CLINICAL STUDY

Association of HLA-DPB1 alleles with type I diabetes mellitus in Slovak population

Stuchlikova M, Kantarova D, Michalkova D, Barak L, Buc M

Department of Immunology, Faculty of Medicine, Comenius University, Bratislava, Slovakia. **martina.stuchlikova@fmed.uniba.sk**

Abstract

Background: **Genes of HLA complex on chromosome 6p21 principally contribute to the genetic risk of insulin-dependent diabetes mellitus type I (T1 DM). Associations of HLA class II loci allelic variants with T1 DM are well established. Another prime candidate, particularly the polymorphic DPB1 gene, has been reported as probably contributing to the disorder, but its relative contribution to the predisposition to the disease is difficult to assess due to strong linkage disequilibrium of HLA alleles. DPB1*0301 and DPB1*0202 have been reported as positively and DPB1*0402 as negatively associated alleles in different Caucasoid populations (predisposing versus protective alleles, respectively).**
Objectives: **The aim of this study was to establish the occurrence rates of HLA-DPB1 alleles in patients suffering from T1 DM and to compare them with those in healthy subjects.**
Methods: **A PCR-SSP method was performed to identify HLA-DPB1 alleles in 61 patients and 160 healthy controls. The exact Fisher's test was used to determine the statistical significance of allele frequency differences between patients and control subjects.**
Results: **The analysis of obtained results has shown a significantly decreased frequency of DPB1*0402 and slightly increased occurrence rates of DPB1*0101 and DPB1*1301, respectively in the investigated group of patients. Neither DPB1*0301 nor DPB1*0202 were observed to be over-represented.**
Conclusions: **The expected significant decrease in the frequency of DPB1*0402 was confirmed, whereas positive associations with DPB1*0301 and DPB1*0202, did not prove to be true, respectively *(Tab. 1, Ref. 19)*.**
Key words: autoimmune diabetes mellitus, HLA-DPB1.

Type I diabetes mellitus (T1 DM) is an immune-mediated disease caused by T-cell destruction of ß-cells of Langerhan's islets in the pancreas. Both genetic and environmental factors influence the susceptibility to this disease. Currently, over 20 regions in the human genome were identified to be associated with the disorder. The major histocompatibility complex (HLA) on chromosome 6p21 is estimated to contribute in over 50 % to the total genetic risk (1, 2, 3, 5). HLA class II region DRB1, DQB1 and DQA1 genes, respectively, play the major role in determining the genetic predisposition to the disease. However, the available evidence suggests that in addition to DRB1 and DQ genes, HLA polymorphism contributes to the susceptibility to diabetes. As the structure and function of DP molecules are similar to those of DR and DQ, the polymorphic DPB1 gene encoding the ß-chain of DP-molecules comes into account as the subsequent susceptibility candidate.

Associations of DPB1 alleles with T1 DM have already been reported. Positive associations with DPB1*0301 and DPB1*0202, respectively (6, 7) as well as the negative association with DPB1*0402 have been described (6, 7, 8). In our previous studies, we concentrated on the investigation of associations of T1 DM to HLA-DR and -DQ molecules and alleles, respectively (4, 9). The aim of the presented study was to enlarge our association

Department of Immunology, Faculty of Medicine, Comenius University, Bratislava, Department of Internal Medicine, Jessenius Faculty of Medicine, Comenius University, Martin, Department of Internal Medicine, 1st Children Hospital, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Address for correspondence: M. Stuchlikova, Mgr, Dept of Immunology, Faculty of Medicine, Comenius University, Spitalska 24, SK-813 72 Bratislava 1, Slovakia.
Phone: +421.2.59357582

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Tab. 1. Occurrence rates of HLA-DPB1 alleles in patients suffering from type I diabetes mellitus.

	T1 DM N=122		Controls N=320				
DPB1*	n	%	n	%	p	OR	CI
0101	8	6.56	8	2.500	0.0851	2.6230	0.9628-7.145
0201	17	13.93	42	13.13	0.8773	1.0620	0.5821-1.936
0202	0	0	1	0.31	1.0000	0.8721	0.0353-21.570
0301	15	12.29	27	8.44	0.2837	1.4570	0.7495-2.833
0302	1	0.82	0	0	0.2777	7.8490	0.3173-194.13
0401	44	36.07	127	39.69	0.6858	0.9087	0.6083-1.358
0402	10	8.2	61	19.06	0.0185	0.4300	0.2134-0.8664
0501	2	1.64	3	0.94	0.6204	1.7490	0.2885-10.597
0601	1	0.82	1	0.31	0.4778	2.6230	0.1627-42.293
0801	1	0.82	0	0	0.2777	7.8490	0.3173-194.13
0901	1	0.82	2	0.63	1.0000	1.3110	0.1178-14.603
1001	0	0	4	1.25	0.5787	0.2907	0.0155-5.443
1101	2	1.64	1	0.31	0.1891	5.2460	0.4711-58.413
1301	4	3.28	2	0.63	0.0553	5.2460	0.9483-29.021
1401	1	0.82	3	0.94	1.0000	0.8743	0.0900-8.491
1501	0	0	1	0.31	1.0000	0.8721	0.0353-21.570
1601	4	3.28	6	1.88	0.4756	1.7490	0.4850-6.305
1701	1	0.82	5	1.56	1.0000	0.5246	0.0606-4.538
Others	10	8.20	26	8.13	–	–	–

Statistically significant p values are shown in bold font, p-values that may be suggestive of weak association (p>0.05 but <0.10) are shown in italics, C – confidence interval, N – total number of alleles (i.e. the number of investigated persons x 2), n – the number of individual alleles, OR – odds ratio

studies to HLA-DPB1 locus alleles and to compare the obtained results with those reported in literature.

Materials and methods

61 unrelated Slovak diabetic patients were recruited from different regions of Slovakia. Their age ranged from 1 to 42 years; the diagnosis in older patients was established no later than at the age of 15. The diagnosis was set up by internationally accepted criteria (10), i.e. according to the clinical symptoms, elevated fasting glucose levels (7.8 mmol/l), levels of C-peptide (<0.3 nmol/l) and to the titer of specific antibodies (anti-glutamic acid 65/GAD65/ >1 IU/ml, anti-insulin/IAA/>1 IU/ml, and anti-tyrosinephosphatase/IA2/>1 IU/ml), respectively. The informed consent has been obtained from patients or their parents and the Ethic Committee of the 1st Children Clinic of the Faculty of Medicine, Comenius University, has approved the study. The control group was formed of 161 unrelated persons of Slovak population (11). All of them were healthy, 19 to 55 years old and included in our panel of volunteer blood donors irrespective of their age or sex.

Peripheral blood samples were collected into tubes containing EDTA serving as anticoagulant solution. The salting-out method and QIAamp spin columns (QIAGEN GmbH, Hilden, Germany) were used to prepare genomic DNA and speed up its purification. PCR-SSP typings of the diabetic group were performed using commercial Olerup SSP AB kits supplied by GenoVision (Olerup SSPTM AB Sweden) according to the Olerup and Zetterquist method (12). DPB1 alleles in healthy subjects were identified by the PCR-RFLP method according to Hviid et al (13) and Olerup SSP AB kits (11). The allele frequencies were determined by direct calculation; the exact Fisher's test was performed to determine the statistical significance of occurrence rates differences between patients and control subjects.

Results

HLA-DPB1-alleles were determined in 61 patients; 26 alleles were typed. Some of them were shown to have different occurrence rates when compared to healthy population. **DPB1*0402** occurred with a significantly decreased frequency of 8.2 % versus 19.065, p=0.0185. The allele was present in patients who had simultaneously possessed DRB1*0401 (70 %) or DRB1*0301 (10 %), respectively; whereas in others (20 %), the allele was associated with different DRB1-alleles. **DPB1*0101** and **DPB*1301,** respectively, were found to have increased occurrence rates; the findings, however, did not reach the statistical significance (DPB1*0101: 6.56 % to 2.5 %, p=0.0851; DPB*1301: 3.28 % to 0.63 %, p=0.0553). The DPB1*0101 allele was present exclusively in patients with DRB1*03-allele. No other deviations in allele frequencies rates were observed (Tab. 1).

Discussion

Autoimmune diabetes mellitus is clearly a genetic disease. Among the genes involved, those of the major histocompatibility complex (HLA) play a paramount importance. The well-known associations between the HLA-complex and T1 DM are related

to the molecules/alleles of HLA-DR and HLA-DQ loci; less attention was, however, paid to HLA-DP associations (4, 14).

Previously we investigated the associations of T1 DM with HLA-DR and HLA-DQ molecules/alleles, respectively (4, 9). The aim of our present study was to extend this study by that of the HLA-DP relationship. The results show that occurrence rates in some of the alleles in the investigated group of patients are deviated when compared to controls: weak positive associations were found with DPB1*0101 and DPB*1301, whereas negative association was found with DPB1*0402. **DPB1*0402** was found significantly underrepresented, the fact of which is in agreement with similar studies reported by Noble et al (6) and Cucca et al (7). The allele was present in patients, the genomes of whom have simultaneously either DRB1*04 (70 %) or DRB1*03 (10 %). As both belong to the predisposing DRB1 alleles, this observation suggests that DPB*0402 may somehow mitigate their susceptible influence.

The frequency of DPB1*0101 was found to be slightly increased; however, it did not reach the statistical significance. Noble et al reported similar results (6). DPB1*0101 was present only in patients, whose DRB1*03 allele was strongly associated with T1 DM, suggesting that both are in linkage disequilibrium; the observed increase in the frequency of DPB1*0101 in our study may therefore simply reflect this biological phenomenon. **DPB1*1301** was found to be increased in the population of patients too, however this finding as well was not statistically significant. Similar results were observed in the ethnically mixed population in Venezuela (15).

DPB1*0301 and **DPB1*0202** were reported to be positively associated with T1 DM (2, 6–8, 15–19). However, neither DPB1*0301 nor DPB1*0202 showed a significant increase in our present study; moreover DPB1*0202 was present in none of the studied patients. These results may have been due to a relatively small number of investigated patients or might be specific for the Slovak population – further studies are needed to clarify the matter.

Our study extends our previous studies and corroborates some reports on the association of type 1 diabetes mellitus and HLA-DP alleles in the Caucasoid population. It also confirms the role of HLA complex in patients predisposed to this disease. As HLA genes are not the only ones involved, future studies should concentrate on their disclosure and elucidation of their relationships

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