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Genetic Aspects of Metabolic Syndrome of Yakut Ethnic Groups

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ABSTRACT

In this article, we have studied polymorphisms in the genes encoding components of carbohydrate metabolism and fibrinolysis system in Yakuts. Genotypes SNPs rs9939609 gene FTO, rs1137101 gene *LEPR*, rs1799889 and rs6046 gene *SERPINE1 F7* gene were identified in the study group patients with metabolic syndrome (n = 100) and healthy (n = 100). We have revealed that the MS group «+» prevailed polymorphic genotype 4G/4G gene *SERPINE1*, associated with obesity (p=0,009). The risk of developing MS in the Yakut population became involved with genotype 4G/4G gene *SERPINE1* (OR = 3,568; CI 95%: 1,534-8,299). In case of polymorphic variants of other genes we studied the risk of MS has not been identified (OR < 1). There was a statistically significant association of gene polymorphism Gln223Arg *LEPR* level of total cholesterol (p = 0,038), triglycerides (p = 0,033) and SC (p = 0,030) in Yakut ethnic group. Association analysis of polymorphism -675 5G/4G gene *SERPINE1* with MS components in the sample revealed significant differences in anthropometric parameters: BMI (p = 0,016), WC (p = 0,001), the ratio of WC to HC (p=0,019).

Keywords: metabolic syndrome, polymorphism, *FTO, LEPR, SERPINE1, F7.*

INTRODUCTION

Currently, WHO experts consider metabolic syndrome (MS) as "pandemic of the XXI century". According to the recommendations of experts All-Russian Scientific Society of Cardiologist metabolic syndrome is characterized by an increase in visceral fat mass, decreased sensitivity of peripheral tissues to insulin and hyperinsulinemia, which cause the development of carbohydrate, lipid, purine metabolism and arterial hypertension (AH). MS is also a prothrombotic state because of endothelial dysfunction, the presence of hypercoagulable imbalance between coagulation factors and substances that regulate fibrinolysis. Violations by the blood coagulation system in the metabolic syndrome characterized by increased levels of fibrinogen and fibrinolysis inhibitor content level - factor VII (F7) , and plasminogen activator inhibitor type 1 (PAI 1) [17].

The study of molecular and genetic factors in the development of metabolic syndrome, the search for susceptibility genes and analysis of their association with various components polymorphisms syndrome are given more attention in research of recent years [9]. In meta-

analyzes of the European population has ties gene associated with fat mass (FTO) with overweight and obesity [4,11]. There are works of the controversial nature related to the Asian population. In Asian populations M. Horikoshi (2007) and H. Li (2008) showed no association of FTO with obesity [26,25], whereas J. Chen (2009), W. Tong (2010) concluded that there is a connection FTO with the metabolic syndrome [13,22]. There are also conflicting results in case of leptin receptor gene polymorphism (LEPR) and obesity. T. Furusawa (2010) et al. revealed that carriers of allele 223 Q (223Arg) had a significantly higher body weight (p = 0.0009) and BMI (p=0,0022) [24]. However, other studies failed to find a positive and significant relationship between obesity and the gene polymorphisms studied *LEPR* [6,16].

-675 5G/4G polymorphism of the gene encoding PAI 1 - *SERPINE1* (rs1799889), is regarded as a risk factor for cardiovascular disease [15]. According to some studies, the 4G-allele carriers are more prone to the development of obesity and MS, but in other works of a similar dependence was identified [5].

Several studies have been shown to increase the concentration of factor VII in patients with type 2 diabetes and its relationship with insulin resistance indices and triglyceride levels [10,14]. The level of factor VII in the blood is determined to a large extent genetic component [21]. Thus, gene polymorphism F7 - rs6046 (Arg353Gln) in exon 9 of the gene is associated with low blood levels of F7. Changes in gene F7 in most cases have a protective effect. Genotype A/A causes F7 decrease enzyme activity by 72%, compared to wild type (genotype G/G) [8].

Studies aimed at identifying genetic polymorphisms associated with metabolic syndrome are continuing. There is a large number of genes with established function and an even greater number of genes - candidates playing defined role in the formation of the main manifestations of MS. Currently, there are few works on studying the association of metabolic syndrome with polymorphic genes in the Yakut ethnic group. But at the same time there are no studies on the association with MS genes encoding components of carbohydrate metabolism and fibrinolysis system in Yakuts, namely gene *FTO* (T/A), *LEPR* (Gln223Arg), *SERPINE 1* (-675 5G/4G), *F7* (Arg353Gln). This paved the way for the study of polymorphisms of these genes in Yakuts with metabolic syndrome.

MATERIALS AND METHODS

The study is included 200 people Yakut with no relationships between them. All patients resided in the Far North - in the Republic of Sakha (Yakutia). According to the clinical, laboratory and instrumental survey, according to the recommendations of All-Russian Scientific Society of Cardiologist in 2006, patients were divided into two groups: those with metabolic syndrome and

healthy people. The main group consisted of 100 patients diagnosed with metabolic syndrome, a hyperbolic (MS «+»). The age of patients ranged from 18 to 70 years, the average age of the group MS «+» 47,63±1,25. Comparison group consisted of healthy volunteers almost corresponding to the group of patients with MS «+» by age, gender, ethnicity, without MS (MS «-»).

All participants signed informed consent. All persons at study performed a comprehensive clinical examination, questioning by a specially developed map of the subject, with the explanation of socio-demographic characteristics, anamnestic data, heredity, physical activity, smoking data, the presence of menopausal women. They have made complaints in the card, such as physical examination, anthropometry: measurement of height, weight, waist circumference (WC), hip circumference (HC), the ratio of WC/ HC, the calculation of body mass index (BMI), blood pressure (BP) and frequency heart rate (HR) and the results of biochemical analysis: glucose, total cholesterol (TC), low density lipoprotein cholesterol (LDL) , high density lipoprotein cholesterol (HDL), triglycerides (TG), atherogenic ratio calculation (ARC) in formula: ARC = (TC - HDL)/HDL, the definition of a single nucleotide polymorphism (SNP) rs9939609 gene *FTO*, rs1137101 gene *LEPR*, rs1799889 and rs6046 gene *SERPINE1* gene *F7*.

DNA was extracted from venous blood leukocytes phenol-chloroform method [3]. Determination of gene polymorphism was performed by polymerase chain reaction (PCR) using the reagent kit for amplification «SNP-Express» production firm «Lytech» thermocycler at ABI 9700. The amplification products were analyzed by electrophoresis in 2% agarose gel stained with ethidium bromide.

We have used a modified criterion $\chi 2$ (p) to check compliance with the empirical frequency distribution of genotypes theoretically expected equilibrium distribution of Hardy-Weinberg, defined using the RxC algorithm. This algorithm allows us to estimate the statistical significance of deviations from the expected frequency distribution when the number of observations for a significant number of classes is less than 5, and the application of the standard criterion $\chi 2$ incompetent. Obtained during the study data were processed using the software package SPSS. Figures in the study groups were described using mean values (M) and standard error (m). Comparison of genotype frequencies in groups of patients and healthy individuals using the chi-square Pearson. Threshold of significance for all statistical tests used accepted meaning of $p < 0,05$. Relative risk for a particular genotype was calculated as the odds ratio (odds ratio - OR) according to the formula: OR = (a x d) / (b x c), where a - genotype frequency in the sample of patients, b - genotype frequency in the control sample, with - the sum of frequencies

other genotypes in a sample of patients; d - the remaining sum of the frequencies of genotypes in the control sample. If one of the indicators is 0, adopted an amendment to the continuity of – 0,5. If OR = 1 - no association, OR> 1 - a positive association with the disease genotype and OR < 1 - negative association.

The project was implemented within the framework of the research work «Metabolic syndrome and chronic non-communicable diseases among residents of Yakutia» (Registration number YSU 11 - 01M.2009.) Department of Internal Medicine and General Practice (Family Medicine) Faculty of Postgraduate Medical Education Medical Institute NEFU. Study was approved by the local ethics committee YSC CMP SB RAMS (Prot № 24 dated June 29, 2010). This work was partially funded by a grant individual NEFU rector for students and young scientists by 2013.

RESULTS AND DISCUSSION

Distribution of polymorphisms studied theoretically corresponds to the equilibrium expected Hardy-Weinberg equilibrium ($p > 0,05$).

The results of the frequency distribution of alleles and genotypes of polymorphic markers of genes are shown in Table. 1. According to the results, when we had compared with a group of MS group healthy frequency distribution we observed differences in genotype frequencies of -675 5G/4G gene *SERPINE1*. As this can be seen from the data presented in the most common group of MS «+» was heterozygous genotype 5G/4G (49%) and gene *SERPINE1* marked predominance of genotype 4G/4G (37%) of the genotype 5G/5G (14%). In the comparison group, we also revealed the prevalence of heterozygous genotype 5G/4G (53%), but there is predominance of genotype 5G/5G (27%) of the genotype 4G/4G (20%). A comparative analysis revealed a statistically significant difference data distribution of genotypes ($p=0,009$). Our findings are consistent with earlier studies, according to which version of 4G/4G polymorphism -675 5G/4G gene *SERPINE1* correlates with central obesity and the relation of options 5G/4G polymorphism with average levels of PAI 1 in blood in the presence of obesity [18,20]. Comparative analysis of the frequency distribution of genotypes of the remaining genes in the test group of patients with MS «+» and the control group showed no significant differences.

The present study has revealed the lowest incidence of carriage "unfavorable" A allele of the *FTO* gene and 223Arg allele of gene *LEPR*, that gives us assume that these alleles are less significant in patients with MS Yakut ethnic population.

The risk of developing MS in the Yakut population became involved with genotype 4G/4G gene *SERPINE1* (OR = 3,568; CI 95%: 1,534-8,299). Similar data were obtained V.H.

Khavinson et al. (2010) on the Russian population. The study showed that the genotype 4G/4G gene SERPINE1, associated with the slowdown of fibrinolysis, systolic hypertension, the risk of acute coronary syndrome, increasing the concentration of glucose and cholesterol in the blood, can be attributed to genetic risk factors of metabolic cardio - vascular syndrome [1].

Speaking about the polymorphic variants of other genes we studied the risk of MS has not been identified (OR < 1). Perhaps in the surveyed Yakut ethnic group gene polymorphisms *FTO, LEPR* and *F7* do not contribute significantly to the development of MS.

For each studied polymorphic variant of the gene was analyzed associations with MS components - blood pressure levels with measures of carbohydrate and lipid profile and anthropometric data.

Passing in review of the contribution of T/A polymorphism of the *FTO* gene in the variability of MS components it was shown that, the polymorphic marker is not associated with any of the studied components of MS. However, a positive trend due to this polymorphic marker with HDL (p = 0,054). Our results are consistent with M.A. Garbuzova (2010), who found no association of rs8050136 and rs9939609 marker gene FTO with anthropometric and metabolic parameters MS: BMI, WC, HC, insulin resistance index, immunoreactive insulin, total cholesterol, HDL cholesterol, LDL cholesterol, TG , TG/HDL, blood glucose levels fasting and after an oral glucose tolerance test (p> 0.05) in the Russian population [2].

There was a statistically significant association of gene polymorphism Gln223Arg *LEPR* level of total cholesterol (p=0,038), triglycerides (p=0,033) and ARC (p=0,030) in Yakut ethnic group. Also, the study G.M. van der Vleuten (2006) found that the carrier 223Gln leptin receptor allele (homozygotes and heterozygotes 223Gln allele) was associated with combined hyperlipidemia, reduced sensitivity to insulin and obesity [23].

Association analysis of polymorphism -675 5G/4G gene *SERPINE1* with MS components in the sample revealed significant differences in anthropometric parameters: BMI (p = 0,016), WC (p = 0,001), the WC/HC (p = 0,019). Regarding the parameters showed a trend toward connection with the polymorphic marker (p = 0,069). This association was showcased the study Zaid H. Al-Hamodi et al. (2012), where the inhabitants of Malaysia polymorphism -675 5G/4G gene *SERPINE1* significantly associated with BMI [19].

The present study revealed no statistically significant association of polymorphism Arg 353 Gln *F7* gene with MS components in the Yakut ethnic group. A number of studies supports our findings. Thus, the study A.P. Reiner et al. (2007) showed that the minor Arg/Arg genotype was associated with a lower BMI, cholesterol and reduced risk of coronary heart disease, but this

association disappeared after adjustment for BMI and HDL [6]. Also not found an association between the level of blood triglycerides and Arg 353 Gln polymorphism F7 studies J.S. Pankow et al. (1998) and A. Lane et al. (1992) [27,12].

CONCLUSION

All things considered in the MC group «+» gene polymorphism 4G/4G genotype *SERPINE1,* associated with obesity played the main part (p=0,009). The risk of developing MS in the Yakut population became involved with genotype 4G/4G gene *SERPINE1* (OR = 3,568; CI 95%: 1,534-8,299). Speaking about the polymorphic variants of other genes we studied that the risk of MS has not been identified (OR<1). There was a statistically significant association of gene polymorphism Gln223Arg *LEPR* level of total cholesterol (p=0,038), triglycerides (p = 0,033) and ARC (p=0,030) and -675 5G/4G gene polymorphism with anthropometric *SERPINE1* BMI (p = 0,016), WC (p=0,001), WC/HC (p = 0.019) in the Yakut ethnic group.

Table 1

Comparative analysis of the distribution of allele and genotype frequencies of polymorphic markers of candidate genes of the metabolic syndrome in the Yakut population

Gene	Allele and genotype	Frequency of alleles (shares) and genotypes (%)		p	OR	
		MC «+» n = 100	MC «-» n = 100		value	CI 95%
SERPINE1 –675 5G/4G (rs1799889)	allele *5G*	0,39	0,54	-	-	-
	allele *4G*	0,62	0,47		-	-
	genotype *5G/5G*	14	27	0,009	0,280	0,121 – 0,652
	genotype *5G /4G*	49	53	-	-	-
	genotype *4G/4G*	37	20	0,009	3,568	1,534 – 8,299
LEPR Gln223Arg (rs1137101)	allele *Gln*	0,87	0,91	-	-	-
	allele *Arg*	0,13	0,9		-	-
	genotype *Gln/ Gln*	86	91	0,277	0,977	0,947- 1,009
	genotype *Gln/ Arg*	12	9		-	-
	genotype *Arg/ Arg*	2	-		-	-
FTO T/A (rs9939609)	allele *T*	0,88	0,89	-	-	-
	allele *A*	0,13	0,11		-	-
	genotype *T/T*	87	89	0,587	0,989	0,967 - 1,011
	genotype *T/A*	12	11		-	-
	genotype *A/A*	1	-		-	-
F7 Arg 353 Gln	allele *Arg*	0,57	0,61	-	-	-
	allele *Gln*	0,43	0,39		-	-

(rs6046)	genotype *Arg/ Arg*	55	52		1,135	0,452 – 2,849
	genotype *Arg/ Gln*	33	38	0,734	-	-
	genotype *Gln/ Gln*	12	10		0,881	0,351 – 2,214

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