UC 577,1; 61 ISSN 0354-3447

Jugoslov Med Biohem 22: 237–242, 2003 *Originalni naučni rad*
Original paper

ASSOCIATION OF LIPOPROTEIN LIPASE GENE ASN291SER DNA POLYMORPHISM WITH PLASMA LIPID LEVELS AND BLOOD PRESSURE LEVELS IN HEALTHY POPULATION OF SERBIA

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Summary: The lipoprotein lipase (LPL) Asn291Ser polymorphism has been determined in 313 healthy subjects from Serbia. LPL variants were detected by mismatch PCR followed by *Rsa*I restriction digestion and 5% polyacrylamide gel electrophoresis. The relationship between LPL Asn291Ser polymorphism and plasma lipid levels, and association between Asn291Ser polymorphism and arterial blood pressure were found. Significant decrease in high-density lipoprotein cholesterol levels ($p<0.05$) and significantly higher values of systolic blood pressure (SBP) ($p<0.01$) were found in females carrying the Ser291 allele. Male carriers of Ser291 allele had significantly lower levels of SBP ($p<0.01$). Findings from our study could be of importance for further research of pathological state regarding lipid metabolism and other risk factors of vascular diseases in Serbian population.

Key words: lipoprotein lipase, lipids, blood pressure

Introduction

Enzyme lipoprotein lipase (LPL) has a central role in lipid metabolism. LPL hydrolysis triglycerides (TG) in the core of circulating chylomicrons and very-low density lipoproteins (VLDL). Lipoprotein triglyceride is hydrolyzed to free fatty acids that are taken-up by tissues and either used as energy or reassembled into triglyceride and stored (1). The major sites of LPL synthesis are the skeletal and cardiac muscle and adipose tissue.

The human LPL gene consists of ten exons, spans about 30kb on chromosome 8p22 (6). About 80 naturally occurring mutations in LPL gene have been described in humans, the majority of which are missense. LPL mutations are spread over most exons; the most frequent sites of these mutations are in exons 5 and 6 (7). Most amino-acid changing mutations in LPL are rare, either restricted to single families or isolated geographic regions (1). Asn291Ser substitution has been identified widely, and it seems to be common in the general population (2, 3). Amino acid change Asn291Ser is a result of A->G transition at position 1127 within exon 6 of LPL gene. Asn291Ser substitution is located in the N-terminal end and may influence the catalytic activity of LPL (4). LPL is most active as a dimmer, composed of two identical subunits arranged in a head to tail configuration (5). Asn291Ser substitution destabilizes homodimmer formation, with a consequent loss of lipolytic activity (3).

Based on a meta-analysis, heterozygote frequencies in population-based studies ranged from 1 7 % (8). The Ser291 variant in general population is associated with an increase in plasma triglycerides, and decrease in high-density lipoprotein (HDL) cholesterol concentrations (9, 10). The aim of this study was to

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Table I Characteristics of a studied population of Serbia

Variable	Total n=313	Men n=169	Women n=144
Age (years)*	40.1 ± 15.3	38.1 ± 15.2	42.5 ± 15.2
Smokers (n (%))	169 (54.0 %)	80 (47.0%)	89 (62.0%)
BMI (kg/m^2)*	25.1 ± 3.85	25.78 ± 3.02	24.40 ± 4.53
TC (mmol/L)*	5.54 ± 1.14	5.33 ± 1.07	5.79 ± 1.18
HDLC (mmol/L)*	1.36 ± 0.29	1.28 ± 0.27	1.45 ± 0.29
LDLC (mmol/L)*	3.52 ± 0.97	3.40 ± 0.92	3.66 ± 1.01
TG (mmol/L)	1.22 ± 0.59	1.21 ± 0.55	1.23 ± 0.63
SBP (mmHg)	132.0 ± 22.7	132.9 ± 18.6	131.0 ± 26.8
DBP (mmHg)*	82.2 ± 11.7	83.6 ± 10.1	80.6 ± 13.3

* $P<0.05$; Non-adjusted values ($\bar{x}\pm$SD) are shown. BMI = body mass index; TC = total cholesterol; HDLC = high density lipoprotein cholesterol; LDLC low density lipoprotein cholesterol; TG = triglycerides, SBP = systolic blood pressure; DBP = diastolic blood pressure.

confirm the presence of Asn291Ser polymorphism in the human LPL gene, to determine genotype and allele frequencies of Asn291Ser polymorphism in Serbian population and relationship between this polymorphism and both plasma lipid and blood pressure levels.

Material and Methods

Subjects and study design

The epidemiological study was performed in a population-based sample of Caucasian subjects from Serbia. Informed consent was obtained from each participant in the study. Personal data (age, sex, weight, height and blood pressure) were obtained from all participants. Blood pressure (BP) was measured in the sitting position after a 15-minute rest. It was read three times by mercury sphygmomanometer and the mean value of these measurements was used. A questionnaire was designed to obtain relevant social (smoking, alcohol consumption, physical activity), medical (general health, cardiovascular state, physician visits), and family (general health and cardiovascular status of parents and siblings) history. All subjects with a personal or family history of cardiovascular diseases, diabetes and thyroid dysfunction were excluded. Individuals taking any drug with lipid lowering effects were also excluded.

Blood samples were obtained from 313 healthy, unrelated individuals (144 women and 169 men), collected from participants after 12 hours of fasting. The total plasma cholesterol and TG levels were determined on Monarch Plus apparatus (Instrumentation Laboratory, Lexington, USA) using enzymatic colorimetric methods. HDL cholesterol was determined after dextran sulfate-Mg^{2+} precipitation of VLDL and low-density lipoprotein (LDL) cholesterol; using CHOD-PAP method. LDL cholesterol was calculated according to the Friedwald's formula (11) for participants with TG levels < 4.5 mmol/L. All reagent kits were provided by Instrumentation Laboratory (Lexington, USA). Characteristics of the population are shown in *Table I*.

DNA analysis

Genomic DNA was purified by the proteinase K/phenol extraction method, as previously described (12), from the whole blood samples collected with EDTA as anticoagulant. DNA segment contained within exon 6 of LPL gene was amplified by mismatch polymerase chain reaction (PCR) in Touch Down™ (Hybaid, Teddington, UK). Genotypes of LPL Asn291Ser polymorphism were determined after *Rsa*I restriction digestion (13), on a 5% polyacrylamide gel (PAA).

Statistical analysis

Frequencies of LPL alleles were estimated by the gene counting method. Chi-square statistics was used to test fit goodness to the Hardy-Weinberg's equilibrium. A one-way analysis of variance (ANOVA) was performed separately in both men and women to test the null hypothesis of equality of lipid and/or BP levels between LPL genotypes. We considered statistical significance to be at the 0.05 levels. Due to skewed distribution of both HDLC and TG, all TG and HDLC analysis were performed on logarithmically transformed values. All lipid and BP values were adjusted to remove the effects of a set of significant covariates (age, body mass index (BMI) and cigarette smoking).

Results

LPL genotype and allele frequencies

Relative frequencies of LPL alleles and genotypes in a sample of 313 healthy subjects from Serbia are shown in *Table II*. Allele frequencies of Asn291/Ser291 were 0.984/0.016. The observed LPL genotype frequencies were in the Hardy-Weinberg's equilibrium.

Table II Distribution of LPL genotype and allele frequencies in a healthy population of Serbia

Allele			Relative frequency	
Asn291			0.984	
Ser291			0.016	
Genotype	Observed		Expected	
	n	%	n	%
Asn291Asn	303	98	303.08	96.83
Asn291Ser	10	2	9.84	3.14
Ser291Ser	0	0	0.08	0.03
Whole sample	313	100	313	100

Association of LPL polymorphism with serum lipid levels and BP values

Adjusted means of serum lipid levels (total cholesterol, HDL cholesterol, LDL cholesterol and TG) according Asn291Ser genotypes are shown in *Table III*. Subjects with genotype Asn291Asn had higher levels of total, LDL cholesterol and HDL cholesterol compared with subjects with Asn291Ser genotype, but statistical significance was not reached ($p>0.05$). There was no significant difference in triglycerides and BP levels between two genotypes in the whole sample ($p>0.05$).

In males with Asn291Asn genotype levels of both total and LDL cholesterol were higher than in those with Asn291Ser genotype ($p>0.05$). No significant difference ($p>0.05$) was found in TG and DBP levels between two genotypes. SBP level was significantly higher in males with Asn291Asn compared to those with Asn291Ser LPL genotype ($p<0.05$).

Significant decrease in HDL cholesterol levels ($p<0.05$) was found in females with Asn291Ser LPL

Table III Plasma lipid levels and BP values in relation to LPL genotypes in a healthy population of Serbia

		LPL genotype		P
		Asn291Asn	Asn291Ser	
Total		303	10	
	TC[a], mmol/L	5.56 ± 1.14	5.05 ± 1.04	0.126
	HDLC[a], mmol/L	1.36 ± 0.29	1.21 ± 0.22	0.110
	LDLC, mmol/L	3.53 ± 0.98	3.29 ± 0.84	0.408
	TGa, mmol/L	1.22 ± 0.58	1.20 ± 0.81	0.162
	SBP[a], mmHg	132.0 ± 22.5	132.0 ±29.4	0.994
	DBP[a], mmHg	82.3 ± 11.8	81.0 ± 9.1	0.707
Men		162	7	
	TC, mmol/L	5.35 ± 1.07	4.80 ± 1.06	0.143
	HDLC[a], mmol/L	1.29 ± 0.27	1.27 ± 0.24	0.964
	LDLC, mmol/L	3.42 ± 0.93	2.98 ± 0.67	0.168
	TG[a], mmol/L	1.21 ± 0.53	1.21 ± 0.98	0.067
	SBP[a], mmHg	133.6 ± 18.6	117.1 ± 9.5	0.009
	DBP[a], mmHg	83.9 ± 10.1	77.9 ± 8.1	0.080
Women		141	3	
	TC[a], mmol/L	5.79 ± 1.19	5.64 ± 0.86	0.901
	HDLC[a], mmol/L	1.46 ± 0.29	1.08 ± 0.04	0.019
	LDLC[a], mmol/L	3.66 ± 1.02	4.03 ± 0.81	0.427
	TG[a], mmol/L	1.23 ± 0.63	1.17 ± 0.20	0.791
	SBP[a], mmHg	130.2 ± 26.3	166.7 ± 32.1	0.009
	DBP[a], mmHg	80.4 ± 13.3	88.3 ± 7.6	0.193

[a] Log-transformed values are used in analysis, but arithmetic means are presented.

genotype. SBP levels were significantly higher ($p<0.01$) in Asn291Ser genotype than in Asn291Asn. Non-significant trend was observed in females: subjects with LPL genotype Asn291Asn had lower values of both LDL cholesterol and DBP.

Discussion

Asn291Ser LPL polymorphism has been determined for the first time in healthy and unrelated subjects from Serbia. It has been found that the frequency of this polymorphism varies in different ethnic groups (14). Genotype frequencies of Asn291Ser polymorphism in our population correspond to those reported for Caucasian populations (9, 15, 16, 18, 20, 21) *(Table IV)*.

The relation between Asn291Ser LPL polymorphism and HDL cholesterol level in healthy individuals has been reported in various studies. In some it has been significantly decreased (10, 16) but in the others insignificantly changed (9). In this study a trend to decrease in HDL cholesterol level among heterozygous carriers of Asn291Ser substitution in the whole population sample and among men, was more pronounced and reached statistical significance in females. The observed more emphasized effect of Asn291-Ser polymorphism on HDL cholesterol in females is in accordance with the results of a previous study, where HDL cholesterol has been found to be significantly decreased (10). Variation of triglyceride levels between individuals with different LPL genotypes in our population was not statistically significant. Previous reports have found significant increase in triglyceride levels of carriers of Asn291Ser polymorphism (9), or only a trend to the increase (16).

The association between lipoprotein lipase gene Asn291Ser polymorphism and BP levels in a healthy (random) population was not examined until now. In our study of Serbian population relationship between LPL Asn291Ser polymorphism genotypes and blood pressure levels was in opposite manner between genders. Recently, a study of pre-eclamptic women revealed a significantly increased frequency in Asn291Ser genotype compared to pregnancy controls (17). In our study female carriers of Ser291 allele had significantly higher levels of SBP ($p=0.009$). On the contrary, in male subjects with Asn291Ser LPL genotype SBP values were significantly lower compared to the other genotype ($p=0.008$). Differences in the effects of Asn-291Ser substitution that were observed among women and men suggest a possible modulation of LPL expression by hormonal or lifestyle factors.

Since the carriers of Asn291Ser genotype are rare in population, future studies require examination of a larger sample. However, data from our study are of importance for further research of pathological state regarding lipid metabolism and other risk factors of vascular diseases in Serbian population.

Acknowledgement: This work was supported by Government Research Grant M2011.

Table IV Asn291Ser polymorphism in different populations

Origin	Number of subjects	Ser291+* (%)	χ^2(p)	References
Serbia	313	3.2		
UK	749	3.2	1	9
Swedish	89	6.7	<0.013	9
German	283	4.9	<0.158	18
Danish	9214	4.9	<0.158	10
ECTIM	723	5.4	<0.084	19
Dutch	215	4.6	<0.235	20
EARS I	958	3.4	<0.851	21
Germany	96	3.1	<0.922	9
Australia	663	3.3	<0.924	16
Canada (Chinese)	321	1.6	<0.029	22
Italy	191	4.2	<0.381	15

*Ser 291+, genotypes Asp291Ser and Ser291Ser

ASOCIJACIJA POLIMORFIZMA DNK ASN291SER U HUMANOM GENU ZA LIPOPROTEIN LIPAZU SA NIVOIMA LIPIDA U PLAZMI I VISINOM KRVNOG PRITISKA U ZDRAVOJ POPULACIJI SRBIJE

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Kratak sadržaj: Genotipovi polimorfizma Asn291Ser u genu za lipoproteinsku lipazu (LPL) su po prvi put određeni u uzorku zdrave populacije sa teritorije Srbije, koji se sastojao od 313 osoba. Genotipizacija je urađena uvođenjem restrikcionog mesta za *Rsa*I u reakciji lančane polimerizacije (PCR). Uočena je asocijacija između Asn291Ser polimorfizma u genu za LPL i nivoa lipida u plazmi, kao i povezanost proučavanog polimorfizma sa vrednostima arterijskog krvnog pritiska. Značajno sniženje HDL holesterola ($p<0,05$) i značajno viši nivoi sistolnog krvnog pritiska. (SBP) ($p<0,01$) su nađeni kod žena koje imaju Ser291 alel. Kod muškaraca, nosioca alela Ser291, uočeni su značajno niži nivoi SBP ($p<0,01$). Podaci naše studije mogli bi biti polazište za utvrđivanje asocijacije ovog polimorfizma i parametara patoloških stanja povezanih sa dislipidemijama.

Ključne reči: lipoproteinska lipaza, lipidi, krvni pritisak

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Received: May 22, 2003

Accepted: June 30, 2003