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S.I. Sofronova, A.N. Romanova, M.P. Kirillina

ASSOCIATION OF IL-6 GENE POLYMORPHISM WITH ARTERIAL HYPERTENSION AND ITS RISK FACTORS IN INDIGENOUS PEOPLES OF YAKUTIA

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A case-control study was conducted in Kolymskoye and Andryushkino of Nizhnekolymsky district of Yakutia to determine the association of IL-6 gene polymorphism with arterial hypertension (AH) and risk factors. The relationship with AH and its risk factors for IL-6 gene polymorphism was obtained, namely, the influence of the G allele of the polymorphic locus C-174G and the C allele of the polymorphic locus C-572G on metabolic factors of atherogenesis. Hypertensive patients- carriers of the G allele of the C-174G polymorphic locus of the IL-6 gene, both in the homozygous and heterozygous versions, showed the highest average systolic blood pressure, a high incidence of abdominal obesity, lipid and carbohydrate metabolism disorders. The study of the polymorphic locus C-572G of the IL-6 gene in patients with hypertension showed the highest incidence of abdominal obesity, lipid and carbohydrate metabolism disorders in carriers of the C allele.

Keywords: gene IL-6, locus C-174G, C-572G, arterial hypertension, risk factors, indigenous people, Yakutia.

The mortality rate from diseases of the circulatory system in Russia in 2018 was 46.3%, of which 52.6% were from IHD [1]. One of the main risk factors for the development of cardiovascular complications is arterial hypertension (AH). AH in 2015 caused more than 10 million deaths and 200 million cases of disability [6]. The leading role in the development of AH is played by not only modifiable, but also non-modifiable and genetic features of the body [2]. The immune system also plays an important role. Thus, the *IL-6* gene encodes an anti-inflammatory cytokine, and therefore an increase in its concentration in the blood leads to an increased susceptibility to the development of atherosclerosis. The role of the immune mediator *IL-6* in the development of hypertension has been proven [8,11,13]. The literature describes that CG genotypes of G-174C polymorphism in the *IL-6* gene promoter increase gene transcription and are significantly associated with an increased content of TC, LDL and SBP [10,12], which determines the relationship of *IL-6* gene polymorphism with metabolic factors of atherogenesis. The results of studies with regard to the association of *IL-6 C-572G* gene polymorphism and the risk of developing IHD, namely the effect of the C allele in the Chinese, are also described [3]. Other studies confirm the association of -572GG and the G allele with the risk of developing ischemic stroke [15].

The study to determine the relationship of variants of the *IL-6 C-174G* and *IL-6 C-572G* genes with hypertension and its risk factors remains relevant, since it has not been previously conducted in indigenous peoples of the North.

Objective: to analyze the association of *IL-6* gene polymorphism with arterial hypertension and its risk factors in indigenous peoples of the Nizhnekolymsky district of Yakutia.

Materials and research methods. The collection of material for the study was carried out under expeditionary conditions in the Arctic territory of Yakutia in places of compact residence of indigenous peoples in the Kolymskoe and Andryushkino villages, Nizhnekolymsky district. A total of 212 people aged 20 to 70 years were examined. Random sampling was formed according to the lists of workers in the village administration. The response was 76%. It should be noted that Kolymskoye village was dominated mainly by the Chukchi, and the Andryushkino village by the Yukagirs. Evens were found equally often in both settlements. The design of the study is presented in the form of "case-control", for which 150 representatives of indigenous peoples of the North were included in the study, which were divided into 2 groups: with AH (case) (n = 73) and without AH (control) (n = 77). The average age of respondents with AH was 53,52 ± 1,12, without AH 39,62 ± 1,26 years.

Inclusion criteria: representatives of indigenous people (Evens, Chukchi, Yukaghirs).

Exclusion criteria: representatives of non-indigenous nationalities, Yakuts.

The research program included the following sections: a survey on the questionnaire to assess the objective state; informed consent of the respondent to conduct research; anthropometric examination with hip and waist measurement; blood sampling for biochemical studies from the cubital vein in the morning on an empty stomach with a 12-hour abstinence from food. Blood sampling from the cubital vein for molecular genetic studies was carried out in a tube with EDTA. Genomic DNA was isolated from peripheral blood leukocytes by the method of phenol-chloroform extraction. Polymorphism of the promoter region of the IL-6 gene was studied at positions C-174G and C-572G. Allelic variants of the IL-6 gene were tested using a polymerase chain reaction with real-time results (real-time PCR). Genotyping of the polymorphic AGT gene was performed with the usage of kits (Lytech R&D LLC, Moscow), on the «Real-time CFX96» amplifier (BioRad, USA) in accordance with the manufacturer's instructions. For quality control, 10% of randomly selected samples were subjected to repeated genotyping.

Laboratory methods of the research included blood lipids test total cholesterol (TC), triglycerides (TG), HDL Cholesterol, LDL Cholesterol, glucose test.

When judging the incidence of disorders of the blood lipid profile in a population, we used the Russian recommendations of the V revision of Society of cardiology of Russian Federation (VNOK), 2012, into account the European recommendations, 2011. Hypercholesterolemia (HCS) is the level of total cholesterol (TC) ≥ 5,0 mmol/l, the high LDL Cholesterol level >3,0 mmol/l, the low HDL Cholesterol level <1,0 mmol/l on men; <1,2 mmol/l on women, the hypertriglyceridemia (HTG) is the TG level is ≥1,7 mmol/l; a hyperglycemia (HG) on an empty stom-

677000, The Republic of Sakha (Yakutia),Yakutsk, Sergelyakhskoe shosse, 4, Yakut science centre of complex medical problems: **SOFRONOVA Sargylana Ivanovna** – PHD, researcher, ORCID: 0000-0003-0010-9850, sara2208@mail.ru, 89841094825; **ROMANOVA Anna Nikolaevna** – MD, director, ORCID: 0000-0002-4817-5315, ranik@mail.ru, 8(4112)32-19-81; **KIRILLINA Marya Petrovna** – PHD, researcher, kirillinamp@mail.ru.

ach (a glucose in a blood plasma on an empty stomach ≥ 6,1 mmol/l) or glucose intolerance (a glucose in a blood plasma in 2 hours after glucose loading within ≥7,8 and ≤11,1 mmol/l).

Blood pressure (BP) was measured twice with an OMRON M2 Basic automatic tonometer (Japan) in a sitting position with calculation of average blood pressure with a margin of permissible measurement error of ± 3 mm Hg. (ESH 2002) according to the instructions for the correct measurement of blood pressure, outlined in the European clinical guidelines for the diagnosis and treatment of hypertension 2017. Hypertension is present at the 140/90 mmHg (2017 ACC/AHA Guideline).

The abdominal obesity (AO) is exposed to the value of the waist measurement (WM) ≥ 80 cm on women, ≥94 cm on (VNOK, 2009) .

The study was conducted according to Ethics Committee protocol YSC KMP on the respondent's informed consent to the processing of personal data and the study.

Statistical data processing was performed using standard methods of mathematical statistics using the SPSS software package (version 19.0). To define the characteristics, the arithmetic mean (M) and the characteristic's standard error of the mean (m) were calculated. Intergroup differences were evaluated using analysis of variance or non-parametric criteria. When comparing the frequencies of genotypes, the standard $\chi 2$ criterion with the Yates correction was used. The relative risk (OR – odds ratio) of disease development at a certain genotype was calculated using the standard formula OR=a/bxd/c, where a and b is the number of patients with and without the mutant genotype, respectively, and d, c is the number of people in the control group with and without the mutant genotype. OR is indicated with a 95% confidence interval. Differences were considered statistically significant at $p < 0.05$.

Results and discussion. This study is exploratory in nature due to the analysis of a small sample of the surveyed population. An attempt was made to analyze the association of *IL-6* gene polymorphism with AH and its risk factors. The effect of heterogeneity in comparisons is possible, which could affect the results.

The frequency of occurrence of genotypes and alleles of *IL-6 C-174G* and *IL-6 C-572G* among the examined groups was compared. All genotype frequencies do not correspond to the Hardy-Weinberg equilibrium, possibly due to a small sample of the studied groups, for *IL-6 C-174G* ($\chi 2=15.12$, $p=0.000$ for the case and $\chi 2=3.36$, $p=0.066$ for control), for *IL-6 C -572G* ($\chi 2=9.18$, $p=0.002$ and $\chi 2=7.76$, $p=0.005$, respectively). In all groups, a high frequency of homozygous GG genotype and G allele was noted; there were statistically significant differences in both the group of patients with AH and the frequency of GG genotype of *IL-6 C-572G* in the control.

We used 2 genetic models (dominant and recessive) to test the relationship between *IL-6 C-174G* and *IL-6 C-572G* with AH. We did not obtain significant differences in the frequencies of genotypes between hypertonics and normotonics, in all cases the dominant model for the minor G allele and the high frequency of the G allele prevailed (Table 1).

Depending on the genotype of the polymorphic locus C-174G of the IL-6 gene, we analyzed the average level of systolic blood pressure (SBP) in hypertensive patients. Some effect of the G allele of *IL-6 C-174G* on the increase in the level of SBP was found. In individuals with hypertension, the average level of SBP in carriers of CC, CG, and GG genotypes was 146.67±8.81, 166.67±10.22, and 160.47±2.28 mmHg, respectively, no significant differences were found ($p> 0.05$). Our data do not coincide with the studies of certain foreign scientists; in particular, the tendency for a more pronounced increase in SBP in carriers of the C allele has been described [10,12]. In normotonics, there were no special differences in the mean values of SBP. As for the polymorphic locus C-572G of the *IL-6* gene, in hypertensive patients with CC, CG and GG genotypes, the mean SBP was 155.71±3.68, 160.67±4.41 and 160.98±2.84 mmHg, respectively, no significant differences were noted. In a number of studies, the relationship of the polymorphic locus C-572G of the *IL-6* gene with the level of SBP was also not obtained [14].

AO plays an important role in the development of hypertension as a component of the metabolic syndrome. In individuals with AH, AO was significantly more often observed than in individuals without AH (78.1% and 29.9%, respectively, $p = 0.001$). We analyzed the distribution frequencies of the genotypes of the polymorphic loci C-174G and C-572G of the *IL-6* gene, depending on the presence of AO in the general population and separately in the "case" and "control". The frequency of AO in the general population of carriers of the CC genotype of the polymorphic locus C-174G was 50%, CG - 58.3%, GG - 53%, no significant differences were noted. In the "case", depending on the relationship to these genotypes, it was 66.7%, 83.3% and 78.1%, respectively ($p> 0.05$), in the "control" - 22.3%, 33.3% and 30.0% ($p> 0.05$). We obtained a contradictory result in comparison with published data, which describes the relationship of the C allele at the -174 locus of the *IL-6* gene with visceral obesity in patients with hypertension [4], possibly associated with a small sample of subjects.

In the general population of carriers of the genotype CC of the C-572G locus, the AO frequency was 50%, CG - 52.8%, GG - 54.1%, the differences were insignificant. Hypertensive patients had a high rate of AO, 95.6%, 86.7% and 72.5%, respectively ($p> 0.05$). Thus, the highest frequency of AO was identified in individuals with AH - carriers of the C allele. In normotonic patients, the occurrence of AO was 22.4%, 28.6% and 34.0%, respectively, the differences are unreliable.

The assessment of the effect of genetic polymorphism on the biochemical blood parameters characteristic of hypertension is of particular interest. We analyzed the association of polymorphism of polymorphic C-174G and C-572G loci of *IL-6* gene with biochemical blood parameters in individuals with and without AH. Compared the average concentration of lipids and blood glucose in individuals with hypertension and without hypertension, depending on their belonging to a particular genotype (Table 2). In all respondents in the "case", the values of lipid metabolism, except for high density lipoprotein cholesterol (HDL cholesterol), were higher compared to the "control". Statistically significant differences were obtained in the average concentrations of low-density lipoprotein cholesterol (LDL cholesterol), TG and blood glucose in homozygous carriers of minor GG genotype *IL-6 C-174G*. In other cases, the indicators had no significant differences. When studying the relationship of *IL-6 C-572G* gene polymorphism with the same blood parameters, we obtained significant differences in hypertensive patients in the average LDL cholesterol values in carriers of a heterozygous genotype and blood glucose in individuals with a mutant homozygous genotype. For other indicators, there were no significant differences. Thus, we obtained the influence of the G allele on a significant increase in the concentration of atherogenic fractions of cholesterol and blood glucose in hypertensive patients.

We determined the frequency of lipid and carbohydrate disorders for indi-

Table 1

Frequency distribution of the *IL-6* C-174G and *IL-6* C-572G genotypes with and without hypertension according to the dominant and recessive models

Genotype		Genotype frequencies		χ2	p	OR	95% CI	Allele	Allele frequencies	
		case	control						case	control
IL-6 C-174G										
Dominant model	CG+GG	0.959	0.987	1.14	>0.05	0.31	0.03-3.02	C	0.082	0.052
	CC	0.041	0.013							
Recessive model	CC+CG	0.123	0.091	0.41	>0.05	1.41	0.49-3.99	G	0.918	0.948
		0.877	0.909							
IL-6 C-572G										
Dominant model	CG+GG	0.904	0.883	0.17	>0.05	1.25	0.44-3.55	C	0.199	0.253
	CC	0.096	0.117							
Recessive model	CC+CG	0.301	0.390	1.29	>0.05	0.68	0.34-1.33	G	0.801	0.747
		0.699	0.610							

viduals with and without hypertension, depending on the carriage of a particular *IL-6 C-174G* genotype. In hypertonic patients, all values exceeded those of normotonic, except for carbohydrate metabolism. We compared the frequencies in relation to a particular genotype separately in individuals in the "case" and "control". In hypertensive patients, there were no differences in the ratio of a specific genotype to lipid and carbohydrate disorders (p> 0.05). In carriers of the CC genotype, the frequency of HCS was 33.3%, CG - 46.9% and GG - 55.6% (p> 0.05); HCS-LDL 33.3%, 50.0% and 59.4%, respectively, p> 0.05. Thus, carriers of the mutant GG genotype showed the highest frequency of lipid metabolism disorders, including the atherogenic fraction. In the literature, there are also confirmations from certain authors about the influence of the mutant homozygous genotype *IL-6 C-174G* on atherogenesis [10]. We have not obtained any special influence of one or another allele on the frequency of lipid and carbohydrate disorders in normotonics.

As for the comparison of the frequencies of occurrence of HCS, HCS-LDL, HTG, Hypo-α-cholesterolemia (Hypo-α-CS), HG by IL-6 C-572G genotypes, significant differences were not obtained in either the "case" or "control". In hypertensive patients, all indicators were also higher compared to the "control", but we found the highest occurrence of HCL in carriers of the C allele (CC - 57.1%, CG - 49.0% and GG - 40.0%, (p>0.05); HCS-LDL - 71.4%, 66.7% and 52.9% (p>0.05); Hypo-α-CS - 42.9%, 40.0% and 21.6% (p>0.05); HG - 14.3%, 8.0% and 6.7%, respectively, p> 0.05. For other parameters in the "case" no difference was noted. In normotonics, the occurrence of all indicators is almost the same.

Conclusion. In the course of our exploratory study, certain results were obtained, the distribution of allelic variants of cytokine genes in patients with hypertension and relatively healthy individuals was revealed. The dominance of a homozygous mutant genotype was found in the polymorphic loci C-174G and C-572G of the *IL-6* gene in two groups.

Hypertonics - carriers of the G allele of the C-174G polymorphic locus of the *IL-6* gene, both in the homozygous and heterozygous versions, showed the highest average systolic blood pressure, a high incidence of abdominal obesity, lipid and carbohydrate metabolism disorders. The study of the polymorphic C-572G locus of the *IL-6* gene in individuals with hypertension showed the highest incidence of AO, lipid and carbohydrate metabolism disorders in carriers of the C allele.

Thus, a relationship was obtained with AH and its risk factors for *IL-6* gene polymorphisms, namely, the influence of the G allele of the polymorphic locus C-174G and the C allele of the polymorphic locus C-572G on metabolic factors of atherogenesis.

All this serves as the basis for further more in-depth research of the indigenous population living compactly in the remote hard-to-reach communities in Russia's Arctic region, with a larger scope of research for targeted selection of therapeutic and preventive measures.

Table 2

The average levels of the lipid and carbohydrate spectrum in people with hypertension and those without hypertension. depending on the genotypes C-174G and C-572G of the IL-6 gene (M ± m)

Genotype frequency		TC	LDL Cholesterol	HDL Cholesterol	TG	Glucose
IL-6 C-174G						
CC	Case	5.49±1.12	2.82±0.52	1.48±0.22	1.33±0.30	5.36±0.18
	p	0.817	0.628	0.393	0.441	0.432
	Control	4.88±0.31	2.23±0.09	1.98±0.21	0.75±0.19	5.60±0.35
CG	Case	5.59±0.35	3.64±0.21	1.70±0.15	0.93±0.11	4.50±0.15
	p	0.395	0.234	0.507	0.583	0.393
	Control	5.09±0.42	3.11±0.35	1.50±0.24	0.84±0.10	4.36±0.30
GG	Case	4.93±0.13	3.16±0.11	1.29±0.04	1.10±0.05	4.96±0.26
	p	0.106	0.039	0.182	0.025	0.007
	Control	4.66±0.10	2.87±0.08	1.37±0.04	0.91±0.05	4.21±0.09
IL-6 C-572G						
CC	Case	5.02±0.15	3.16±0.33	1.16±0.11	1.07±0.10	5.02±0.60
	p	0.729	0.513	0.074	0.080	0.143
	Control	4.66±0.25	2.91±0.20	1.41±0.07	0.78±0.10	4.11±0.22
CG	Case	4.87±0.29	3.14±0.21	1.26±0.12	1.08±0.11	5.39±0.82
	p	0.326	0.049	0.215	0.236	0.153
	Control	4.56±0.16	2.70±0.12	1.45±0.08	0.88±0.10	4.33±0.16
GG	Case	4.82±0.38	3.14±0.12	1.38±0.04	1.09±0.06	4.73±0.21
	p	0.376	0.512	0.761	0.153	0.047
	Control	4.83±0.13	3.03±0.11	1.35±0.05	0.95±0.06	4.23±0.11

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