aims to assess the TG serum concentration and distributions of three LPL single nucleotide polymorphisms (SNPs), specifically D9N, HINDIII and S447X, in a nationally representative sample of Iranian adolescents.

Material and methods

Study population

This project was conducted as a sub-study of the national survey of school student high risk behaviors, which was the third survey of a nationwide school-based surveillance system entitled CASPIAN-III Study. This health survey was conducted in 2009–2010 in 27 provinces of Iran.

Ethics committees and other relevant national regulatory organizations approved the study. Our team obtained written informed consent from parents and oral assent from participants. The details of the data collection and sampling were published previously, and here we present these in brief.¹⁷

The main survey included 5,528 students aged 10–18 years who were recruited by multistage random cluster sampling from urban and rural areas. For the current study, we randomly selected 750 frozen samples (girls = 364, boys = 386) stored at -70°C . As no uniform cutpoint exists to define elevated TG in the pediatric population, we

used two definitions to categorize TG levels. As one definition, we considered the highest quartile ($> 75^{\text{th}}$ percentile) of TG in the population studied as elevated TG, and the rest as normal TG. As another definition, we used the cut-points suggested by the guidelines of the National Heart, Lung, and Blood Institute (NHLBI) for individuals aged 10–19 years, i.e. TG < 90 mg/dL as acceptable, 90–129 mg/dL as borderline-high, and ≥ 130 mg/dL as high levels, respectively.¹⁸

Physical examination and biochemical measurements

A team of trained health care professionals and physicians recorded the information and conducted the physical examination under standard protocols and by using calibrated instruments. Body mass index (BMI) was calculated as the weight (kg) divided by the height squared (m^2). For blood sampling, participants were invited to the nearest health center to the school, where fasting venous blood was taken and analyzed for plasma levels of glucose, lipid profile, and liver function tests. In each county, the biochemical analysis was performed in the central provincial laboratory that met the standards of the National Reference laboratory, a collaborating center of the World Health Organization in Tehran.¹⁷

Genetic studies

DNA extraction

DNA was extracted from peripheral blood by a QIAamp DNA Blood Mini kit (Qiagen, Germany) according to the manufacturer's protocol. Real-time PCR and high-resolution melt analysis were performed in a Corbett rotor-gene 6000 instrument (Corbett Research Pty Ltd, Sydney, Australia).

High resolution melt analysis

Primers were designed by Beacon Designer 7.91 (PREMIER Biosoft International, USA) to flank the genomic regions and were synthesized by TIB MOLBIOL (Germany). The primer sequences are shown in Appendix 1.

Amplicons were generated under the following conditions using a Type-it HRM kit (Qiagen, Germany): one cycle at 95°C for 15 min; 40 cycles at 95°C for 15 s, 60.0°C for 15 s, 72°C for 15 s, one cycle of 95°C for 1 s, 72°C for 90 s and a melt from 70 to 95°C rising at 0.1°C per s. The amplification mixture of a total volume of 25 μL included 12.5 μL of HRM PCR master mix, 1.75 μL of 10 μM primer mix, 2 μL of genomic DNA as a template and 8.25 μL of RNase-free water. For each genotype reaction, we included sequence-proven major and minor allele homozygote and heterozygote controls. The HRM analysis was performed by instrument software, which allows for

APPENDIX 1

Primer Sequence 5'-3'	
F: GCAGAAAGGAAAGGCACCTGCGG R: AGCTCAGGATGCCAGTCAGCT	S447X
F: TCCAAGATAATCTCAACCT R: TAACAATAACAGCACACTATA	HINDIII
F: ATAGCATCAGCGGTGGTT R: GGAATGAGGTGGCAAGTG	D9N
F: TGAGGATCTACCTGCCAG R: CAAGGCTCCCCAGACAAG	Apo A5

R – reverse; F – forward.

clustering of the samples into groups based on a difference plot obtained by analyzing the differences in melting curve shape between the known controls and samples.

Statistical analysis

Statistical analysis was performed with SPSS 20.0 (Chicago, USA) with a nominal significance level of $p < 0.05$. The deviations of genotype frequencies from those predicted by the Hardy-Weinberg equation were tested by χ^2 analysis. Pairwise linkage disequilibrium for single nucleotide polymorphism at the same locus was determined using correlation coefficients as described before.¹⁹ We used pairwise χ^2 analysis, one-way analysis of variance (ANOVA) and Bonferroni post-hoc tests to assess the allele frequency differences between TG categories.

Results

This study comprised 746 participants, with mean $\pm$ SD age of 14.6 ± 2.5 years. Table 1 presents the general characteristics (mean $\pm$ SD) of participants with normal or elevated TG. It shows that the mean values of BMI

and SGOT were higher in those with elevated TG than in those with normal TG levels; without significant difference in other variables. The BMI standard deviation score (BMI-SDS) was also significantly higher in those with elevated TG than in their other counterparts (0.4 vs. -0.2 , respectively, $p = 0.01$). The individuals were genotyped for three SNPs in the D9N, Hind III and S447X genes, i.e. rs1801177, rs320 and rs328. The distribution of these SNPs in participants with TG higher and lower than the 75th percentile is presented in Table 2. The distribution of the GG genotype of D9N (rs1801177) polymorphism in both individuals with elevated and normal TG is zero, and the differences of AA and AG genotypes were not significant between these groups ($p = 0.64$). For the HINDIII (rs320) polymorphism, the frequency of the TT genotype was 17.7% and 18.9% in the groups with elevated and

Table 2. Pairwise comparisons of the genotype and allele frequency in participants with normal and elevated triglycerides levels: The CASPIAN-III study

Genotype	TG > 107 mg/dL (n = 186)	TG < 107 mg/dL (n = 560)	p-value
D9N (rs1801177)			
AA	174 (93.5)	529 (94.5)	0.64
AG	12 (6.5)	31 (5.5)	
GG	0	0	
G	0.032	0.028	
HINDIII (rs320)			
GG	80 (43)	185 (33)	0.05
GT*	73 (39.2)	269 (48)	
TT*	33 (17.7)	106 (18.9)	
T	0.37	0.42	
S447X (rs328)			
CC	143 (76.9)	448 (80)	0.50
CG	40 (21.5)	100 (17.9)	
GG	3 (1.6)	12 (2.1)	
G	0.12	0.11	

* – significantly different with GG group.

Table 1. General characteristics of participants with normal and elevated triglycerides levels: the CASPIAN-III Study

Characteristics	TG > 107 mg/dL (n = 186)	TG < 107 mg/dL (n = 560)	p-value
Age, years	14.7 ± 2.4	14.6 ± 2.6	0.63
Body mass index (kg/m ²)	20.3 ± 4.2	18.8 ± 3.8	< 0.0001
Systolic blood pressure (mm Hg)	102.5 ± 12.5	102.8 ± 13.3	0.79
Diastolic blood pressure (mm Hg)	65.9 ± 9.5	66.1 ± 10.3	0.78
Triglycerides (mg/dL)	152.5 ± 51.4	73.8 ± 18.5	< 0.0001
HDL-C (mg/dL)	45.7 ± 22.6	50.5 ± 21.5	0.01
LDL-C (mg/dL)	82.3 ± 32.5	81.9 ± 29	0.89
FBG (mg/dL)	86.9 ± 13.5	86.3 ± 13.2	0.52
SGOT (U/L)	31.4 ± 18.0	24.8 ± 12.0	< 0.0001
SGPT (U/L)	21.1 ± 17.6	19.5 ± 12.5	0.28

*Data is presented as mean $\pm$ SD. TG – triglycerides; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; FBG – fasting blood glucose; SGOT – serum glutamic oxaloacetic transaminase; SGPT – serum glutamic-pyruvic transaminase.

Table 3. Comparisons of the genotype and allele frequency in participants with triglyceride levels categorized according to the NHLB guidelines (18): the CASPIAN-III Study

Genotype	TG < 90 mg/dL (n = 442)	TG = 90–129 mg/dL (n = 194)	TG > 130 mg/dL (n = 110)	p-value
D9N				
AA	419	182	102	0.67
AG	23	12	8	0.68
HINDIII				
GG	146	65	54	0.02
GT	210	90	42	0.03
TT	86	39	14	0.01
S447X				
CC	350	152	89	0.79
CG	81	39	20	0.76
GG	11	3	1	0.56

TG – triglycerides; NHLB – National Heart, Lung, and Blood Institute.

normal TG, respectively. In the HINDIII (rs320) polymorphism, the differences between the two TG groups are near the significant value ($p = 0.05$). The same comparison conducted among participants with TG levels categorized according to the NHLB revealed similar results; it showed that the only significant difference existed between the genotypes of the HINDIII polymorphism (Table 3).¹⁸ Furthermore, as shown in Table 4, the mean TG was not significantly different according to the genotype distribution. Table 5 presents the comparison of different genotype significations. The observed genotype frequen-

cies for two polymorphisms, D9N (rs1801177) and HINDIII (rs320), were in the Hardy-Weinberg equilibrium in individuals with normal TG levels ($p = 0.5$, $X^2 = 0.45$ for D9N (rs1801177); $p = 0.6$, $X^2 = 0.21$ for HINDIII (rs320)), but the S447X (rs328) polymorphism did not follow the Hardy-Weinberg equilibrium.

Discussion

In this study, which to the best of our knowledge is the first of its kind in the MENA region, we presented the results on polymorphisms of SNPs, rs1801177, rs328 and rs320, in LPL encoding lipoprotein in a nationally representative sample of Iranian adolescents. We did not find any difference in the distribution of the GG genotype of the D9N (rs1801177) polymorphism in individuals in the upper quartile of TG and their other counterparts; we also observed that the differences of the AA and AG genotypes were not significant in these two groups. Regarding the HINDIII (rs320) polymorphism, the differences between two groups the genotype distribution differences for S447X (rs328) polymorphism were significant in these groups. The same comparison among participants with normal, borderline-high, and high TG levels showed that the only significant differences existed between genotypes of HINDIII polymorphism.¹⁸

LPL is the major enzyme responsible for the hydrolysis of TG-rich lipoprotein in circulating lipoproteins.⁵ Genetic defects in LPL are responsible for the reduction in TG-rich lipoprotein clearance, and mutations in the LPL gene play important roles in the development of hypertriglyceridemia in the general population.^{20–23} To date, approximately 143 different mutations have been found in the human LPL gene, 90% of which occur in the coding regions and affect LPL functions through catalytic activity, dimerization, secretion, and heparin bonding.²⁴

Some previous studies have shown that SNPs located in the LPL gene are associated with TG levels. It is also documented that rs2083637 and rs10096633 are related to TG

Table 4. Mean value of triglycerides in different genotypes: the CASPIAN-III Study

Genotype	Alleles	Number	TG (mg/dL) mean $\pm$ SD
D9N	AA	703	92.92 $\pm$ 44.62
	AG	43	101.12 $\pm$ 58.78
HIND3	GG	265	100.49 $\pm$ 52.43
	GT	342	90.77 $\pm$ 42.37
	TT	139	86.31 $\pm$ 36.64
S447x	CC	591	94.27 $\pm$ 47.00
	CG	140	91.77 $\pm$ 40.02
	GG	15	73.80 $\pm$ 30.80

SD – standard deviation; TG – triglycerides.

Table 5. Comparison of different genotype significations: the CASPIAN-III Study

	Genotype	TG (mg/dL) Mean $\pm$ SD	p-value
HINDIII	GG	100.49 $\pm$ 52.4	0.004a, 0.01b, 0.03c
	GT	90.77 $\pm$ 42.37	
	TT	86.31 $\pm$ 36.6	
S447X	CC	94.27 $\pm$ 47	0.2d, 0.84e, 0.22f
	CG	91.77 $\pm$ 40	
	GG	73.80 $\pm$ 30.8	

TG – triglycerides; SD – standard deviation; a – ANOVA test used to compare (GG, GT and TT); results of Bonferroni post-hoc test; b – p-value to compare (GG, TT); c – p-value to compare (GG, GT); d – ANOVA test used to compare (CC, CG and GG); e – p-value to compare (CC, CG); f – p-value to compare (CC, GG).

levels.¹¹ Moreover, in African-Americans and European-American populations, rs326, rs328 and rs13702 are associated with HDLC and TG levels.²⁵

Our findings are consistent with another study that did not find any relationship between the HINDIII allele and higher LPL activity.²⁶ However, contradictory results are reported from studies conducted in different populations, for instance in Tunisian patients, the mutated HINDIII genotype was significantly associated with increased TG and ApoB/ApoAI ratio, as well as with decreased HDL-C. Significant genetic differences are also reported among Mexican-American and non-Hispanic Caucasians; moreover, in Saudi patients, homozygosity for LPL haplotype1 had a protective role against CVDs. In all these studies, LPL gene variants were associated with dyslipidemia.^{27–29}

The S447X variant has a different function relevant to the lipid profile.³⁰ However, the major function of the S447X variant is TG hydrolysis.³¹ The S447X variant is associated with a number of lipid parameters. The LPL S447X variant is mostly associated with lower TG and higher HDL-C levels, as well as lower CVD risk.^{32,33}

CVD is a complex problem resulting from several interactions between various genetic and environmental factors. After secretion, LPL binds to the luminal surface of endothelial cells and shows promising effects in the catabolism of TG in circulation.³⁴ The genetic risk of CVD is not fully understood perhaps because of different genes involved in its mechanism. LPL can be a possible target for the treatment of CVD; however multiple LPL gene variants and several mutations are engaged in the pathophysiology of CVD.³⁵

The main limitation of this study is its cross-sectional nature; moreover we could only study a limited number of genetic factors. The strengths of this study are the novelty in the pediatric age group, including a nationally representative sample of participants, and being the first of its kind in the MENA region, where hypertriglyceridemia is highly prevalent.

In the current study, most of the LPL gene variants were not significantly different in adolescents with normal and elevated TG. The mean TG was not significantly different according to the genotype distribution. As the first evidence from the pediatric population of the MENA region, the current findings might be used in international comparisons. Our findings might suggest that the high prevalence of hypertriglyceridemia in Iranian adolescents is likely to be caused by lifestyle rather than genetic factors.

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