ASSOCIATIONS BETWEEN HLA CLASS II ALLELES AND TYPE 1 DIABETES MELLITUS IN THE SLOVAK POPULATION

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Objectives. Several associations between HLA complex and diabetes mellitus type 1A were found in various groups of patients of Caucasoid population. This study was therefore prompted to be conducted in Slovak population, since any such has not yet been performed in Slovak population.

Methods. Patients suffering from DM-1A originated from all regions of Slovakia. Their age ranged from 1 to 42 years; but the criterion for including the subject to the study was the definition of diagnosis in older patients before their age of 15 (Table 1). The diagnosis was set up according to internationally accepted criteria. A total of 460 patients was typed for HLA-DQB1 alleles, among them 97 also for HLA-DQA1 and 146 for HLA-DRB1 alleles. HLA-typing was performed by a PCR-SSP method. Control group consisted of 196 (DQA), 143 (DQB1) and 130 (DRB1) unrelated blood donors aged 19-55 years old irrespective of their age or sex. The data obtained were expressed in a 2 x 2 contingency table and statistical significance was calculated by the Fisher exact test.

Results. Among 11 HLA-DQB1 alleles tested DQB1*0302 was the most frequent in DM-1A patients (30.33 % vs. 5.59 % in healthy subjects (HS), followed by DQB1*0201 (22.93 % vs. 12.94 %, respectively). In contrast, the frequency rate of DQB1*0301 (10.66 % vs. 24.48 %), DQB1*0602 (2.17 % vs. 10.14 %) and DQB1*0603 (2.5 % vs. 8,39 %) were decreased in DM-1A patients.

Out of 14 DQA1 alleles the highest occurrence rate showed DQA1*0301 (30.93 % vs. 17.09) and DQA1*0501 (34.02 % vs. 25.76 %), while DQA1*0102 (8.76 % vs. 16.58 %) and DQA1*0201 (6.18 % vs. 13.51 %7), respectively, were found to be the least frequent. Among 13 HLA-DRB1 alleles tested, the most common occurrence rates showed DRB1*03 (26.37 % vs. 9.62 %) and DRB1*04 (7.19 % vs. 14.23 %), while the least frequent alleles were DRB1*15 (2.74% vs. 12.31 %), DRB1*07 (7.19 % vs. 14.23 %), and DRB1*11 (2.74 % vs. 20.38 %).

The alleles DQB1*0302 and DQA1*0301, respectively, were present in the same individual in all DRB1*04 positive patients, suggesting that they belong to the haplotype. Similar situation was observed with the alleles DQB1*0201, DQA1*0501, and DRB*0301, respectively, forming the second HLA haplotype so characteristic for DM1A.

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Type 1 diabetes mellitus (DM-1A) is an autoimmune disease belonging to the most frequent chronic diseases of the childhood and young adults. It is a genetically determined disease as proved by family studies on disease concordance among siblings. A probability to contract the disorder appeared of app. 40 % for monozygotic and 5 % for dizygotic siblings. However, if the latter are HLA-identical, this number increases to 20 %.

Table 1
Characteristic of investigated DM-1A groups of patients

	Groups of investigated patients		
Patients	DQB1	DQA1	DRB1
Total N-	460	97	146
Boys	227	43	78
Girls	233	54	68
Age 1-10 years	76 (38/38)	17 (7/6)	19 (11/8)
Age 11-18 years	214 (105/109)	63 (34/29)	90 (47/43)
Age > 19* years	170 (84/86)	21 (13/8)	37 (20/17)

*the diagnosis of DM1A was established into the age 15

These facts indicate a substantial role of the major histocompatibility complex in man (HLA) in immunopathogenic processes leading to the disease development (Thomas and Powers 1996; Robles and Eisenbarth 2001, Martinka 2004, Buc 2005).

Previous relevant associations between the HLA-complex and DM-1A, which are however still valid and used in a clinical practice for prediction of a disease development, were related to HLA-DR molecules. DM-1A is associated with HLA-DR3 coded for by DRB1*0301 and HLA-DR4, coded for by DRB1*04, respectively. Later more significant associations were described – those to HLA-DQ2 (DQA1*0501, DQB1*0201) and HLA-DQ8 (DQA1*0301, DQB1*0302), respectively (Nepom 2000). These associations were found in various groups of patients of the Caucasoid population, however no such study was conducted in the Slovak population which prompted us to perform it.

Materials and Methods

Patients suffering from DM-1A originated from all regions of Slovakia. Their age ranged from 1 to 42 years; but the criterion for including the subject to the study

Table 2
Occurrence rates of HLA-DQB1 alleles in patients suffering from type 1 diabetes mellitus

DQB1*	DM-1A (%) N- 920	Controls (%) N- 286	P ≤	OR	95% CI
0201	22.93 (211/920)	12.94 (37/286)	0.0002	2.003	1.372-2.923
0202	7.83 (72/920)	8.04 (23/286)			
0301	10.66 (98/920)	24.48 (70/286)	0.0001	0.3679	0.2615-0.5176
0302	30.33 (279/920)	5.59 (16/286)	0.0001	7.345	4.351-12.400
0303	3.26 (30/920)	4.19 (12/286)			
040x	3.37 (31/920)	4.55 (13/286)			
050x	15.98 (147/920)	19.58 (56/286)			
0601	0.54 (5/920)	0.35 (1/286)			
0602	2.17 (20/920)	10.14 (29/286)	0.0001	0.1969	0.1096-0.3540
0603	2.50 (23/920)	8.39 (24/286)	0.0001	0.2799	0.1554-0.5041
0604	0.43 (4/920)	1.75 (5/286)			
	100,0 %	100,00 %			

CI – confidence interval, N – the number of alleles (i.e. the number of investigated persons x 2), OR – odds ratio

Table 3
Occurrence rates of HLA-DQA1 alleles in patients suffering from type 1 diabetes mellitus

DQA1*	DM-1A (%) N-194	Controls (%) N-392	P ≤	OR	95% CI
0101	9.28 (18/194)	13.78 (54/392)			
0102	8.76 (17/194)	16.58 (65/392)	0.0111	0.4832	0.2748-0.8497
0103	3.61 (7/194)	7.65 (30/392)			
0104	0.00 (0/194)	1.78 (7/392)			
0105	0.00 (0/194)	0.26 (1/392)			
0106	0.00 (0/194)	0.26 (1/392)			
0201	6.18 (12/194)	13.51 (53/392)	0.0077	0.4217	0.2197-0.8095
0301	30.93 (60/194)	17.09 (67/392)	0.0002	2.172	1.452 – 3.248
0302	1.03 (2/194)	0.26 (1/392)			
0303	1.03 (2/194)	0.26 (1/392)			
0401	4.12 (8/194)	2.29 (8/392)			
0501	34.02 (66/194)	25.76 (101/392)	0.0413	1.486	1.023-2.158
0503	0.52 (1/194)	0.26 (1/392)			
0601	0.52 (1/194)	0.26 (1/392)			
	100,00 %	100,00 %			

CI – confidence interval; N- – the number of alleles, OR – odds ratio

was the definition of diagnosis in older patients before their age of 15 (Table 1). The diagnosis was set up by internationally accepted criteria (Lambert et al. 2004), i.e. according the clinical symptoms, elevated fasting glucose levels (7.8 mmol . l^{-1}), levels of C-peptide (<0.3 nmol . l^{-1}) and titer of specific antibodies (anti-glutamic acid 65 /GAD65/ > 1 IU . ml^{-1}, anti-insulin (IAA) >1 IU . ml^{-1}, and anti-tyrosinephosphatase (IA2) >1 IU . ml^{-1}), respectively. The informed consent was obtained from patients or their parents and an ethic committee of the 1st Children Clinic Comenius University School of Medicine approved the study. 460 patients were typed for HLA-DQB1 alleles, 97 out of them also for HLA-DQA1 and 146 for HLA-DRB1 alleles, respectively. The HLA-typing was performed by a PCR-SSP method. Primer sets originated from the GenoVision (Olerup SSP™ AB Sweden). A control group represented 196 (DQA), 143 (DQB1) and 130 (DRB1) unrelated persons of the Slovak population, respectively (Čechová et al. 1988). All of them were healthy, 19 to 55 years old and included into our panel of volunteer blood donors irrespective of their age or sex. The data obtained were expressed in a 2 x 2 contingency table and statistical significance was calculated by the Fisher exact test.

Results

DQB1*0302 was found to be the most frequent in the group of investigated patients (30.33 % compared to 5.59 % in the healthy population; p< 0.0001; odds ratio (OR) = 7.345), followed by DQB1*0201 (22.93 % vs. 12.94 %; p=0.0002; OR=2.003) among 11 HLA-DQB1 alleles tested. On the contrary, the occurrence rate of DQB1*0301 (10.66 vs. 24.48 %; p<0.0001, OR=0.3679), DQB1*0602 (2.17 % vs. 10.14 %; p<0.0001, OR=0.1969), and DQB1*0603 (2.5 % vs. 8,39 %; p<0.0001, OR=0.2799) were decreased (Table 2).

Out of 14 DQA1 alleles tested, DQA1*0301 showed the highest occurrence rate (30.93 % compared to 17.09 % in the healthy population; p<0.0002; OR=2.172) followed by DQA1*0501 (34.02 % compared to 25.76 % in the healthy population; p<0.0413; OR=1.486). On the opposite site, the occurrence rate of DQA1*0102 (8.76 % vs. 16.58 %, p=0.0111, OR=0.4832) and DQA1*0201 (6.18 % vs. 13.51 %; p=0.0077, OR=0.4217), respectively, were found to be the least frequent (Table 3).

Among 13 HLA-DRB1 alleles tested, the most common occurrence rates showed DRB1*03 (26.37 % vs. 9.62 %; p<0.0001, OR=3.367) and DRB1*04 (34.93 % vs. 11.16 %; p<0.0001, OR=4.276), respectively. The least frequent alleles were DRB1*15 (2.74 % vs. 12.31 %; p<0.0001, OR=0.2007), DRB1*07 (7.19 % vs. 14.23 %; p=0.0081, OR=0.4670), and DRB1*11 (2.74 % vs 20.38 %; p<0.0001, OR=0.1698), respectively (Table 4).

The alleles DQB1*0302 and DQA1*0301, respectively, were present in the same individual in all DRB1*04 positive patients, suggesting that they belong to the haplotype HLA-DQA1*0301-DQB1*0302-DRB1*04. The same situation was observed with the alleles DQB1*0201, DQA1*0501, and DRB*0301, respectively, forming the haplotype HLA- DQA1*0501-DQB1*0201-DRB1*0301, and with the alleles DRB1*1501, DQA1*0102, and DQB1*0602 forming the haplotype HLA-DQA1*0102-DQB1*0602-DRB1*1501, respectively.

Discussion

Type 1 diabetes mellitus is an autoimmune disease characterised by a destruction of beta-cells of the Langerhans islets. The autoimmune process involves both the cellular and humoral arms of the immune system; however, cell mediated processes prevail. An initiating event remains still unknown (Thomas and Powers 1996; Chowdhury et al. 1999; Notkins et al. 2004; Buc 2005).

Autoimmune diabetes mellitus is clearly a genetic disease. Susceptibility is inherited in a polygenic fashion, 18 IDDM–genes were shown to be involved in its genetic determination until now (Robles and Eisenbarth 2001). Among the genes involved, those of the major histocompatibility complex in man, HLA (IDDM1) play a paramount importance. The first associations between the HLA-complex and DM-1A were related to the HLA-B8 and HLA-B15 molecules; these findings were overcome by more significant, to those of the HLA-DR locus: DM-1A is associated with HLA-DR3 coded for by the allele DRB1*03 and HLA-DR4, determined by DRB1*04, respectively. Later even more significant associations were disclosed – those to HLA-DQ2 (DQA1*0501, DQB1*0201) and HLA-DQ8 (DQA1*0301, DQB1*0302), respectively (Busova et al. 1995; Thomson 1984; Lee et al. 2001; Novota et al. 2004).

Slovak patients suffering from DM-1A were typed for class II HLA alleles. Positive associations to alleles HLA-DQB1*0302, DQB1*0201, DQA1*0301, DQA1*0501, DRB1*03, DRB1*04 and negative to DQB1*0602, DQA1*0102, DQA1*0201[1], and DRB1*07, DRB1*15, respectively were found. Both, positively and negatively associated alleles were found to be an inherent part of the HLA-haplotypes characteristically associated with DM-1A: DQA1*0301-DQB1*0302-DRB1*04, DQA1*0501-DQB1*0201-DRB1*03, DQA1*0102-DQB1*0602-DRB1*15, respectively.

Our findings corroborate similar reports in the Czech (Cinek et al. 2001, Cerna et al. 2003), the Polish (Kubryn et al. 2003), and other Caucasoid (Nepom 2000) DM1A-populations, respectively. However, any findings of negative associations to DQA1*0301, DQA1*0603, and DRB*11, respectively, in Caucasoid were not yet reported by other investigators. As our panel of investigated patients was relatively large enough to obtain relevant results, the observation can either mean that these associations are specific for the Slovak population or that the groups of investigated patients in other studies were not sufficient. Further studies are needed to clarify the matter.

[1] The alleles DQA1*0102 and DQB1*0602, respectively, code for the molecule DQ6.

Table 4
Occurrence rates of HLA-DRB1 alleles in patients suffering from type I diabetes mellitus

DRB1*	DM-1A (%) N- 292	Controls (%) N- 260	P ≤	OR	95% CI
01	6.5 (19/292)	7.31 (19/260)			
03	26.37 (77/292)	9.62 (25/260)	0.0001	3.367	2.067-5.483
04	34.93 (102/292)	11.16 (29/260)	0.0001	4.276	2.713-6.740
07	7.19 (21/292)	14.23 (37/260)	0.0081	0.4670	0.2657-0.8211
08	8.56 (25/292)	4.62 (12/260)			
09	1.03 (3/292)	0.38 (1/260)			
10	0.00 (0/292)	2.31 (6/260)			
11	2.74 (/292)	20.38 (53/260)	0.0001	0.1698	0.07865-0.3666
12	0.34 (8/292)	1.53 (4/260)			
13	4.45 (13/292)	8.07 (21/260)			
14	0.68 (2/292)	2.69 (7/260)			
15	2.74 (8/292)	12.31 (32/260)	0.0001	0.2007	0.0907-0.4441
16	4.46 (13/292)	5.38 (14/260)			
	100,00 %	100,00 %			

CI – confidence interval, N – the number of alleles, OR – odds ratio

Our study corroborates also previous reports on an association between type 1 diabetes mellitus and HLA class II alleles in the Caucasoid population and confirms a role of the HLA complex in a disease predisposition. As HLA genes are not the only ones involved, future studies should be focused to disclose them and elucidate their relationships.

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