Distribution of Alleles at DQCAR Microsatellite Locus in the Croatian Population

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Aim. To investigate the polymorphism of DQCAR alleles and their association with HLA-DRB1, -DQA1, -DQB1 haplotype associations in the Croatian population.

Methods. Blood samples were collected from 135 healthy unrelated donors from Zagreb area previously typed for HLA class II alleles (DRB1, DQA1, DQB1). The DQCAR samples were run on a standard denaturing sequencing gel in a DNA sequencer and the sequences were analyzed and compared.

Results. Among 10 different DQCAR alleles found in the population of Croatia, the most frequent were DQCAR 103 bp (41.5%), 121 bp (13.7%), 111 bp (11.9%), and 99 bp (10.7%). DQCAR alleles 101 bp, 115 bp, 123 bp, and 125 bp were not observed. Comparison of DQCAR allele frequencies between Croatsians and other populations did not reveal any significant difference. The study proved a little diversity in DQ1 haplotype associations. Among 141 examined DQ1 associations, 120 were DQCAR 103 bp, whereas the remaining 21 were DQCAR 107 bp. The DRB1*07 haplotype showed the highest diversity of DQCAR alleles (111 bp, 113 bp, 117 bp, 119 bp, and 121 bp). Three unusual haplotype combinations were found: HLA-DRB1*0401, -DQA1*0301, -DQB1*0302, -DQCAR119bp; HLA-DRB1*0408, -DQA1*0301, -DQB1*0304, -DQCAR117bp; and HLA-DRB1*0701, -DQA1*0201, -DQB1*02, -DQCAR 105bp.

Conclusion. Specific DQCAR alleles observed in association with common Caucasoid haplotypes are also found in the Croatian population, but in new and unusual associations. These associations have not been reported in other populations, which suggests that they might be characteristic for Croatsians.

Key words: Croatia; evolution; haplotypes; HLA-DQ antigens; linkage disequilibrium; microsatellite markers; polymorphism

Microsatellite loci are relatively short (<100 bp) repeats of 1-6 bp DNA fragments (1). So far, there are over 100 known microsatellite loci within the HLA region and their number is still increasing (2).

A high level of polymorphism and easy way of typing microsatellite loci make these loci an ideal marker for linkage analysis and anthropological studies (3). One of the well characterized microsatellite loci is the DQCAR locus, located 1-1.5 kb centromeric to HLA-DQB1 (4). This microsatellite locus is an object of great interest because of its location in the Human Leucocyte Antigen (HLA) class II region. It is well known that a large number of autoimmune diseases is associated with HLA-DR and -DQ genes (5,6). Because of a high degree of linkage disequilibrium between DR and DQ alleles, it is not always possible to establish whether the primary association is to DR or DQ alleles, or other genes in this region influence it (7). Linkage disequilibrium is a value that refers to the presence of two alleles at different loci occurring together in the same haplotype or haplotype association more frequently than it would be expected to happen by chance. Haplotype associations are combinations of alleles at neighboring loci which are inherited together on the same chromosome. For that reason, it is recommended to use additional polymorphic markers within DR-DQ region and to perform a refined analysis of association between HLA and diseases (8). Data about DQCAR polymorphism have indicated a good correlation between DQB1 and DQCAR allelic motifs, which on the other hand depends on the absence of an-
ces tral cross overs between these two loci (9). More than 10 alleles have been detected on this microsatellite locus and all of them are strongly associated with the spe cific DQB1 al leles (10-12).

The aim of the present study was to investigate the poly morphism of DQCAR alleles, their relation to HLA class II genes, and their associations with common and rare DR-DQ haplotypic associations in the Croatian population. The DQCAR polymorphism can be used as an additional marker in immunogenetic studies of diseases as well as in transplantation studies. Haplotypic associations are useful in the discrimination of populations and these data represented a reference point for further research on possible roles of associations in the pathology of diseases. This study is a continuation of our previous work on HLA class II polymorphism in the Croatian population (13-15).

Materials and Methods

Blood samples were collected from 135 healthy, unrelated donors from the wider area of the Cro atian capital city, Zagreb. Genomic DNA was isolated from peripheral blood leu kocytes by a standard salt ing-out method (16).

HLA-DRB1, -DQA1, and -DQB1 alleles were determined in all samples using a high-resolution poly merase chain reaction (PCR) with sequence-specific oligo probes (PCR-SSOP) method, as described by Kimura et al (17). PCR amplification was carried out in an ap pro pri ate buffer using 1 µg of genomic DNA in the presence of Taq polymerase, a pair of specific primers for the second exons of DRB1, DQA1, or DQB1 genes, and dNTPs. After the amplification, PCR products were checked by agarose gel electrophoresis, blotted on a nylon membrane, and hybridized with biotinylated probes, as specified in the Xth International Histocompatibility Workshop (17). Forty-two DRB1 alleles, 8 DQA1 alleles, and 13 DQB1 alleles were tested, representing the most common Caucasoid HLA class II alleles.

Amplification of DQCAR microsatellite locus was performed as previously described (18). The forward primer (GAA ACA TAT ATT AAC AGA GAC AGA CAA A) was labeled at the 5' end with CTP, and the reverse primer (CAT TCT TCT TCT TTA TCA CTT CAT A) was not labeled. After the amplification, PCR products were analyzed by agarose gel electrophoresis, blotted on a nylon membrane, and hybridized with biotinylated probes, as specified at the Xth International Histocompatibility Workshop (17). Forty-two DRB1 alleles, 8 DQA1 alleles, and 13 DQB1 alleles were tested, representing the most common Caucasoid HLA class II alleles.

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In the case of an unusual haplotypic association, resequencing was performed for all tested loci.

The frequency of DQCAR alleles was estimated by direct counting when and when a single DQCAR allele was observed. Ten different DQCAR alleles could be identified by the differences in the length of the amplified fragments. Five possible alleles (DQCAR 101 bp, 115 bp, 123 bp, 125 bp, and 127 bp) were not detected in our sample. The most common DQCAR allele in Croats was 103 bp, with the frequency of 41.5%. The frequencies of all other DQCAR alleles were less than 15% for each allele. DQCAR alleles 105 bp and 109 bp had the low frequency (0.4%).

The analysis of extended haplotypic associations showed that 13 distinct combinations were present more than 5 times (Table 2). They represented 89.6% (242 out of 270) of all combinations of alleles. The most frequent association (HLA-DRB1*0701, -DQA1*0201, -DQB1*0201, -DQCAR*0301, -DQCAR*0502, -DQCAR*103 bp) was followed by HLA-DRB1*04, -DQA1*0301, -DQB1*0302, -DQCAR*111 bp, -HLA-DRB1*0101, -DQA1*0101, -DQCAR*0501, -DQCAR*103 bp and HLA-DRB1*0301, -DQCAR*0501, -DQCAR*02, -DQCAR*99 bp. The frequency of all other associations was less than 10%.

Among 7 haplotypic associations that appeared only once (data not shown), 3 unusual haplotypic associations were found. The most frequent association was HLA-DRB1*0701, -DQA1*0201, -DQB1*0201, -DQCAR*0301, -DQCAR*0502, -DQCAR*103 bp, followed by HLA-DRB1*04, -DQA1*0301, -DQB1*0302, -DQCAR*111 bp, HLA-DRB1*0101, -DQA1*0101, -DQCAR*0501, -DQCAR*103 bp and HLA-DRB1*0301, -DQCAR*0501, -DQCAR*02, -DQCAR*99 bp. The frequency of all other associations was less than 10%.

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Discussion

In our study, DQCAR polymorphism in the Croatian population was analyzed for the first time. The prevalence of DQCAR 103 bp allele was similar to those reported for other populations (4). The longest alleles (123 bp, 125 bp, 127 bp), rare in Norwegians, North Americans, and Japanese, were
not observed in Croats (4) at all, whereas the 105 bp allele appeared rarely. This is in concordance with the results obtained for other Caucasian populations (Norwegians, North Americans) (4). This study confirmed the absence of 101 bp allele in the Croatian population (4).

In the previous population study, the distribution of HLA-DRB1, -DQA1, -DQB1 haplotypic associations showed a great number of usual associations (24 distinct combinations) as well as 14 unusual haplotypic associations (13), which was expected. Certain DRB1 alleles (*0101, *0301, *04, *1001, *1101, *1104, and *14) were exclusively associated with one specific DQA1-DQB1 combination, whereas some other DRB1 alleles were associated with more than one DQA1-DQB1 combination. For example, DRB1*0701 was always found with DQA1*0201, but with two different DQB1 alleles, *02 and *0303. DRB1*15 and DRB1*13 haplotypic associations showed the greatest heterogeneity, since 10 different associations have been observed for each of these two alleles (14,15).

All DRB1*0101, *1501, *1601, *1301, and *1302 haplotypic associations shared the same DQCAR allele, DQCAR 103 bp. This is in concordance with the results of Macaubas et al (4). They reported on 13 different DQA1/DQB1 allelic DQ1 associated combinations, in which DQCAR 103 bp allele was present in all but 3 cases (4,12). Two haplotypic associations (DRB1*1502 and *14) from DQ1 associated haplotypes were not found with this DQCAR allele, but were observed exclusively with the DQCAR 107 bp allele. Also, the shortest DQCAR allele observed, DQCAR 99 bp, was found in all individuals with HLA-DRB1*0301, -DQA1*0501, -DQB1*02 combination of alleles. These findings are consistent with previous research.

Table 2. The distribution of thirteen HLA-DRB1, -DQA1, -DQB1, -DQCAR haplotypic associations present five times or more in a sample (N=135) of Croatian population

<table>
<thead>
<tr>
<th>DQCAR</th>
<th>DQB1</th>
<th>DQA1</th>
<th>DRB1</th>
<th>HF (%)a</th>
<th>LD × 100b</th>
<th>p c</th>
</tr>
</thead>
<tbody>
<tr>
<td>103</td>
<td>0501</td>
<td>0101</td>
<td>0101</td>
<td>11.4</td>
<td>11.3</td>
<td>0.00749</td>
</tr>
<tr>
<td>103</td>
<td>0602</td>
<td>0102</td>
<td>1501</td>
<td>8.6</td>
<td>8.5</td>
<td>0.00311</td>
</tr>
<tr>
<td>107</td>
<td>0601</td>
<td>0103</td>
<td>1502</td>
<td>2.1</td>
<td>1.9</td>
<td>0.00612</td>
</tr>
<tr>
<td>103</td>
<td>0502</td>
<td>0102</td>
<td>1601</td>
<td>12.5</td>
<td>12.3</td>
<td>0.00407</td>
</tr>
<tr>
<td>99</td>
<td>02</td>
<td>0501</td>
<td>0301</td>
<td>11.1</td>
<td>10.9</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>111</td>
<td>0302</td>
<td>0301</td>
<td>04</td>
<td>11.8</td>
<td>11.7</td>
<td>0.00251</td>
</tr>
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<td>119</td>
<td>0303</td>
<td>0201</td>
<td>0701</td>
<td>3.2</td>
<td>3.0</td>
<td>0.03416</td>
</tr>
<tr>
<td>121</td>
<td>0301</td>
<td>0501</td>
<td>1101</td>
<td>4.3</td>
<td>4.2</td>
<td>0.01540</td>
</tr>
<tr>
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<td>0301</td>
<td>0501</td>
<td>1104</td>
<td>2.1</td>
<td>2.0</td>
<td>0.10812</td>
</tr>
<tr>
<td>121</td>
<td>0301</td>
<td>0501</td>
<td>1104</td>
<td>6.1</td>
<td>5.9</td>
<td>0.00554</td>
</tr>
<tr>
<td>103</td>
<td>0603</td>
<td>0103</td>
<td>1301</td>
<td>5.4</td>
<td>5.3</td>
<td>0.11070</td>
</tr>
<tr>
<td>103</td>
<td>0604</td>
<td>0102</td>
<td>1302</td>
<td>3.6</td>
<td>3.3</td>
<td>0.08696</td>
</tr>
<tr>
<td>107</td>
<td>0503</td>
<td>0101</td>
<td>14</td>
<td>5.4</td>
<td>5.3</td>
<td>0.05184</td>
</tr>
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</table>

aHF – haplotypic association frequency.
bLD x 100 – linkage disequilibrium x 100.
cChi-square test.

Table 3. Unusual HLA-DRB1, -DQA1, and -DQB1, haplotypic associations with -DQCAR in a sample (N=135) of Croatian population

<table>
<thead>
<tr>
<th>Haplotypic association</th>
<th>DQCAR</th>
<th>DQB1</th>
<th>DQA1</th>
<th>DRB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>105</td>
<td>02</td>
<td>0201</td>
<td>0701</td>
<td></td>
</tr>
<tr>
<td>117</td>
<td>0302</td>
<td>0301</td>
<td>0401</td>
<td></td>
</tr>
<tr>
<td>117</td>
<td>0304</td>
<td>0301</td>
<td>0408</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. The distribution of HLA-DRB1*07, -DQA1*0201, -DQB1*02 haplotypic association in a sample (N=135) of Croatian population

<table>
<thead>
<tr>
<th>Haplotypic association</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>111</td>
<td>02</td>
</tr>
<tr>
<td>113</td>
<td>02</td>
</tr>
<tr>
<td>117</td>
<td>02</td>
</tr>
<tr>
<td>119</td>
<td>02</td>
</tr>
<tr>
<td>121</td>
<td>02</td>
</tr>
</tbody>
</table>

aNone of the associations was statistically significant (chi-square test).
ports (10) and suggest that haplotypes bearing shorter DQCAR alleles display little additional variation. It is interesting that HLA-DRB1*0701, -DQA1*0201, and -DQB1*0202 haplotypic association showed the greatest DQCAR polymorphism. On the other hand, the other common DRB1*0701 association (DRB1*0701, -DQA1*0201, -DQB1*0303) showed an exclusive association with DQCAR 119 bp allele. The variation in the association with DQCAR alleles (117 bp, 119 bp, and 121 bp) was also observed within DRB1*11 haplotypic associations. The DQ2 or DQ3 associated haplotypes exhibited a lower degree of diversity in the DQA1 and DQB1 genes, if compared to the DQ1 associated haplotypes. Therefore, the association between the DQCAR alleles and DQ combination of alleles can not be explained by the allelic diversity found in the DQA1 and DQB1 genes themselves. Some authors tried to explain the polymorphism detected in the DQCAR locus by mutation via slippage during the replication and/or chromosomal cross-over within the DQ region (21,22). The rate of strand slippage, as suggested by Schlotterer et al. (23), depends on the length of the dinucleotide sequence. The longer the sequence, the more susceptible it gets, as in the case of the DQ2 or DQ3 associated haplotypes (24).

Three haplotypic associations listed in Table 3 are really uncommon combinations of alleles. They were found in individuals heterozygous for all 4 investigated loci and bearing one common haplotypic association. The rest of 4 associations, though common, were observed only once because the frequency of DRB1 alleles (e.g., DRB1*0102, *1602, *1304, and *1305) in this sample of Croatian population was low.

The most significant value (p<0.0001) of linkage disequilibrium was ob served for HLA-DRB1*0301, -DQA1*0501, -DQB1*0202, -DQCAR 99 bp haplotypic association. Strong linkage disequilibrium was found in all haplotypic associations with shorter DQCAR alleles (103 bp, 107 bp, and 111 bp) in Cro atian as well as in other populations reported so far. Among long DQCAR alleles, only DQCAR 121 bp in combination with HLA-DRB1*1104, -DQA1*0501, -DQB1*0301 showed a very significant linkage disequilibrium p value (p=0.003).

In conclusion, our study showed that specific DQCAR alleles observed in association with common Caucasian haplotypes can also be found in the Croatian population. The polymorphism of the DQCAR microsatellite described in this study will also serve as a referent panel for the future HLA-associated disease studies. A comparative analysis of the DQCAR alleles on HLA matched haplotypes between the patients and the controls may indirectly reveal the ex tence of a novel disease-associated polymorphism. Finally, the existence of 3 unusual haplotypic associations in our sample should be taken into account in future, especially in studies carried out on other neighboring populations, which will show whether these haplotypic associations are indeed characteristic for Croatians.

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References


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