ORIGINAL ARTICLE

Population Genetics

Determination of T1D-Susceptible HLA DR-DQ Haplotypes in Yakuts Using Three Single Nucleotide Polymorphisms

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Abstract

The purpose of this study was to determine T1D-susceptible HLA DR-DQ haplotypes in Yakuts using 3 single nucleotide polymorphisms (rs3104413C/G, rs2854275G/T and rs9273363A/C). A total of 92 patients with a verified diagnosis of T1D (Group 1) were examined. The comparison group consisted of 210 people (Group 2) without autoimmune diseases and without hereditary predisposition. All participants were Yakuts by ethnicity.

According to the results of genotyping, it was established that the haplotype *DRB1**03:01-*DQA1**05:01-*DQB1**02:01 corresponds to the rs3104413C/C-rs2854275T/T combination and the rs3104413C/G-rs2854275G/T-rs9273363C/C combination. *DRB1**04:01–*DQA1**03:01-*DQB1**03:02 haplotype (DR4-DQ8) is the rs3104413G/G-rs9273363A/C combination, the rs3104413C/G-rs2854275G/G-rs9273363A/C combination, and the rs3104413C/G-rs2854275G/G-rs9273363A/A combination. DR3/4-DQ8 haplotype is the rs3104413C/G-rs2854275G/T-rs9273363A/A combination and the rs3104413C/G-rs2854275G/T-rs9273363A/C combination. *DRB1**04:01-*DQA1**03:01-*DQB1**03:01 haplotype is the rs3104413G/G-rs9273363C/C combination, the rs3104413C/G-rs2854275G/G-rs9273363C/C combination, and the rs3104413C/G-rs2854275G/T-rs9273363C/C combination. A combination of rs3104413C/C with rs2854275G/G was characterized by the absence of DR3 and DR4, which may be due to the presence of a different genetic variant in these individuals. A combination of rs3104413C/C with rs2854275G/T was characterized by the presence of only one type of DR3 and a variant of the type that does not belong to either DR3 or DR4. An analysis of the evaluation of the effectiveness of a diagnostic test using the Youden index demonstrated significance for all combinations of genotypes of the studied polymorphisms. (**International Journal of Biomedicine. 2019;9(3):216-219.**)

Key Words: haplotype • single nucleotide polymorphism • Yakuts • type 1 diabetes

Introduction

Type 1 diabetes (T1D), a multifactorial disease with a strong genetic component, is caused by the autoimmune destruction of pancreatic β cells.[1,2] The major T1D susceptibility locus maps to the class II loci *HLA-DRB1* and *HLA-DQB1* on chromosome 6p21. The DR-DQ haplotypes conferring the highest risk are *DRB1**03:01-*DQA1**05:01-*DQB1**02:01 (abbreviated "DR3") and *DRB1**04:01/02/04/05/08-*DQA1**03:01-*DQB1**03:02/04 (or *DQB1**02; abbreviated "DR4").[3] More than 80% of T1D patients are carriers of one or both haplotypes. It is important that the polymorphism of HLA genes can also have a pronounced protective effect.[4,5] HLA genes can also have an impact on the clinical features of the disease, such as the age of onset or the outcome of active cellular autoimmunity. Therefore, the haplotype *DRB1**03:01-*DRB1**04:01(DR3/4) not only strongly predisposes to the development of T1D, but also accelerates the onset of the disease (i.e. occurs much more frequently among patients with the onset of T1D in early childhood).[6] In the Russian population, high-risk genotypes (DQ2/DQ8) were significantly more common in children who were sick before the age of 5 (33%), compared

with children in whom T1D manifested at the age of 10 years (23%).[7] The results obtained in Yakutia will be in demand in the development of a personalized approach to diagnosis (screening) and prevention of T1D.

The purpose of this study was to determine T1D-susceptible HLA DR-DQ haplotypes in Yakuts using 3 single nucleotide polymorphisms (rs3104413C/G, rs2854275G/T and rs9273363A/C).

Materials and Methods

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems (YSC CMP). The HLA-DR/DQ genotyping was performed in the laboratory of molecular genetics at YSC CMP. A total of 92 patients with a verified diagnosis of T1D (Group 1) were examined. The comparison group consisted of 210 people (Group 2) without autoimmune diseases and without hereditary predisposition. All participants were Yakuts by ethnicity. The ethnic origin was considered to the third generation. The study was performed after participants signed a written, informed consent; for participants under the age of adulthood, the informed consent was signed by parents. For each study participant, an individual genetic map was developed, containing clinical, functional and laboratory parameters, as well as genealogical data of the participant. Relatives were excluded from the analysis.

Genomic DNA samples were isolated from the peripheral blood leukocytes using a commercial DNA kit, Excel biotech (Yakutsk). Studies of HLA-DR and HLA-DQ gene variants were carried out using an allele-specific PCR (AS-PCR), according to Cao Nguyen (2013).[8] Characteristics of single-nucleotide polymorphisms rs3104413, rs2854275, rs9273363 are presented in Table 1. SNP selection was based on a study conducted by Cao Nguyen and colleagues, who analyzed 19,035 SNPs of 10,579 subjects (7,405 from a discovery set and 3,174 from a validation set) from the Type 1 Diabetes Genetics Consortium and developed a novel machine learning method to select as few as 3 SNPs that could define the HLA-DR and HLA-DQ types accurately. Thus, 3 SNPs were proposed to accurately determine the types of HLA-DR and HLA-DQ. The high accuracy is consistent with the proximity of the SNPs to the class II genes (rs3104413 is located in the intergenic region between *HLA-DRB1* and *HLA-DQA1*, rs2854275 is within the *HLA-DQB1* gene, and rs9273363 is in close proximity to the *HLA-DQB1* gene) and with the strong linkage disequilibrium between HLA genes.[8]

Table 1.

Characteristics of single-nucleotide polymorphisms rs3104413, rs2854275, rs9273363

Gene	SNP	Localization (hg19)	Allele	Method	MAF
HLA-DQA1	rs3104413	chr6: 32582650	C/G	RT-PCR	0.13(G)
HLA-DQB1	rs2854275	chr6: 32660651	G/T	AS-PCR	0.06(T)
HLA-DQB1	rs9273363	chr6: 32658495	A/C	AS-PCR	0.24(A)

MAF is the minor allele frequency; hg19 is the reference genome

At the first stage, for definition of HLA-DR types, rs3104413 SNP was used. Further research tactics were determined depending on the genotype established at the first stage. Thus, individuals homozygous for rs3104413C (C/C) were further investigated by rs2854275, homozygous for rs3104413G (G/G) by rs9273363, and C/G heterozygous for rs3104413 (C/G) were investigated for both SNPs.

To determine compliance with the HLA-DR and HLA-DQ haplotypes with the studied SNPs (rs3104413, rs2854275, and rs9273363), a commercial HLA-Type kit (BAG Health Care GmbH, Germany) was used. The genotyping of HLA DRB1 and DQB1 alleles was carried out according to previously described methods.[9,10] Haplotype combinations of the three analyzed polymorphisms were used for defining the HLA types relevant to T1D (Nguyen et al, 2013),[8] distinguishing the highest-risk DR4- DQ8 and DR3/4-DQ types.

Statistical analysis was performed using the Statistica 8.0 software package (StatSoft Inc, USA).The frequencies of the alleles and genotypes of SNPs (rs3104413, rs2854275, and rs9273363) were determined by direct counting, using generally accepted formulas. To determine the effectiveness of the diagnostic test, the Youden index was calculated using the following formula: $J = Sen + Spe - 1$,

J – the Youden index, Sen – sensitivity, Sp –specificity

Indicators Sen and Spe were calculated using the following formulas: $Sen = TP/(TP+FN)$ and $Spe = TN/(TN+FP)$,

TP–true positive value, FP – false positive value, TN – true negative value, FN – false negative value.

Results and Discussion

Depending on the distribution of genotypes of the investigated polymorphisms, in patients of Group 1 with rs3104413C/C, rs2854275G/T prevailed, which corresponds to the HLA DR3/X genotype. rs2854275T/T associated with adverse haplotype *DRB1*03-DQA1*05:01-DQB1*02:01* was found in 21.9% of cases in this group. Patients with rs2854275G/G (12.5%) were characterized by the absence of DR3 and DR4, which may be due to the presence in these individuals of another allelic variant (Table 2).

Among patients with rs3104413C/G, 50% had rs2854275G/T, associated with haplotypes *DRB1*03-DQA1*05:01-DQB1*02:01* and DR3/4-DQ8, and 50% had rs2854275G/G, associated with haplotype DR4/4-DQ8.

Among patients with rs3104413G/G, rs9273363A/C associated with the DR4-DQ8 haplotype was found in 100% of cases. Among patients with rs3104413C/G, rs9273363A/C was prevalent, which in 37.5% of cases correspondents to DR4-DQ8 and in 25% - DR3/4-DQ8.

The results of the distribution of HLA gene alleles among T1D patients are consistent with the results of E.V. Titovich (2009).[5] In a comparative analysis of the predisposing alleles distribution frequencies among the Yakut population and the Russian population of Moscow, it was shown that among T1D patients in the Yakut population, alleles of *DRB1*04 (DR4) and *DRB1*17(03) are more common. The authors also showed that *DRB1*17(03) is the most predisposing allele in the Yakut population.

Table 2.

Distribution of haplotypes in the group of patients with T1D depending on the distribution of SNP genotypes

SNP	rs3104413, n=92														
Genotype, n (%)	C/C 64 (69.6)			C/G 16 (17.4)									G/G 12 (13.0)		
SNP	rs2854275			rs2854275			rs9273363						rs9273363		
Genotype	T/T	G/T	G/G	G/T	G/G	T/T	A/A	A/A	A/C	A/C	C/C	C/C	A/A	A/C	C/C
n (%)	14(21.9)	42(65.6)	8(12.5)	2(12.5)**	6(37.5)	8(50)	0	2(12.5)	2(12.5)	6(37.5)***	4(25)	2(12.5)	0	12(100)	0
Haplotypes obtained with Histotype Kit	*DRB1*03-*DQA1*05:01-*DQB1*02:01*	DR3/X	DRX/X	*DRB1*03-*DQA1*05:01-*DQB1*02:01*	DR3/4-DQ8	DR4-DQ8	-	DR3/4-DQ8	DR4-DQ8	DR4-DQ8	DR3/4-DQ8	*DRB1*03-*DQA1*05:01-*DQB1*02:01*	-	DR4-DQ8	-

DR/X – lack of DR3 and DR4; DR3/X – variant carriers of one type DR3 and a type without relation to DR3 and DR4

** - carriers of rs9273363C/C and rs2854275G/T ; *** - carriers of rs2854275G/G and rs9273363A/C

Table 3.

Distribution of haplotypes in the control group depending on the distribution of SNP genotypes

SNP	rs3104413, n=210												
Genotype, n (%)	C/C 76 (36.2)			C/G 46 (21.9)							G/G 88 (41.9)		
SNP	rs2854275			rs2854275				rs9273363			rs9273363		
Genotype	T/T	G/T	G/G	T/T	G/T	G/T	G/G	A/A	A/C	C/C	A/A	A/C	C/C
n (%)	0	8(10.5)	68(89.5)	0(0)	2(4.4)**	4(8.7)	40(86.9)	2(4.4)	0(0)	44(95.6)	0	0	88(100)
Haplotypes obtained with Histotype Kit	-	DR3/X	DRX/X	-	DR3/4-DQ8	DR4-DQ7	DR4-DQ7	DR3/4-DQ8	-	DR4-DQ7	-	-	DR4-DQ7

DR/X – lack of DR3 and DR4; DR3/X – variant carriers of one type DR3 and a type without relation to DR3 and DR4

** - carriers of rs2854275G/T and rs9273363C/C

Table 4.

Identification of HLA haplotypes and genotypes using SNPs (rs3104413, rs2854275, rs9273363)

Haplotypes obtained with Histotype Kit	The number of people with corresponding haplotype	The combination of genotypes, #	rs3104413	rs2854275	rs9273363	The number of people with corresponding genotype for SNP, total	The number of people with corresponding genotype but different haplotype	Sen	Spe	J, (Sen+Spe -1)
*DRB1*03:01-*DQA1*05:01-*DQB1*02:01*	16	1	C/C	T/T	-	14	0	1.000	0.993	0.993
		2	C/G	G/T	C/C	6	4	0.333	0.953	0.287
*DRB1*04:01–*DQA1*03:01-*DQB1*03:02* (DR4-DQ8)	20	3	G/G	-	A/C	12	0	1.000	0.972	0.972
		4	C/G	G/G	A/C	6	0	1.000	0.953	0.953
		5	C/G	G/G	A/A	2	0	1.000	0.940	0.940
DR3/4-DQ8	8	6	C/G	G/T	A/A	4	0	1.000	0.987	0.987
		7	C/G	G/T	A/C	4	0	1.000	0.987	0.987
DR3/X	50	8	C/C	G/T	-	50	0	1.000	1.000	1.000
*DRB1*04:01-*DQA1*03:01-*DQB1*03:01* (DR4-DQ7)	132	9	G/G	-	C/C	88	0	1.000	0.794	0.794
		10	C/G	G/G	C/C	40	0	1.000	0.649	0.649
		2	C/G	G/T	C/C	6	2	0.667	0.570	0.237
DRX/X	76	11	C/C	G/G	-	76	0	1.000	1.000	1.000

Analysis of haplotype distribution in Group 2 demonstrated the following results: in carriers of rs3104413C/G, the vast majority of individuals had rs2854275G/G associated with the low-risk genotype DR4-DQ7.[8] Evaluation of rs9273363 SNP among individuals with rs3104413G/G and rs310441C/G demonstrated the prevalence of rs9273363C/C associated with the DR4-DQ7 genotype, rs9273363A/C was completely absent (Table 3).

In order to determine the sensitivity and specificity of rs3104413, rs2854275, and rs9273363 with respect to HLA haplotypes and genotypes, the Youden index was evaluated (Table 4). For most of the analyzed combinations of genotypes of the studied polymorphisms, the Youden index varied significantly from 0.649 to 1, with the exception of option #2. The highest value of the Youden index for the genotype DR3/X was established for combinations #8 and the lack of a carrier state of DR3 and DR4 - combination #11 (Table 4).

Conclusion

According to the results of genotyping, it was established that the haplotype *DRB1**03:01-*DQA1**05:01-*DQB1**02:01 corresponds to the rs3104413C/C-rs2854275T/T combination and the rs3104413C/G-rs2854275G/T-rs9273363C/C combination.

The haplotype *DRB1**04:01–*DQA1**03:01-*DQB1**03:02 (DR4-DQ8) is the rs3104413G/G-rs9273363A/C combination, the rs3104413C/G-rs2854275G/G-rs9273363A/C combination, and the rs3104413C/G-rs2854275G/G-rs9273363A/A combination.

DR3/4-DQ8 haplotype is the rs3104413C/G-rs2854275G/T-rs9273363A/A combination and the rs3104413C/G-rs2854275G/T-rs9273363A/C combination.

DRB1*04:01-*DQA1**03:01-*DQB1**03:01 haplotype is the rs3104413G/G-rs9273363C/C combination, the rs3104413C/G-rs2854275G/G-rs9273363C/C combination, and the rs3104413C/G-rs2854275G/T-rs9273363C/C combination.

A combination of rs3104413C/C with rs2854275G/G was characterized by the absence of DR3 and DR4, which may be due to the presence of a different genetic variant in these individuals. A combination of rs3104413C/C with rs2854275G/T was characterized by the presence of only one type of DR3 and a variant of the type that does not belong to either DR3 or DR4.

An analysis of the evaluation of the effectiveness of a diagnostic test using the Youden index demonstrated significance for all combinations of genotypes of the studied polymorphisms.

Competing Interests

The authors declare that they have no competing interests.

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