CLINICAL STUDY

HLA class II allele frequencies in type 1A diabetes mellitus Slovak patients

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Abstract

Background: **Diabetes mellitus type 1A (DM-1A) is an autoimmune disease in which the immune response is directed to pancreatic islet cells. DM-1A occurs in genetically predisposed individuals. Among type 1A diabetes associated genes, those of the HLA region have the greatest effect.**
Objectives: **The aim of our study was to obtain a comprehensive survey of the HLA-DRB1 and HLA-DQB1 allele frequencies in Slovak patients suffering from DM-1A.**
Methods: **HLA class II genotyping was performed on genomic DNA by the PCR-SSP method according to the 12th Workshop protocol.**
Results: **Our report gives the first presentation of the distribution of HLA-DRB1 alleles (including complete DRB1*04 subtypes) and that of HLA-DQB1 alleles in the Slovak diabetic patients diagnosed at 0–18 years of age. Susceptibility is significantly associated with the alleles DQB1*0302 (OR=7.8), DRB1*04 (OR=4.9), DRB1*0301 (OR=4.2) and DQB1*02 (OR=2.2), whereas the alleles DQB*0602 (OR=0.05), DRB1*11 (OR=0.2), DRB1*15 (OR=0.2) and DQB1*0301 (OR=0.3) were found to be protective.**
Conclusions: **Our results, consistent with other studies, show increased frequencies of known positively associated HLA class II alleles in our type 1A diabetes mellitus patients compared to the general (nondiabetic) population. The protective effect of previously reported alleles was confirmed as well. Results of our population-based study serve in clinical practice for the identification of subjects at risk of developing DM-1A among the first-degree relatives *(Tab. 2, Ref. 12)*.**
Key words: type 1A diabetes mellitus (DM-1A), HLA class II alleles, Slovak population.

Type 1A diabetes mellitus is a genetically determined disease resulting in the immune-mediated destruction of pancreatic beta cells. It is one of the most common diseases of children and young adults. The HLA class II region of chromosome 6 is well known to carry the most important genetic factors in the susceptibility to type 1A diabetes (1, 2). Studies on HLA association with DM-1 together with the development of immunological markers for anti-islet cell autoimmunity provide a genetic basis for understanding the mechanisms leading to islet cell destruction as well as for the identification of subjects at risk of developing the disease. The risk conferred by particular HLA-alleles as well as the incidence of DM-1A varies across different ethnical groups and geographical regions (3). Slovakia is a central European country with ethnically homogenous Caucasoid population. Gypsies who constitute a fraction of about 5 % were not included in this study. Slovakia belongs to countries with moderate DM-1 incidence rates with approximately 11 new cases per 100,000 per year. Because of the severeness of type 1A diabetes mellitus, international population-based studies documenting the genetic epidemiology have been initiated (4).

The aim of our study was to obtain a survey of the HLA-DRB1 and -DQB1 allele distribution in Slovak DM-1A children

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Tab. 1. HLA-DRB1 and DQB1 alleles in DM-1A patients and controls.

HLA-DRB1		DM-1A patients n=184		Control subjects n=260			
	count	%	count	%	OR	95 % CI	p_c*
*0101-4	11	6.0	19	7.3			
*1501-2	5	2.7	32	12.3	0.2	0.07-0.52	<0.003
*1601-8	6	3.3	14	5.3			
*0301	57	31.0	25	9.6	4.22	2.52-7.08	<0.001
*0302-11	0	0.0	0	0.0			
*0401-14	70	38.0	29	11.2	4.89	3.00-7.96	<0.001
*1101-31	8	4.3	53	20.3	0.18	0.08-0.38	<0.001
*1201-5	2	1.1	4	1.5			
*1301-32	3	1.6	21	8.8			
*1401-30	4	2.2	7	2.6			
*0701	9	4.9	37	14.2			
*0801-17	7	3.8	12	4.6			
*0901	2	1.1	1	0.3			
*1001	0	0.0	6	2.3			
HLA-DQB1		n=184		n=286			
	count	%	count	%	OR	95 % CI	p_c*
*0501-4	23	12.5	56	19.6			
*0601	0	0.0	1	0.3			
*0602	1	0.5	29	10.1	0.05	0.01-0.35	<0.001
*0603	3	1.6	24	8.4			
*0604	0	0.0	5	1.7			
*0605-14	0	0.0	0	0.0			
*0201-3	67	36.4	60	20.9	2.15	1.42-3.26	<0.004
*0301	16	8.7	70	24.5	0.29	0.16-0.52	<0.001
*0302	58	34.2	16	5.6	7.77	2.84-9.39	<0.001
*0303	4	2.1	12	4.2			
*0304-8	4	2.1	0	0.0			
*0401-2	3	1.6	13	4.5			

Legend: n – number of alleles, OR – odds ratio, 95 % CI – 95 % confidence interval, p_c – p value corrected for number of alleles considered, * – only significant OR are presented (p_c<0.001).

and young adults as a contribution to the precise definition of HLA class II-associated predisposing and protective alleles in different Caucasian populations.

Subjects and methods

Subjects. 92 unrelated DM-1 patients recruited by the Slovak Children Diabetes Centre were investigated. All patients were selected on the basis of absolute insulin dependency according to the WHO criteria. Age at disease onset ranged from 0–18 years (mean 8.5 years). The sex ratio M/F was 1.1 (48 males, 44 females). A family history of DM-1A was reported in 15 out of 92 patients (16.3 %).

The reference group consisted of 183 unrelated nondiabetic individuals randomly recruited among blood and bone marrow donors from various parts of Slovakia. Data on the control subjects have been reported elsewhere (5). An informed consent was obtained from all subjects participating in this study and investigations were carried out in accordance with the principles of the Declaration of Helsinki.

HLA typing. Genomic DNA was extracted by the salting-out procedure (6). HLA-DRB1 and -DQB1 alleles were identified by the PCR-SSP method according to the 12th Workshop protocol (7, 8) using group-specific primers (Olerup SSP™ low resolution, Sweden). Assignment of DRB1*04, *03 as well as DQB1*03 and *06 subtypes was performed by Olerup SSP™ high-resolution primer sets. Amplification reactions were done according to manufacturers' protocols.

Statistical analysis. Allele frequencies were determined in the DM-1A patient group and the nondiabetic control group, respectively. Associations of particular alleles with DM-1A were expressed as odds ratios (OR) with 95 % confidence intervals (CI). Odds ratios were calculated according to Woolf's formula. The p-values were defined by the two-sided Fisher's exact test and corrected for the number of alleles considered at each locus (p_c) according to Bonferroni. The level of significance was set to p_c 0.01.

Tab. 2. HLA-DRB1*04 subtypes in DM-1A patients and controls.

HLA-DRB1	DM-1A patients n=67		Control subjects n=62				
	count	%	count	%	OR	95 % CI	p_c*
*0401	49	73.1	29	46.8	3.1	1.48-6.46	<0.028
*0402	4	6.0	6	9.7			
*0403	3	4.4	6	9.7			
*0404	5	7.5	10	16.1			
*0405	2	2.9	2	3.2			
*0406	0	0.0	0	0.0			
*0407	0	0.0	5	8.1			
*0408	4	6.0	4	6.4			

Legend: n – number of alleles, OR – odds ratio, 95 % CI – 95 % confidence interval, p_c – p value corrected for number of alleles considered, * – only significant OR are presented (p_c<0.05).

Results

The HLA class II allele frequencies found in 92 DM-1A patients and in 183 healthy controls are given in Table 1. Odd ratios are indicated only when statistically significant, i.e. when the p-value corrected for the number of alleles considered at each locus is <0.01. Out of the HLA-DRB1 alleles the DRB1*04 (OR=4.9, p_c<0.001) and DRB1*0301 (OR=4.2, p_c<0.001) were significantly positively associated with the disease. As opposed to the latter, the DRB1*11 (OR=0.2, p_c<0.001) and DRB1*15 (OR=0.2, p_c<0.003) were found to be negatively associated with the disease. Out of the DQB1 alleles, the DQB1*0302 (OR=7.8, p_c<0.001) and DQB1*02 (OR=2.2, p_c<0.004) had a significant predisposing effect, whereas the DQB*0602 (OR=0.05, p_c<0.001) and DQB1*0301 (OR=0.3, p_c<0.001) alleles were negatively associated with DM-1A.

The distribution of HLA-DRB1*04 subtypes, determined in both, patients and controls, is given in Table 2. No statistically significant differences were found. Out of the HLA-DRB1*03 alleles, only the DRB1*0301 subtype was observed, however this was the case in the healthy population, too.

Discussion

The known positive association with DRB1*03 and 04, as well as with DQB1*0302 and DQB1*02 in Caucasians was confirmed in the Slovak DM-1A patients. Highest DM-1A risk observed in our study was conferred by the DQB1*0302 allele (OR=7.8).

There seems to be a considerable heterogeneity in the distribution of DRB1*04 subtypes associated with DM-1A among different Caucasian groups (3, 9, 10, 11). Out of the DRB1*04 subtypes studied, the allele DRB1*0401 seems to be more relevant than others (73.1 % in the patients vs. 46.8 % in the controls, OR=3.1), however the difference was on the edge of statistical significance (p_c<0.026). The predisposing or protective effect of other DRB1*04 alleles reported in some other European populations was not confirmed. Our preliminary findings do not support the hierarchic influence of different DRB1* alleles as suggested by Undlien (12). However, for a precise analysis of different DRB1*04 subtypes effects on susceptibility to DM-1, a larger study needs to be done.

The strongest protection against DM-1A in our patients was conferred, in decreasing order by the alleles DQB*0602, DRB1*11, DRB1*15, DQB1*0301.

In summary, the classical DM-1A associated HLA class II alleles were observed in our patient group. Population-based case-control studies like this one serve as the foundation for analytic investigations of DM-1A risk factors. In clinical practice they are helpful in identifying the subjects at risk of developing DM-1A. Our results are currently being used in the prediction of potential DM-1A patients among the first-degree relatives.

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