Association of Cold Receptor *TRPM8* Gene Polymorphism with Blood Lipid Indices and Anthropometric Parameters in Shorians

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We analyzed single nucleotide polymorphisms of the cold receptor *TRPM8* gene as genetic markers of blood serum lipid indices in Shorians. Associations were found between rs11562975 (L250L) *TRPM8* gene mononucleotide polymorphism with total cholesterol and LDL cholesterol and between rs28901637 (P249P) and HDL cholesterol. No associations of P249P and L250L with triglyceride level were found. L250L polymorphism was associated with anthropometric parameters characterizing lipid metabolism (hip and waist circumferences). The *TRPM8* gene is likely to be involved in the regulation of lipid metabolism.

Key Words: *TRPM8 gene; genotypes; association; cholesterol; anthropometric indices*

Elevated serum LDL cholesterol (CH) and triglyceride (TG) content and low HDL CH level are risk factors for cardiovascular diseases [1,4,7,13]. Recent studies revealed association of polymorphous markers of certain genes with blood lipid phenotypes: *APOE/PCSK9* genes with LDL CH, *CETP/LIPC/LPL* gene with HDL CH and *APOA5/LPL* with TG [3,5,6,8,11,14].

In this study, *TRPM8* gene (2q37.1) encoding cold receptor sensitive to low temperature and some chemical agents such as menthol and icilin was chosen as a candidate gene [12]. Expression of *TRPM8* was detected in sensory neurons (cells of the trigeminal nerve nucleus and sympathetic ganglia cells), epithelial cells of various organs, smooth muscle cells of blood vessels, and cancer cells [9,12]. Activation of TRPM8 protein isoform of human lung epithelium enhances expression of some cytokine genes controlling inflammation process [10]. The *TRPM8* gene can also be involved in the regulation of other physiological functions.

Here we studied possible association of single nucleotide polymorphisms (SNP) rs28901637 (P249P) and rs11562975 (L250L) of *TRPM8* gene with blood serum lipid indices and anthropometric parameters in Shorians. The studied SNP are localized in the coding region of the gene (exon 7) and are responsible for synonymous substitution: P249P polymorphism consists in A→T substitution, which does not lead to proline 249 substitution with another amino acid and L250L (G→C) does not lead to leucine 250 replacement. Less widespread alleles of these SNP are frequent in Shorians: 0.112 (T-allele P249P) and 0.344 (C-allele L250L) [2].

MATERIALS AND METHODS

A sample of Shorians formed by residents of Mezhdurechensky district of the Kemerovo region included 133 individuals (112 women and 21 men); mean age was 44.29 years for women (18-72 years) and 47.72 years for men (23-72 years).

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Genomic DNA was extracted from the blood using standard procedure of phenol-chloroform extraction. Genotyping was performed using allele-specific PCR technique.

We used the following primers for fragment amplification: for SNP rs28901637 (P249P): 5'-ccgatgacttcacaagagataca-3' (A-allele), 5'-ccgatgacttcacaagagatact-3' (T-allele), 5'-ccctaaccactgaccttgaata-3' (common); for SNP rs11562975 (L250L): 5'-tttgtggttgttgtccaggatattc-3' (G-allele), 5'-ttgtggttgttgtccaggatattg-3' (C-allele), 5'-atataggattctgggaggaggcat-3' (common).

The following PCR conditions were used: denaturation at 95°C for 1 min, annealing at 57°C (P249P) or 60°C (L250L) for 1 min, synthesis at 72°C for

TABLE 1. Blood Lipid Level and Anthropometric Parameters in Individuals with Different L250L SNP Genotypes of *TRPM8* Gene in Shorians ($M \pm m$)

Index	Mean values for different genotypes			*p*	
	GG	GC	CC		
Women					
N	52	48	12		
Total CH	4.98±0.20	5.68±0.20	5.23±0.3	0.017	0.005 (GG/GC)
LDL CH	3.07±0.20	3.71±0.20	3.37±0.30	0.036	0.010 (GG/GC)
TG	1.33±0.10	1.32±0.10	1.04±0.20	n.s.	n.s.
HDL CH	1.31±0.00	1.37±0.00	1.36±0.10	n.s.	n.s.
WC	69.29±0.90	67.27±0.90	66.67±1.90	n.s.	n.s.
HC	90.00±1.00	88.10±1.10	84.75±2.10	0.078	0.030 (GG/CC)
BWI	22.87±0.40	22.59±0.40	23.22±0.80	n.s.	n.s.
Men					
N	9	11	1		
Total CH	5.08±0.60	5.35±0.50	5.10	n.s.	n.s.
LDL CH	3.33±0.60	3.44±0.50	2.90	n.s.	n.s.
TG	1.00±0.20	1.23±0.10	1.65	n.s.	n.s.
HDL CH	1.3±0.1	1.33±0.10	1.49	n.s.	n.s.
WC	77.89±1.60	72.91±0.40	70.00	0.057	0.030 (GG/GC)
HC	95.78±1.70	87.91±1.50	86.00	0.007	0.003 (GG/GC)
BWI	23.89±0.60	24.19±0.50	18.80	n.s.	n.s.
Total					
N	61	59	13		
Total CH	5.0±0.2	5.62±0.20	5.21±0.30	0.031	0.009 (GG/GC)
LDL CH	3.11±0.20	3.66±0.20	3.36±0.30	0.049	0.020 (GG/GC)
TG	1.28±0.80	1.31±0.60	1.09±0.50	n.s.	n.s.
HDL CH	1.31±0.20	1.36±0.60	1.37±0.20	n.s.	n.s.
WC	70.56±0.80	68.32±0.90	66.92±1.48	0.084	0.069 (GG/GC)
					0.078 (GG/CC)
HC	90.85±0.90	88.07±0.90	84.85±2.00	0.011	0.037 (GG/GC)
					0.007 (GG/CC)
BWI	23.03±0.30	22.89±0.30	22.88±0.80	n.s.	n.s.

Note. Here and in Table 2: WC (waist circumference, cm), HC (hip circumference, cm), BWI (body weight index, kg/m²), *p*: level of significance (between three genotypes and for pairwise comparison), n.s.: difference is not significant.

1 min; 30 cycles. Amplification mixture in a volume of 25 μl contained 75 mM tris-HCl pH 9.0, 20 mM $(NH_4)_2SO_4$, 0.01% Tween-20, 1.5 mM (P249P) or 2.5 mM (L250L) $MgCl_2$, 0.2 mM of each dNTP, 0.5 μM of each primer, 1.25 U Taq-polymerase, and 0.5 μg genomic DNA. PCR products 161 b.p. (P249P) and of 207 b.p. (L250L) were analyzed by 4% PAAG electrophoresis, stained with ethidium bromide, and visualized in transmitted UV light.

Indices of blood serum lipid profile were determined using enzymatic methods. Association of genotypes of the studied markers with blood lipid profile indices and anthropometric parameters were estimated using Fisher test.

RESULTS

Results of association analysis showed statistically significant differences between L250L SNP genotypes of the *TRPM8* gene in women (p=0.017) an in pooled sample (p=0.031; Table 1). Comparison of mean total CH values in individuals with different genotypes revealed its higher content in heterozygotes (GC) compared to homozygotes (GG): 5.68 mmol/liter *vs.* 4.98

TABLE 2. Blood Lipid Level and Anthropometric Parameters in Individuals with Different P249P SNP Genotypes of *TRPM8* gene in Shorians (M±m)

Index	Mean values of indices for different genotypes			*p*	
	AA	AT	TT		
Women					
N	79	16	3		
Total CH	5.21±0.10	5.31±0.30	5.23±0.70	n.s.	n.s.
LDL CH	3.25±0.40	3.55±0.30	3.2±0.7	n.s.	n.s.
TG	1.32±0.10	1.25±0.20	1.4±0.4	n.s.	n.s.
HDL CH	1.36±0.30	1.19±0.80	1.4±0.2	0.125	0.045 (AA/AT)
WC	68.4±0.7	65.69±1.60	74.0±3.8	n.s.	n.s.
HC	88.67±0.80	87.12±1.90	92.33±4.40	n.s.	n.s.
BWI	22.97±0.30	21.84±0.70	23.47±1.60	n.s.	n.s.
Men					
N	14	5	0		
Total CH	5.08±0.40	5.42±0.70	—	n.s.	n.s.
LDL CH	3.18±0.40	3.69±0.70	—	n.s.	n.s.
TG	1.17±0.20	1.06±0.30	—	n.s.	n.s.
HDL CH	1.37±0.10	1.24±0.10	—	n.s.	n.s.
WC	74.71±1.40	77.8±2.3	—	n.s.	n.s.
HC	92.14±1.70	93.2±2.9	—	n.s.	n.s.
BWI	23.38±0.60	24.04±1.00	—	n.s.	n.s.
Total					
N	93	21	3		
Total CH	5.19±1.30	5.33±1.30	5.23±0.80	n.s.	n.s.
LDL CH	3.24±1.30	3.58±1.40	3.2±0.7	n.s.	n.s.
TG	1.3±0.1	1.21±0.10	1.4±0.4	n.s.	n.s.
HDL CH	1.36±0.30	1.2±0.2	1.39±0.20	0.068	0.022 (AA/AT)
WC	69.35±0.70	68.57±1.50	74.0±4.0	n.s.	n.s.
HC	89.19±0.80	88.57±1.60	92.33±4.30	n.s.	n.s.
BWI	23.03±0.30	22.37±0.60	23.47±1.60	n.s.	n.s.

mmol/liter in women (p=0.005) and 5.62 mmol/liter *vs.* 5.00 mmol/liter (p=0.009). Mean total CH level was also higher in men with heterozygous GC phenotype (5.35 mmol/liter *vs.* 5.08 mmol/liter), but the difference between the genotypes was insignificant.

Total CH level indicates total concentration of blood lipids, including a number of fractions with different atherogenic properties. Elevation of total CH level was produced by its major and most atherogenic fraction, LDL CH (Table 1). Significant differences in LDL CH level between L250L SNP were found in women (p=0.036) an in pooled sample (p=0.049). In this case individuals with heterozygous genotype again had higher LDL CH levels compared to individuals with GG genotype: 3.71 mmol/liter *vs.* 3.07 mmol/liter (p=0.010; women) and 3.68 mmol/liter *vs.* 3.11 mmol/liter (p=0.020; pooled sample). No associations of L250L SNP with TG and HDL CH content were found. These values varied insufficiently among genotypes.

A tendency to association of *TRPM8* gene P249P polymorphism with HDL CH in the pooled sample (p=0.068; Table 2) was revealed. Pairwise comparison of genotypes showed significant differences in this index between AT heterozygotes and AA homozygotes in women (p=0.045) an in pooled sample (p=0.022). In individuals with AT genotype, HDL CH level was lower than in individuals with AA genotype: 1.19 mmol/liter *vs.* 1.36 mmol/liter (women) and 1.20 mmol/liter *vs.* 1.36 mmol/liter (pooled sample), respectively. Lower values were observed in men with heterozygous genotype (1.24 mmol/liter) compared to AA genotype (1.37 mmol/liter), but the difference was insignificant. No associations of P249P SNP with total CH, LDL CH, or TG were found.

Comparison of mean values of anthropometric parameters revealed significant difference between two L250L SNP genotypes in measurement round the hips in men (p=0.007) an in pooled sample (p=0.011), and also a tendency for association in women (p=0.078; Table 1). Pairwise comparison showed difference between individuals with following genotypes: GG/CC (p=0.030) in women, GG/GC (p=0.003) in men, and GG/CC (p=0.007) and GG/GC (p=0.037) in pooled sample. In these groups, mean hips circumference in GG homozygotes was higher than in heterozygotes and CC homozygotes. Comparison of the mean waist circumference revealed significant difference between the individuals with L250L SNP GG and GC genotypes in men (p=0.030) and a tendency to association in pooled sample (p=0.084). Difference between L250L SNP genotypes in body weight index was statistically insignificant. No associations of P249P SNP with anthropometric parameters were revealed (Table 2).

Thus, our analysis revealed association of L250L *TRPM8* SNP with total CH and LDL CH level and association of P249P SNP with blood serum HDL CH level. Mean level of total CH and LDL CH was higher in individuals with heterozygous genotype of L250L SNP as compared to the GG genotype. Individuals with heterozygous genotype in P249P SNP had lower HDL CH level than individuals with the AA homozygotes (more prevalent allele). We revealed an association of L250L SNP with anthropometric parameters (waist and hip circumference) characterizing lipid metabolism disturbances. *TRPM8* gene is probably involved in the regulation of lipid metabolism. L250L and P249P *TRPM8* gene polymorphisms can be used as genetic markers of blood lipid profile in Shorians.

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