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DETERMINATION OF HLA ALLELES USING SINGLE NUCLEOTIDE POLYMORPHISM OF RS3104413 HLA-DQA1 GENE AMONG PATIENTS WITH TYPE 1 DIABETES OF THE REPUBLIC SAKHA (YAKUTIA)

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In this paper, we analyzed the polymorphism rs3104413 of the *HLA-DQA1* gene among patients with type 1 diabetes and a control sample of the population of the Republic of Sakha (Yakutia). Type 1 diabetes (diabetes mellitus type 1) among all forms of diabetes is no more than 10-15%, and type 1 diabetes is related to the most important medical and social problems associated with the often occurring childhood and adolescence, severe course, early disability and mortality. In the study of rs3104413 polymorphism, a significantly lower frequency of the C allele (47.1) was observed in the group of healthy individuals compared with the group of people with type 1 diabetes (78.3%). The calculation of the odds ratio showed that the frequency of the C allele in the group of people with type 1 diabetes was significantly higher (OR - 4.036; 95% CI: 2.71-6.02; p <0.001). The study revealed 6 haplotypes and genes. The haplotype DRB1 * 03: 01-DQA1 * 05: 01-DQB1 * 02: 01, DR3 / X, DRX / X was associated with the allele C of the rs3104413 polymorphism. The allele G of rs3104413 polymorphism was associated with the haplotype carrier DRB1 * 04: 01 – DQA1 * 03: 01-DQB1 * 03: 02 (DR4-DQ8) and DRB1 * 04: 01-DQA1 * 03: 01-DQB1 * 03: 01 (DR4-DQ7). Thus, the data obtained can be used as biological predictors of the development of type 1 diabetes in order to carry out timely personalized preventive measures.

Keywords: type 1 diabetes mellitus, HLA typing, *HLA-DQA1* gene, polymorphism.

Type 1 diabetes is a disease in which the body is not able to properly metabolize carbohydrates and to a lesser extent other components of food. This disease is caused by a lack of insulin, a hormone that is produced by the pancreas and that is required by the body to convert glucose from other food components into energy. The most severe form of diabetes is type 1 diabetes (type 1 diabetes mellitus). Despite the fact that its share among all forms of diabetes is no more than 10-15%, it is type 1 diabetes that is considered to be the most important medico-social problem of health care, since this disease often occurs in childhood and adolescence, characterized by severity, early disability and mortality [3].

According to modern data, a large number of genes are involved in the development of type 1 diabetes [3], more than half of the genetic risks are due to the participation of polymorphic variants of HLA genes located on the short arm of chromosome 6 (6p21). The main genetic contribution to the susceptibility to type 1 diabetes is made by genes of the HLA system encoding class II molecules of the main human histocompatibility complex, especially the DR and DQ genes of the HLA class, whose association with the development of type 1 diabetes has been shown in numerous publications for various population groups [1, 4].

In Russia, studies on the definition of HLA alleles using single nucleotide polymorphisms are not carried out. In this regard, today there is a need for research aimed at developing a regionally-adapted method for the HLA-typing of type 1 diabetes using single nucleotide polymorphisms (SNP).

Objective: to determine HLA alleles using single nucleotide polymorphism of rs3104413 *HLA-DQA1* gene and its association with type 1 diabetes.

Material and research methods: An experimental part of the work on the genotyping of the rs3104413 polymorphism, the *HLA-DQA1* gene, was carried out in the laboratory of hereditary pathology of the Department of Molecular Genetics of the Yakutsk Scientific Center for Complex Medical Problems (YSC CMP). DNA samples from the collection of the YSC CMP biomaterial are used for the study - a unique scientific installation "The Genome of Yakutia" (reg. No. US_507512). The sample of patients consisted of 92 patients of the Yakut Scientific Center for Complex Medical Problems, state autonomous institution of the Republic Sakha (Yakutia) "Republic Hospital №1 National Center of Medicine » and Endocrinology Department of Yakutsk Clinical Hospital, Yakutsk. The sample of patients included 92 patients with a diagnosis of type 1 diabetes, aged 4 to 56 years, living in the RS (Ya), Yakuts by ethnicity. By gender there were 44 (47.8%) male and 48 (52.2%) female. The average age of patients was 23.04 ± 0.27 years (from 4 to 56 years), the average age of male patients was 20.5 ± 2.3 years (from 5 to 40 years), and female - 25.11 ± 2 , 59 years old (from 4 to 56 years old). The control sample consisted of 210 Yakuts who did not suffer from type 1 diabetes. Ethnicity counted to the third generation. Ethnicity counted to third generation.

Amplification of the *HLA-DQA1* gene region containing the single nucleotide polymorphism rs3104413 was performed during real-time PCR using primer pairs and allele-specific probes for DNA amplification described in Serr I. et al. [6] Primers and probes were synthesized by the company Biotech-Industry (Lumiprobe) LLC (Moscow, Russia). The sequence of primers: Forward primer 5'-CAGCTGAGCACTGAGTAG-3 ', reverse primer 5'-GCAGTTGAGAAGTGAGAG-3'. Probes structure: FAM - Probe rs3104413 LPC [6FAM] CAGCCT [+ G] CT [+ C] TC [+ C] TA [+ T] TGG [BHQ1], HEX - Probe rs3104413 LPG [HEX] CAGCCT [+ G] CT

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Table 1

Temperature polymorphism amplification program rs3104413

Stages	Temperature, °C	Time	Cycles
First denaturation	95	10 s	1
Denaturation	95	30 s	50
Annealing	55	1 min	

[+ G] TC [+ C] TA [+ T] TGG [BHQ1].

Amplification was carried out according to the temperature program below (Table 1):

The fluorescence signal was measured at the second stage of the reaction (55 ° C - 1 min). The detection of fluorescence was carried out "at the end point" according to the protocol of the device "Real-time CFX 96 Touch" ("Biorad", USA). An example of the distribution of clouds of genotypes of PCR and detection of fluorescence "at the end point" is presented in Figure 1.

Statistical analysis of the results of the medical genetic study was conducted using the program: "Office Microsoft Excel 2010", "Statistics 8.0". The frequencies of alleles and rs3104413 genotypes were determined by direct counting. Results were considered significant when the “p” value was less than 0.05 (p <0.05).

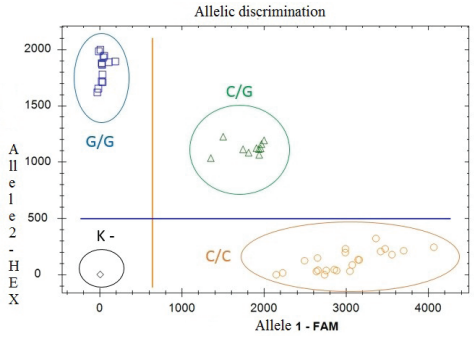

Figure 1. Distribution of genotype clouds of rs3104413 polymorphism of the *HLA-DQA1* gene. Note: “K–” - negative control, C / C - homozygous for ancestral allele C. C / G - heterozygote, G / G - homozygote for mutant allele G. Compliance with fluorescent dyes: allele C - FAM channel, allele G - channel HEX.

Genotyping of *HLA DRB1* and *DQB1* alleles was carried out with the commercial HISTOTYPE kits, the HLA alleles of *DRB1 * 03: 01 (DR3), DRB1 * 04: 01 (DR4), DQB1 * 02: 01 (DQ2), DQA1 * 05: 01* were progenotyped.

The amplification parameters were optimized for a total reaction volume of 10 μl. PCR was performed according to the manufacturer’s instructions in an MJ Mini Gradient Thermal Cycler (BioRad) thermal cycler (Table 2).

The results of the amplification were fractionated in 2% agarose gel, with ethidium bromide, at a voltage of 120-300 V, for 45-120 minutes. Documentation and visualization of PCR amplification was performed by photographing in UV light using a Vil-ber Lourmat gel-documenting instrument (Fig. 2).

The interpretation of the results of genotyping according to the HISTOTYPE kits (updated 01 / 2015_3.19.0 (6.2)) was carried out on the basis of the assessment chart: for HISTOTYPE, specific bands have sizes of 220, 200, 800, 150 and 235 bp. In all lanes without an allele-specific amplification, an internal control of 429 or 1070 bp should be clearly visible. Evaluation of the bands was carried out using a DNA marker “Step 100”

Table 2

Temperature polymorphism amplification program HISTOTYPE

Steps	Temperature, °C	Time	Кол-во циклов
First denaturation	96	5 min	1
Denaturation	96	20 s	5
Annealing and Elongation	68	1 min	
Denaturation	96	20 s	10
Annealing	64	50 s	
Elongation	72	45 s	
Denaturation	96	20 s	15
Annealing	61	50 s	
Elongation	72	45 s	
Final elongation	72	5 min	1

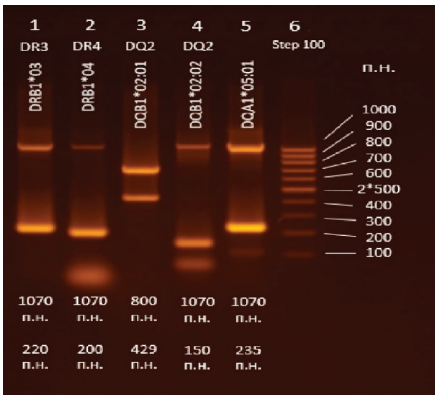

Figure 2. Electrophoregram of the *HLA DRB1* and *DQB1* amplification product on 2% agarose gel (*HLA DRB1* and *DQB1* genotyping using commercial HISTOTYPE kits). Note: b.p.. - base pair. 1 - *DRB1 * 03* (220 bp); 2, - *DRB1 * 04* (200 bp); 3 - *DQB1 * 02: 01* (800 bp); 4 - *DQB1 * 02: 02* (150 bp); 5 - *DQA1 * 05: 01* (235 bp). Internal control: 1070 b.p. (1, 2, 4, 5) and 429 bp (3). 6 - DNA marker "Step100".

(Biolabmix LLC, Novosibirsk, Russia).

Results and discussion. The results of the analysis of the frequency distribution of alleles and genotypes of the rs3104413 polymorphism among patients with type 1 diabetes and control sample are presented in table 3.

In the study of rs3104413 polymorphism, a significantly lower frequency of the C allele (47.1) was observed in the group of healthy individuals compared with the group of people with type 1 diabetes (78.3%). The calculation of the odds ratio showed that the frequency of the C allele in the group of people with type 1 diabetes was significantly higher (OR - 4.036; 95% CI: 2.71-6.02; p <0.001).

Analysis of the distribution of genotypes showed that the most common genotype of the studied polymorphism in the group of people with type 1 diabetes is C / C (69.6%), and in the group of people not suffering from type 1 diabetes is the homozygous genotype G / G (41.9%).

The calculated conjugacy coefficient of Pearson (C) allele C (0.278) shows the average strength of the connection between the carriage of the allele C and type 1 diabetes. The normalized value of the Pearson coefficient (C ') indicates the average relationship between the carriage of the allele C (0.393) and type 1 diabetes.

The analysis revealed 6 haplotypes and genes. The haplotype *DRB1 * 03: 01-DQA1 * 05: 01-DQB1 * 02: 01, DR3 / X, DRX / X* was associated with the allele C of the rs3104413 polymorphism. The allele G of rs3104413 polymorphism was associated with the haplotype carrier

Table 3

The frequency of occurrence of genotypes and alleles of the rs3104413 polymorphism in the group of patients with type 1 diabetes and control sample

Haplotypes, alleles	Patient with T1D (n = 92),abs. (%)	control sample (n = 210), abs.(%)	X^2	OR (95% CI) For alleles	Significance, p
C/C	64 (69.6)	76 (36.2)	32.099	4.036 (2.708-6.017)	<0.001*
C/G	16 (17.4)	46 (21.9)			
G/G	12 (13.0)	88 (41.9)			
C	144 (0.783)	198 (0.471)	49.184		<0.001**
G	40 (0.217)	222 (0.529)			

Note. The achieved level of significance when comparing the distribution of genotypes (*) and allele frequencies (**) in comparison groups 1 and 2 is the number of samples, X^2 with the Yeats amendment.

Table 4

Identification of HLA haplotypes and genotypes using single nucleotide polymorphism rs3104413

Haplotype by genes HLA	The number of people with this haplotype	Genotype, %			Allele *C*	Allele *G*
		C/C	*C/G*	*G/G*		
*DRB1*03:01-DQA1*05:01-DQB1*02:01*	16	87.5	12.5	0	0.938	0.063
*DRB1*04:01–DQA1*03:01-DQB1*03:02 (DR4-DQ8)*	20	0	40	60	0.200	0.800
DR3/4-DQ8	8	0	100	0	0.500	0.500
DR3/X	50	100	0	0	1.000	0.000
*DRB1*04:01-DQA1*03:01-DQB1*03:01 (DR4-DQ7)*	132	0	33.3	66.7	0.167	0.833
DRX/X	76	100	0	0	1.000	0.000

Note: DRX / X - the absence of both DR3 and DR4; DR3 / X - carrier variant 1 of type DR3 and type DR3, not related to DR4

*DRB1 * 04: 01 – DQA1 * 03: 01-DQB1 * 03: 02 (DR4-DQ8) and DRB1 * 04: 01-DQA1 * 03: 01-DQB1 * 03: 01 (DR4-DQ7)*. Table 4.

According to the literature, more than 90 percent of patients with type 1 diabetes are carriers of either *HLA-DR3, DQB1 * 0201 (DR3-DQ2), or DR4, DQB1 * 0302 (DR4-DQ8)*. About 30% of patients have the combined genotype DR3 / 4, which is associated with the greatest susceptibility to the disease [2]. Associated previously with a low risk of developing the disease is the haplotype *DRB1 * 04: 01-DQA1 * 03: 01-DQB1 * 03: 01 (DR4-DQ7*) [5].

Thus, the study of this rs3104413 polymorphism in determining the haplotype is insufficient and requires further research in combination with other SNPs.

Conclusion

The study revealed 6 haplotypes and genes. The haplotype *DRB1 * 03: 01-DQA1 * 05: 01-DQB1 * 02: 01, DR3 / X, DRX / X* was associated with the allele C of the rs3104413 polymorphism. The allele G of rs3104413 polymorphism was associated with the haplotype carrier *DRB1 * 04: 01 – DQA1 * 03: 01-DQB1 * 03: 02 (DR4-DQ8) and DRB1 * 04: 01-DQA1 * 03: 01-DQB1 * 03: 01 (DR4-DQ7)*.

There is a significantly low frequency of allele C (47.1) in the control sample compared with patients with type 1 diabetes (78.3%) in the rs3104413 polymorphism. The calculation of the odds ratio showed that the frequency of the C allele in the group of people with type 1 diabetes was significantly higher (OR - 4.036; 95% CI: 2.71-6.02; p <0.001).

Thus, the data obtained can be used as biological predictors of the development of type 1 diabetes in order to carry out timely personalized preventive measures.

The study was conducted in the framework of research on the study of the genetic structure and burden of hereditary pathology of populations of the Republic of Sakha (Yakutia).

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