CLINICAL STUDY

Analysis of extended human leukocyte antigen haplotype association with Addison’s disease in three populations

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Abstract

Objective: Addison’s disease is an organ-specific autoimmune disorder with a polygenic background. The aim of the study was to identify non-class II human leukocyte antigen (HLA) susceptibility genes for Addison’s disease.

Design and methods: Addison’s disease patients from three European populations were analysed for selected HLA–DR–DQ alleles and for 11 microsatellite markers covering ~4 Mb over the HLA region. Subjects were 69 patients with Addison’s disease from Estonia (24), Finland (14) and Russia (31). Consecutively recruited healthy newborns from the same geographical regions were used as controls (269 Estonian, 1000 Finnish and 413 Russian). Association measures for HLA–DRB1, DQB1, DQA1 and 11 microsatellites between D6S273 and D6S2223 were taken. A low-resolution full-house typing was used for HLA class II genes, while microsatellite markers were studied using fluorescence-based DNA fragment sizing technology.

Results: We confirmed that the HLA–DR3–DQ2 and the DQB1*0302–DRB1*0404 haplotypes confer disease susceptibility. In Russian patients, we also found an increase of DRB1*0403 allele, combined with DQB1*0305 allele in three out of six cases (P < 0.0001). Analysis of 11 microsatellite markers including STR MICA confirmed the strong linkage in DR3–DQ2 haplotypes but DRB1*0404–DQB1*0302 haplotypes were diverse. MICA5.1 allele was found in 22 out of 24 Estonian patients, but results from Finnish and Russian patients did not support its independent role in disease susceptibility.

Conclusion: HLA–DRB1*0403 was identified as a novel susceptibility allele for Addison’s disease. Additionally, we found no evidence of a non-class II HLA disease susceptibility locus; however, the HLA–DR3–DQ2 haplotype appeared more conserved in patient groups with high DR–DQ2 frequencies.

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Introduction

Addison’s disease is an organ-specific autoimmune disease characterized by the damage of adrenal cortex and insufficiency of corticosteroid production (1). Steroidogenic P450 cytochrome enzymes have been identified as target antigens of the autoimmune response (2, 3) and circulating antibodies against 21-hydroxylase are a sensitive diagnostic marker of the disease with a tendency to decrease in percentage when adrenal gland destruction progresses (4). The disease is often found as a part of polyendocrinopathy syndromes combining it with other organ-specific autoimmune diseases, thyroid disease and type 1 diabetes (T1D) being the most frequent associates (5).

Human leukocyte antigen (HLA) association of the disease with the common ‘autoimmune’ HLA–DR3 haplotype has been known for decades (6) and some studies have also found an increased frequency of HLA–DR4 among patients (7, 8). An association with specific HLA–DR4-positive haplotype, HLA–DQB1*0302–DRB1*0404, was described among Addison’s disease patients and diabetic children positive for 21-hydroxylase autoantibodies (9). More recently, analysis of microsatellites as well as some candidate genes within class I region in Addison’s disease (10) and other autoimmune diseases (11) has given a basis to suggest that there might be other susceptibility genes outside of DR–DQ region. Among the candidates for association with Addison’s disease is the major histocompatibility complex class I polypeptide-related sequence A (STR MICA), of which allele MICA5.1 was reported to be strongly associated with Addison’s disease (12) or progression to Addison’s disease among diabetic persons with antibodies against 21-hydroxylase (13).
To further explore the HLA-associated susceptibility, we analysed Addison’s disease patients from three European populations for selected HLA–DRB1 and HLA–DQB1 alleles and for 11 microsatellite markers covering ~4 Mb over the HLA region.

Materials and methods

Sixty-nine Addison’s disease patients, including 24 from Estonia, 14 from Finland and 31 from Russia, were studied. Most of the patients were adults (66 out of 69). None of these patients had type 1 polyendocrinopathy syndrome but type 2 autoimmune polyendocrinopathy syndrome was present in 20 out of 69 patients (29.0%). The 21-hydroxylase antibodies were measured by radiobinding assay as described elsewhere (14). Altogether, 70% of studied patients from Estonia and Finland were antibody positive, including seven out of eight patients with type 2 autoimmune polyendocrinopathy syndrome. We have further clinical information only from Finnish and Estonian patients. Autoimmune polyglandular syndromes were present in six Finnish and two Estonian patients. No reasonable further analyses can be based on this incomplete information. As a control we used background population frequencies of the class II haplotypes established in a previous study of the same populations (15).

For genotyping of the HLA class II genes HLA–DRB1, HLA–DQA1 and HLA–DQB1, we used a panel of oligonucleotide probes designed for low-resolution full-house typing, including a more detailed genotyping of the HLA–DR4 subtypes (16). Microsatellite markers (D6S273, TNFa, C12A, STR MICA, MB, C125, C143, C245, C3211, MOCc and D6S2223) were studied using PCR primers labelled with fluorescent dyes (17) and C245, C3211, MOGc and D6S2223) were studied using (D6S273, TNFa, C12A, STR MICA instructions (MegaBace 1000). Commonly referred allele names were used for the TNFa and STR MICA microsatellites. For the other microsatellite loci, allele numbers corresponded to the length of the PCR product (estimated by sequencing of homozygous samples).

Results

Table 1 shows haplotype frequencies among patients and controls in each population. The HLA–DR3 haplotype was associated with Addison’s disease in all analysed populations but there was a considerable variation in the strength of this association. Eleven out of 14 (78.6%) of Finnish patients were positive for this haplotype when compared with 12 out of 24 (50.0%) Estonian and only 11 out of 31 (35.5%) Russian patients (P = 0.03; df = 2). In addition to the increased frequency of HLA–DR3 haplotype, we also found a significant increase in DQB1*0302–DRB1*0404 haplotype among Estonian and Russian patients (P = 0.0056 and 0.0051 respectively). In the Finnish population, this haplotype was also more common among patients than controls, although the difference did not quite reach statistical significance.

Among Russian patients, we also found that the DRB1*0403 allele was significantly more common than among controls (6 out of 31 and 2 out of 413 respectively, P < 0.0001). It was present in six patients, in three cases conventionally with DQB1*0302 and in three cases combined with the DQB1*0305 allele. Results of microsatellite analysis are shown in Fig. 1 together with individual HLA–DQB1 and HLA–DQA1 genotypes. Microsatellite markers present on both chromosomes are shown. Markers are ordered from centromere to telomere and sorted to demonstrate the DQB1*0302–DRB1*0404 (DR3–DR2) haplotype and microsatellite markers characterizing it. The presentation also offers a way to analyse the possible contribution to disease risk by the other haplotype. The allele associated with the extended haplotype in each locus is marked using shading. We see the known strong conservation of 8.1 haplotype in the class III region but not in the telomeric class I region, which is most pronounced in Finnish samples. All 11 microsatellites share D6S273 and TNFa alleles, two differ at

Table 1 Distribution of Addison’s disease-associated haplotypes of the HLA class II genes in Estonians, Finns and Russians.

<table>
<thead>
<tr>
<th>Population</th>
<th>Haplotype</th>
<th>Patients N (%)</th>
<th>Controls N (%)</th>
<th>OR</th>
<th>P</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finns</td>
<td>DR3, DQB1<em>02–DQA1</em>05</td>
<td>11 (78.6)</td>
<td>202 (20.2)</td>
<td>14.5</td>
<td>&lt;0.0001</td>
<td>3.7–41.4</td>
</tr>
<tr>
<td></td>
<td>DR4, DQB1<em>0302–DRB1</em>0401</td>
<td>2 (14.3)</td>
<td>124 (12.4)</td>
<td>–</td>
<td>NS</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>DR4, DQB1<em>0302–DRB1</em>0404</td>
<td>3 (20.0)</td>
<td>62 (6.2)</td>
<td>4.1</td>
<td>0.078</td>
<td>0.9–16.5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>14</td>
<td>1000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estonians</td>
<td>DR3, DQB1<em>02–DQA1</em>05</td>
<td>12 (60.0)</td>
<td>60 (22.3)</td>
<td>3.5</td>
<td>0.0056</td>
<td>1.4–8.8</td>
</tr>
<tr>
<td></td>
<td>DR4, DQB1<em>0302–DRB1</em>0401</td>
<td>0 (0)</td>
<td>12 (4.5)</td>
<td>–</td>
<td>NS</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>DR4, DQB1<em>0302–DRB1</em>0404</td>
<td>7 (29.2)</td>
<td>25 (9.2)</td>
<td>4.0</td>
<td>0.0058</td>
<td>1.4–11.6</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>24</td>
<td>269</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Russians</td>
<td>DR3, DQB1<em>02–DQA1</em>05</td>
<td>11 (35.5)</td>
<td>65 (15.7)</td>
<td>2.9</td>
<td>0.0102</td>
<td>1.3–6.8</td>
</tr>
<tr>
<td></td>
<td>DR4, DQB1<em>0302–DRB1</em>0401</td>
<td>3 (9.7)</td>
<td>31 (7.5)</td>
<td>–</td>
<td>NS</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>DR4, DQB1<em>0302–DRB1</em>0404</td>
<td>7 (22.6)</td>
<td>28 (6.8)</td>
<td>4.0</td>
<td>0.0051</td>
<td>1.4–10.9</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>31</td>
<td>413</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P values were calculated by χ²-test with continuity correction.
### Table 1

<table>
<thead>
<tr>
<th>DQA1</th>
<th>DQB1</th>
<th>D65273</th>
<th>TNFα</th>
<th>C12A</th>
<th>STR MICA</th>
<th>MIB</th>
<th>C12S</th>
<th>C143</th>
<th>C245</th>
<th>C0311</th>
<th>MOD3c</th>
<th>O652229</th>
</tr>
</thead>
</table>
| Finns  
03, 05 | 02, 0302 137 141 2 11 242 256 5.1 5.1 336 350 194 198 417 449 436 436 195 199 130 148 170 170  
03, 05 | 02, 0302 135 141 2 6 236 256 5 4.1 336 346 194 208 441 449 436 468 195 209 132 132 168 168  
03, 05 | 02, 0302 137 141 2 5 256 256 5.1 5.1 336 350 194 198 441 449 436 436 204 207 132 145 170 170  
03, 02 | 02, 04 135 141 2 6 242 256 5.1 5.1 332 350 194 194 449 449 436 436 209 217 122 148 170 170  
03, 02 | 03, 0202 137 141 2 6 250 256 5.1 5.1 350 350 194 194 445 445 458 468 195 195 132 146 170 170  
03, 02 | 03, 0202 141 141 2 11 242 256 5.1 5.1 336 350 194 198 449 449 436 436 207 217 132 148 170 170  
05, 05 | 02, 04 135 141 2 6 242 256 5.1 5.1 332 350 194 194 449 449 436 436 209 217 122 148 170 170  

Estonians  
05, 05 | 02, 04 135 141 2 13 238 256 5.1 5.1 336 350 194 204 433 449 432 432 195 201 136 148 170 170  
05, 05 | 03, 0202 137 141 2 11 242 256 5.1 5.1 336 350 194 198 417 449 436 436 195 199 130 148 170 170  
03, 05 | 03, 0202 137 141 2 6 250 256 5.1 5.1 336 350 194 198 417 449 436 436 195 199 130 148 170 170  
03, 05 | 03, 0202 135 141 2 10 254 256 5.1 9 332 350 194 208 421 449 436 436 211 215 122 148 170 170  
03, 05 | 03, 0202 137 141 2 6 250 256 5.1 5.1 336 350 194 200 425 449 436 436 195 195 130 134 170 170  
03, 05 | 03, 0202 135 139 2 6 250 256 5.1 5.1 350 350 194 194 425 449 436 436 195 195 132 134 170 170  

Russians  
03, 05 | 02, 0101 137 141 2 11 242 256 5.1 5.1 336 350 194 198 417 449 436 436 195 199 130 148 170 170  
03, 05 | 02, 0101 137 141 2 11 242 256 5.1 5.1 336 350 194 198 417 449 436 436 195 199 130 148 170 170  
03, 05 | 02, 0101 131 135 2 10 254 256 4 8 350 350 201 208 441 449 436 436 209 217 132 148 170 170  
03, 03, 0501 131 135 1 5 236 256 5 9 350 350 190 218 429 441 432 432 205 211 130 130 170 170  
03, 04, 0402 135 137 11 13 238 242 5.1 5.1 336 350 194 194 423 445 435 435 207 215 130 132 170 170  

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**Figure 1** Extended HLA haplotypes in Finnish, Estonian and Russian patients with Addison's disease. Conserved haplotype structures are marked in grey.

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C12A and one more at STR MICA. MIB allele 350 is found again in 13 out of 14 cases but only three haplotypes seem to extend to C3211. A similar type of pattern is seen in the Estonian patients, conserved haplotypes extend even further to C3211 where 7 of the original 11 DR3 haplotypes may continue and 4 may be extended up to MOC marker. In the Russian haplotypes, there is slightly more heterogeneity in loci adjacent to HLA–DQ, at D6S273 and TNFa locus, where 8 out of 11 patients share the common allele 2.

Of the markers found on the haplotypes other than the DR3 haplotype, MICA5.1 is conspicuous especially on Estonian samples, less so in Finnish and Russians. In the analysis of 166 haplotypes non-transmitted in Finnish families to a diabetic child and therefore representing a background Finnish population, MICA5.1 allele was the most common allele present on 51.2% of all haplotypes (unpublished results). These haplotypes represent the Finnish background population and the results demonstrate that the high frequency of MICA5.1 allele is to be expected. Similarly, the most telomeric D6S2223 marker is hardly informative due to the high frequency of 170 bp allele.

Similar analysis of DQB1*0302–DRB1*0404 haplotypes revealed very poor linkage disequilibrium in them. TNFa11 was the most common allele at that locus but it was found (together with linked D6S273 allele) only in one of the three Finnish, two of the seven Estonian and four of the seven Russian haplotypes. Absence of TID-associated DQB1*0302–DRB1*0404–B1*39 haplotype (18) was also confirmed by the lack of HLA–B*39 allele except in one Finnish patient.

No association between any of studied haplotypes or microsatellite markers and antibodies against 21-hydroxylase or type 2 autoimmune polyendocrinopathy syndrome was revealed among patients.

**Discussion**

Our results confirm the association of both DQB1*02–DQA1*05 (DR3–DQ2) and DQB1*0302–(DQA1*03)–DRB1*0404 haplotypes with Addison’s disease but there were remarkable differences between the populations. DR3–DQ2 association was very strong in the Finnish population, whereas the effect of the DRB1*0404 association was in fact stronger among Estonians and Russians when estimated by odds ratio.

These differences could not only be related to patient selection, but might also reflect genetic differences between two more mixed populations and the ‘outlier’ Finnish population characterized by genetic isolation and strong founder effects. Susceptibility gene alleles associated with Addison’s disease might therefore also be more diverse in Estonians and Russians than among Finns. The difference in the frequency of the DR3 haplotype between Finnish and Russian patients cannot be explained by the frequency in the background populations as these are similar.

Haplotype analysis using microsatellite markers did not provide statistical support to the importance of HLA regions other than HLA–DR–DQ loci. However, we found that the HLA–DR3–DQ2 haplotype appears more conserved in Finns and Estonians, than among Russians. MICA5.1 allele was very common among patients. In the Finnish cases, it was found in 11 of 14 cases but this is the same percentage as was positive for DQB1*02–DQA1*05 combination and the allele is in strong linkage with these DQ alleles and also found in more than 50% of random selection of haplotypes. Results of Estonian patients might be interpreted to support the role of MICA5.1 allele, as this was found in all but two cases. However, among Russians it was again found in about half of the DR3-negative ones and the linkage of it with DR–DQ was not particularly strong in DR3 haplotypes.

The diversity of DRB1*0404–DQB1*0302-positive haplotypes and the fact that DRB1*0401–DQB1*0302 haplotype does not show any association with the disease emphasizes the role of the DRB1*0404 molecule itself. In this respect, it is very interesting that DRB1*0403 was found increased among Russian patients. It was not found among Estonians and Finns but the frequency of DRB1*0403 is very low among these populations and only DRB1*0401 and DRB1*0404 are the common DR4 alleles found in DR4–DQ8 haplotypes. This is also the case in most populations where DRB1*0404 association of Addison’s disease has been described (8, 9). Because families were not available, the origin of each allele cannot be deduced by certainty, but exclusion of the presence of markers in the haplotype can be done.

More data should be collected in populations where DRB1*0403 and/or the structurally similar allele DRB1*0406 as well as other DRB1 alleles *0402 and *0405 are common to test which of the DR4 subtypes in fact show a disease association. The identification of three DQB1*0305 alleles combined with DRB1*0403 was also intriguing. This haplotype has been mainly reported in individuals from Sardinia but there is probably a reporting bias as it is not necessarily detected by traditional probe panels (19).

In conclusion, for the first time, we found evidence that the HLA–DRB1*0403 allele confers susceptibility to Addison’s disease. Additionally, we propose that on the DRB1*0404–DQB1*0302 haplotype the DRB1*0404 allele is more important in conferring disease susceptibility than the DQB1 allele. We found no evidence of a non-class II HLA disease susceptibility locus for Addison’s disease; however, the HLA–DR3–DQ2 haplotype appeared more conserved in patient groups with high DR–DQ2 frequencies.

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References


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