Association between genotype rs1378942 of the CSK gene with hypertriglyceridemia among young northerners

Asociación del genotipo rs1378942 del gen CSK con hipertrigliceridemia entre jóvenes del norte

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Abstract

The objective of this work was to identify possible associative links between genotype rs1378942 of the *CSK* gene with hypertriglyceridemia among the young residents living in northern region for a long time. A prospective cohort study was conducted among 882 patients with metabolic syndrome living in the city (245 people), 354 people in the village, and 283 Khanties, the indigenous population of the north. The results of hypertriglyceridemia and genotype to locus rs1378942 of the *CSK* gene association analysis are presented. Among young people, the prevalence of hypertriglyceridemia was 88.7%, while among the indigenous population (92.7%) more often than among non-indigenous people (85.6%). The heterozygous genotype GT rs1378942 of the *CSK* gene prevailed in the cohort in 50.7% of cases, the mutant G allele was found in 19.8% of the study participants. The obtained results reflect the significant protective effect of the mutant G allele with respect to hypertriglyceridemia among young residents of the north, which indicates the influence of genetic and environmental factors on the development of cardiovascular diseases.

Keywords: rs1378942 of *CSK* gene, hypertriglyceridemia

Resumen

El objetivo de este trabajo fue identificar posibles vínculos asociativos entre el genotipo rs1378942 del gen *CSK* con hipertrigliceridemia entre los jóvenes residentes que viven en la región norte durante mucho tiempo. Se realizó un estudio de cohorte prospectivo entre 882 pacientes con síndrome metabólico que viven en la ciudad (245 personas), 354 personas en el pueblo y 283 Khanties, la población indígena del norte. Se presentan los resultados del análisis de la asociación entre hipertrigliceridemia y el genotipo del locus rs1378942 del gen *CSK*. Entre los jóvenes, la prevalencia de hipertrigliceridemia fue del 88,7%, mientras que entre la población indígena (92,7%) fue más frecuente que entre los no indígenas (85,6%). El genotipo heterocigoto GT rs1378942 del gen CSK prevaleció en la cohorte en el 50,7% de los casos, el alelo G mutante se encontró en el 19,8% de los participantes del estudio. Los resultados obtenidos reflejan el efecto protector significativo del alelo G mutante con respecto a la hipertrigliceridemia entre los residentes jóvenes del norte, lo que indica la influencia de los factores genéticos y ambientales en el desarrollo de las enfermedades cardiovasculares.

Palabras clave: rs1378942 del gen *CSK*, hipertrigliceridemia.

Introduction

Hypertriglyceridemia, both independently and in combination with obesity, metabolic syndrome (MS), diabetes mellitus (DM) is a risk factor for the development of cardiovascular diseases (CVD)[1;2]. Triglycerides (TG) enter the blood plasma from the liver as part of very-low-density lipoproteins (VLDL) and with food in combination with the chylomicrons. As a result of a decrease in lipoprotein lipase activity, there is a TG increase in blood serum. Hereditary hypertriglyceridemia has a connection with the increased levels of VLDL.

Src kinases are a family of nonreceptor tyrosine kinases that are widely expressed in human tissues. To date, at least 14 members have been identified, of which 60-kDa c-Src is the prototype. Of the many Src kinases, c-Src is highly expressed in vascular smooth muscle cells. It is also rapidly activated by angiotensin II and has a key role in signalling events associated with vascular smooth muscle cell contraction, growth and migration. Src non-receptor tyrosine kinase also mediates oxidative stress, proliferation of cells, and ex-

pression of inflammatory genes as a result of growth factors, biologically active lipids such as lysophosphatidic acid, and receptor ligands for glycation end products in the muscular wall of vessels[3]. There is evidence that the Src kinase can regulate pro-inflammatory reactions as a result of oxidized LDL and its components[4-6]. *CSK* gene encodes tyrosine kinases. The gene-associated single-nucleotide polymorphism (SNP) rs1378942 is located in the first intron of CSK (c-src tyrosine kinase) at the long arm of the 15th chromosome at the locus 15q23–25[7,8].

Thus, in the present study we evaluated the possible associative links between genotype rs1378942 of the *CSK* gene with hypertriglyceridemia among young residents who live for a long time in Northern region.

The Materials and Methods

The formation of patient groups occurred based on the next medical institutions: "Fedorovskaya city hospital", a branch of the hospital in d. Russkinskaya, "Surgut city clinical polyclinic No. 1". All patients signed informed consent to participate in the study. For the period 2015-2018, we selected all in all 882 people age 18-44 (average age 36.62 ± 5.12 years). The non-indigenous residents (n=599) included the urban population of 245 (146 women and 99 men) and the rural population of 354 (108 men and 246 women). The Small Indigenous Peoples of the North (SIPN) were 283 of the Khanty, as well as 72 men and 211 women[9]. We conducted: survey, anthropometric survey: measurement of height, body weight, body mass index (BMI=kg/m²), waist circumference (WC). The lipid metabolism was assessed in serum samples which were obtained from the ulnar vein in women and men with 12 hours of fasting. The levels of lipoprotein of high and low density (HDL cholesterol and LDL cholesterol), total cholesterol (TC), and triglycerides (TG) were determined. Hypertriglyceridemia was considered when the concentration of TG was higher than 1.7 mmol/l[9].

All the data were preprocessed and analysized by the R 3.5.3 environment for statistical computing (R Foundation for Statistical Computing) and additional packages of the BioConductor repository (HardyWeinberg 1.6.3, SNPassoc 1.9-2).

We present the descriptive statistics for categorical variables in percents, as for quantitative variables - in the form of a median (1st and 3rd quartiles of the sample distribution).

To detect deviations of the observed genotype frequencies from the theoretical frequencies by the Hardy-Weinberg equilibrium, and the χ2 test for the cohort as a whole and the groups of participants (urban, rural, and the Khanty), it was considered the deviations as statistically significant at p<0.05. To analyze the joint frequency distribution of genetic markers pairs, we used a good-of-fit strategy (тест2 test) to test the hypothesis of an equilibrium coupling between the genetic markers. We rejected the null hypothesis at p<0.05. As a measure of the genetic linkage effect, it was applied to D-statistics as p(AB) - p(A)×p (B).

To indicate the association of genotypes with the quantitative and categorical variables, we used generalized linear models for dominant, codominant, and recessive inheritance types (relative to the rarest allele) with consideration of the available information from the SNPedia regarding the single-nucleotide polymorphisms (SPN) under study. It was used the information about the gender and age of the participants under study within the models as covariates. We considered the association of the genotype with the dependent variable as statistically significant at p<0.05. To evaluate the effect size, we referred to odds ratios (with corresponding 95% confidence intervals) for categorical dependent variables and the absolute difference between average values (with corresponding 95% CI) for quantitative variables. We applied to marginalized estimates as the assessment of the average value (in the case of quantitative variables) and the probability of an outcome (in the case of binary categorical variables) with consideration of the covariate distributions within the regression model.

Results

Table 1. Distribution of rs1378942 genotypes and alleles of the CSK gene among the patients under examination

	Cohort	City	Village	the Khanty	p-value
GG	174 (19.8%)	63 (22.8%)	58 (17.2%)	53 (19.9%)	
GT	446 (50.7%)	134 (48.6%)	186 (55.2%)	126 (47.4%)	0.1985
TT	259 (29.5%)	79 (28.6%)	93 (27.6%)	87 (32.7%)	
G	794 (45.2%)	260 (47.1%)	302 (44.8%)	232 (43.6%)	0.4989
T	963 (54.8%)	292 (52.9%)	372 (55.2%)	300 (56.4%)	

As shown in Table 1, the mutant GG allele represent a 19.8% of the participants under study, slightly more often among urban residents (22.8%), less often among rural residents (17.2%), and among the Khanty (19.9%). Table 2 and figure 1 present the forecast assessment of the hypertriglyceridemia development in the population as a whole and the study groups in accordance with the genotype at the rs1378942 locus of the *CSK gene.* Figure 2 shows The Level of Service assessment of triglyceride concentrations regardless of gender and age from the genotype at the rs1378942 locus of the *CSK* gene.

Table 3 shows the analysis results of the association of hypertriglyceridemia and genotype at the rs1378942 locus of the *CSK*gene. The findings reflect a significant protective effect of the mutant G allele in relation to hypertriglyceridemia among the indigenous people – the Khanty) - a 2.4-fold reduction in the chance of heterozygotes and 4.4 of homozygotes. There was no significant association of this polymorphism with the risk of hypertriglyceridemia among the urban and rural residents. We obtained the corresponding results (table 4) for TG concentration values regardless of gender and age: decrease in TG concentration by 0.251 mmol/l on average with heterozygotes and 0.359 with homozygotes. The analysis results of the polymorphism association for this locus and the above-mentioned variables are also in Figures 3 and 4.

Figure 1. The forecast assessment of hypertriglyceridemia based on the genotype at the rs1378942 locus of the CSK gene.

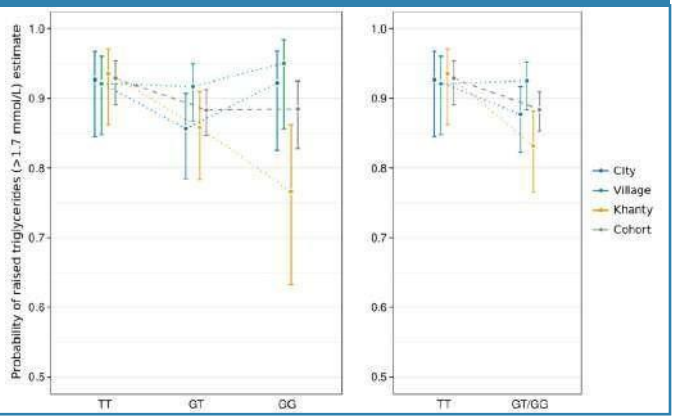


Figure 2. The LS assessment of triglyceride concentration based on the genotype by the locus rs1378942 of the CSK gene.

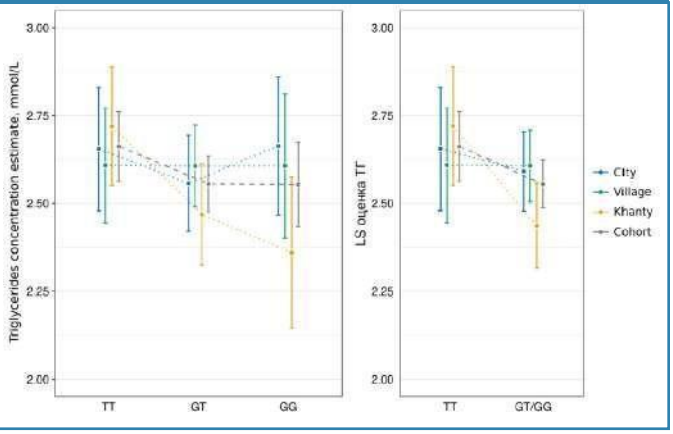


Table 2. The forecast assessment of hypertriglyceridemia (TG concentration >1.7 mmol/l) based on the genotype at the rs1378942 locus of the CSK gene within the target population, urban, rural, and indigenous residents.

Group	Genotype	Risk assessment	95% CI
Codominant model			
City	TT	0.927	0.845-0.967
	GT	0.856	0.784-0.907
	GG	0.922	0.825-0.967
Village	TT	0.921	0.848-0.961
	GT	0.917	0.867-0.950
	GG	0.950	0.856-0.984
the Khanty	TT	0.935	0.862-0.971
	GT	0.858	0.783-0.910
	GG	0.766	0.632-0.862
Cohort	TT	0.929	0.890-0.954
	GT	0.884	0.847-0.912
	GG	0.885	0.827-0.925
Dominant model			
City	TT	0.927	0.845-0.967
	GT/GG	0.877	0.822-0.917
Village	TT	0.921	0.848-0.961
	GT/GG	0.925	0.884-0.953
the Khanty	TT	0.935	0.862-0.971
	GT/GG	0.832	0.765-0.882
Cohort	TT	0.929	0.890-0.954
	GT/GG	0.884	0.853-0.909

Table 3. The forecast ratio assessment of hypertriglyceridemia chances development between genotypes at the rs1378942 locus of the CSK gene in the target population, urban, rural, and Khanty populations.

	Contrast	Chances assessment	95% CI	P-value
Codominant model (AIC = 607.23)				
City	TT - GT	2.122	0.810-5.554	0.1256
	TT - GG	1.069	0.309-3.690	0.9165
	GT/GG	0.504	0.179-1.417	0.1938
Village	TT - GT	1.056	0.433-2.574	0.9049
	TT - GG	0.611	0.155-2.413	0.4824
	GT/GG	0.579	0.162-2.067	0.4000
the Khanty	TT - GT	2.403	0.918-6.287	0.0740
	TT - GG	4.421	1.558-12.545	0.0052
	GT/GG	1.840	0.831-4.072	0.1324
Cohort	TT - GT	1.716	0.902-3.265	0.0492
	TT - GG	1.692	0.780-3.673	0.1116
	GT/GG	0.986	0.518-1.877	0.9596
Dominant model (AIC = 606.09)				
City	TT - GT/GG	1.769	0.694-4.506	0.2318
Village	TT - GT/GG	0.945	0.397-2.246	0.8973
the Khanty	TT - GT/GG	2.935	1.176-7.326	0.0210
Cohort	TT - GT/GG	1.709	1.019-2.867	0.0422

Table 4. The assessment of the difference between the average values of triglyceride concentrations between genotypes at the rs1378942 locus of the CSK gene among the target population, urban residents, rural residents, and the Khanty regardless of gender and age.

	Contrast	Absolute difference	95% CI	P-value
Codominant model				
City	TT - GT	0.098	-0.123-0.319	0.3850
	TT - GG	-0.008	-0.271-0.254	0.9496
	GT/GG	-0.106	-0.344-0.132	0.3809
Village	TT - GT	0.002	-0.196-0.200	0.9849
	TT - GG	0.001	-0.259-0.262	0.9913
	GT/GG	-0.000	-0.234-0.233	0.9969
the Khanty	TT - GT	0.251	0.034-0.468	0.0237
	TT - GG	0.359	0.089-0.630	0.0094
	GT/GG	0.109	-0.146-0.364	0.4019
Cohort	TT - GT	0.100	-0.000-0.300	0.0864
	TT - GG	0.100	-0.100-0.300	0.1644
	GT/GG	0.000	-0.200-0.200	0.9810
Dominant model				
City	TT - GT/GG	0.064	-0.143-0.271	0.5464
Village	TT - GT/GG	0.002	-0.188-0.191	0.9870
the Khanty	TT - GT/GG	0.283	0.079-0.486	0.0065
Cohort	TT - GT/GG	0.100	0.000-0.200	0.0688

Figure 3. The risk assessments of hypertriglyceridemia in the context of codominant (A) and dominant (B) inheritance models. The black dots refer to the forecast assessment of a metabolic syndrome development, the blue line is 95% CI, and the red line is the comparison of genotypes within the corresponding models and subgroups.

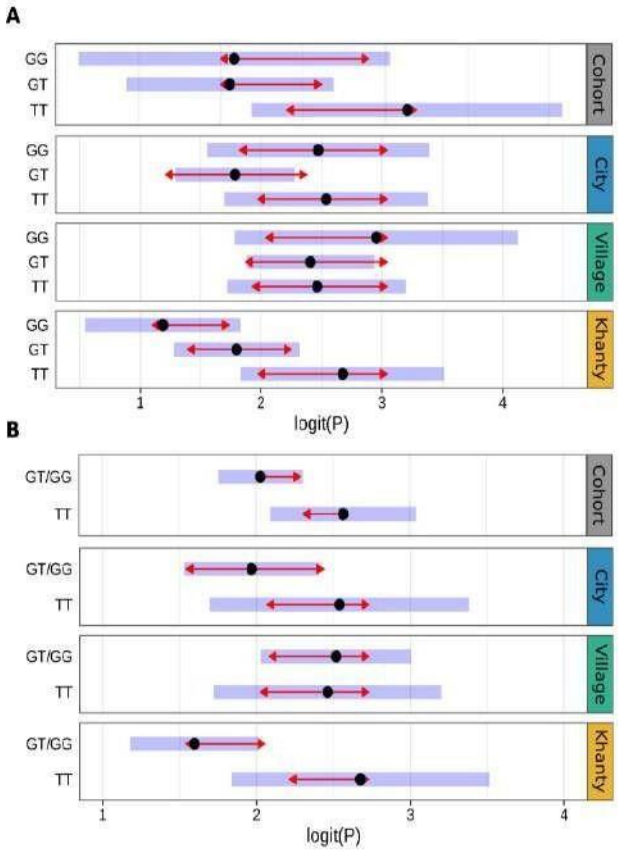
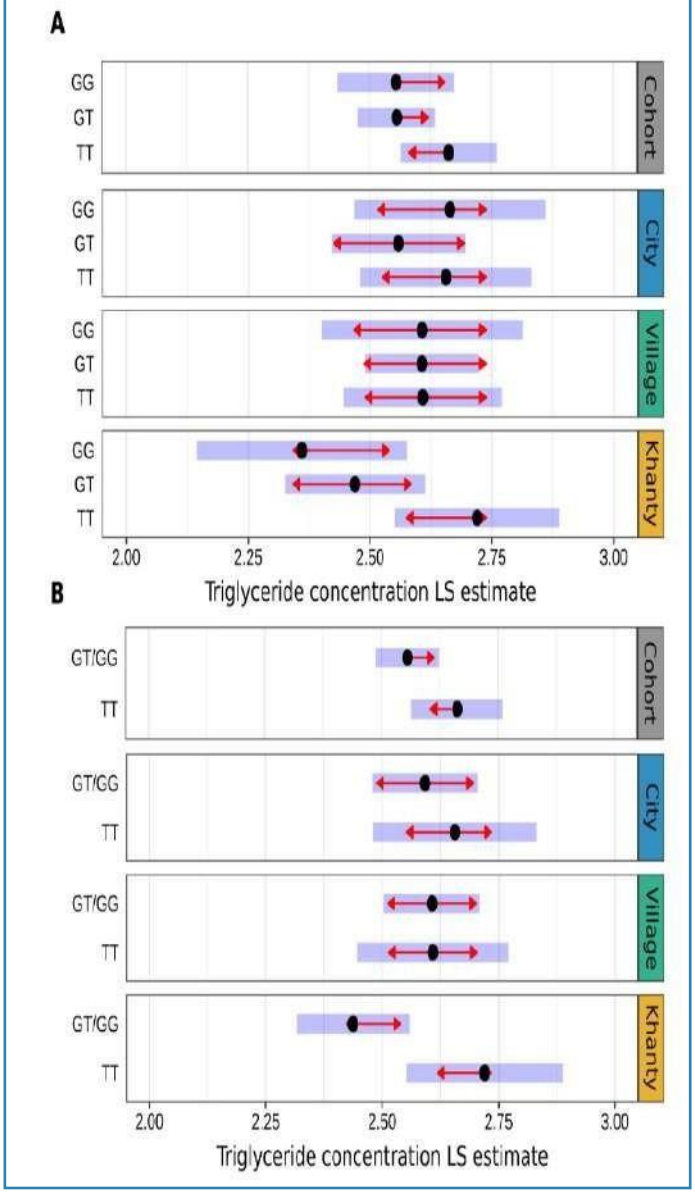


Figure 4. The LS assessments of the average triglyceride concentrations in the context of codominant (A) and dominant (B) inheritance models. The black dots refer to the forecast assessment of a metabolic syndrome development, the blue line is 95% CI, and the red line is the comparison of genotypes within the corresponding models and subgroups.



Discussion

Among people of young age, the prevalence rate of hypertriglyceridemia is 88.7%, while among the indigenous population (92.7%) it is more often than among the non-indigenous population (85.6%). We determined the association of hypertriglyceridemia with the carrier of the rs1378942 genotype under the *CSK* gene, these results are consistent with numerous studies[1,2,5,7,9]. Whereby, one determined the predominance of the heterozygous genotype GT rs1378942 of the *CSK* gene in the cohort of 50.7% of cases, with the predominance within the group of rural residents – 55.2%. We found the mutant GG allele among 19.8% of the survey participants under study, slightly more often among urban residents (22.8%), less often among rural residents (17.2%), and among the Khanty (19.9%). The association of the heterozygous genotype GT rs1378942 of the *CSK* gene with hypertriglyceridemia among young residents of the North indicates the influence of genetic and environmental factors on the development of cardiovascular diseases, Numerous studies confirm these results[2,3,6,8].

Conclusion

The molecular genetics study was conducted at the Scientific Research Institute of Therapy and Preventive Medicine - branch of FSBSI "Federal Research Center Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences". The genomic DNA was obtained from venous blood by phenol-chloroform extraction. The rs1378942 poly-

morphism of the CSK gene was tested by PCR with RFLP. The obtained results reflect the significant protective effect of the mutant G allele with respect to hypertriglyceridemia among young residents of the north, which indicates the influence of genetic and environmental factors on the development of cardiovascular diseases.

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