Brief Communication

Identification of a novel DQB1 allele (*0609) segregating in an Ashkenazi Jewish family: Implications for DQB1 typing systems


The class I and class II loci of the mammalian major histocompatibility complex (MHC (HLA in humans)) exhibit a high degree of coding sequence polymorphism. Most of the sequence-defined polymorphism in the class II genes is located in the second exon, which encodes the peptide binding groove of the cell surface heterodimer on antigen-presenting cells. Recognition of peptides derived from foreign and self-antigens bound to the appropriate class II molecule results in the activation of CD4+ T cells. Identification of HLA class II allelic sequence polymorphism has become a valuable tool in the areas of tissue transplantation (1), disease susceptibility or resistance (2-4), forensics (5, 6), and molecular evolution and anthropology (e.g. 7, 8, 9). Most methods for detecting HLA sequence variation use the polymerase chain reaction (PCR) (10, 11) to amplify DNA for analysis using sequence-specific oligonucleotide probes, direct sequencing methodologies, or allele-specific amplification strategies. Here, we present an updated sequence-specific oligonucleotide probe (SSOP) typing system for the DQB1 locus, which has allowed us to identify a novel DQB1*06 allele (*0609) segregating in an Ashkenazi family of triplets being typed for tissue transplantation. This new DQB1 allele (*0609), which has also been identified recently in a cell line (12), was found in 6 members of this family, including the father, oldest daughter, grandson, and three triplet (fraternal) sibs (Fig. 1).

Cells from buccal cell swabs were collected and extracted according to recently published procedures (9). Thirty microliters of supernatant were PCR-amplified and HLA class II-typed with HRP-labelled oligonucleotide probes as previously described for DRB1 (13), DQA1 (14), and DPB1 (15-17). Genomic DNAs were amplified for DQB1 and hybridized to the SSO probes listed in Table 1, which include probes that have been redesigned from previously published methods (18, 19) to allow specific hybridization under the same reaction conditions. Two new SSO probes (ET0604 and ET0605) specific for the variant sequences surrounding codon 14 of DQB1 were also employed, according to the conditions listed in Table 1, to screen the family. Unusual DQB1 probe hybridization patterns for some individuals within this family signalled the presence of a potential novel DQB1*06 allele. Genomic DNA from four of the anomalous DQB1*06 samples was amplified for DQB1, cloned into M13mp18 (20) and three to six
I. DRB1*1302-DQA1*0102-DQB1*0609-DPB1*0301
   B. DRB1*0501-DQA1*0101-DQB1*0501-DPB1*0201
   C. DRB1*1302-DQA1*0501-DQB1*0301-DPB1*0401
   D. DRB1*1301-DQA1*0103-DQB1*0603-DPB1*1001

II. E. DRB1*0101-DQA1*0101-DQB1*0501-DPB1*0201
    F. DRB1*13-DQA1*0501-DQB1*0301-DPB1*0301

III. A. E D
     B. A D C
     C. A C A C
     D. A B D E

Figure 1. HLA class II haplotypes of an Ashkenazi Jewish family of triplets carrying DQB1*0609.

Table 1. Sequence-specific oligonucleotide probes and conditions for HLA-DQB1 allele typing

<table>
<thead>
<tr>
<th>PROBE</th>
<th>AMINO ACID SEQUENCE</th>
<th>HRP-SSO PROBE NUCLEOTIDE SEQUENCE 5'-3'</th>
<th>HYB/WASH SSPE CONC.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB53</td>
<td>LGLPA (52-57)</td>
<td>TGCTGGGGCTGCCCTGCC</td>
<td>1x/0.1x</td>
</tr>
<tr>
<td>DB176</td>
<td>LGPPD (53-57)</td>
<td>CTGGGGCCGCTGAC</td>
<td>1x/0.1x</td>
</tr>
<tr>
<td>DB238</td>
<td>QRD (DW-2) (55-60)</td>
<td>GCCGGCTGTAGCTGCCGA</td>
<td>1x/0.1x</td>
</tr>
<tr>
<td>DB269</td>
<td>QRD (DW-12) (54-60)</td>
<td>GCAGGGGGCGXCTGACXCGG</td>
<td>1x/0.1x</td>
</tr>
<tr>
<td>DB272</td>
<td>LGPPA (54-60)</td>
<td>GGCCGGCTGCCGCC</td>
<td>1x/0.1x</td>
</tr>
<tr>
<td>DB430</td>
<td>ERVRL (22-26)</td>
<td>GAGCCTGCTGCTTGTAA</td>
<td>1x/0.1x</td>
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<tr>
<td>DB448</td>
<td>DVEVY (42-47)</td>
<td>GACGTGGAGGGTGACC</td>
<td>1x/0.1x</td>
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<tr>
<td>DB449</td>
<td>DVGY (43-47)</td>
<td>CGGATACACCCCCACGTC</td>
<td>1x/0.1x</td>
</tr>
<tr>
<td>DB451</td>
<td>TRYTY (26-33)</td>
<td>TCTCGGTTATAGAGCTCTGGTCAC</td>
<td>1x/0.1x</td>
</tr>
<tr>
<td>DB454</td>
<td>LGRLD (53-58)</td>
<td>CGTCAGGCCGGCCCAG</td>
<td>1x/0.1x</td>
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<td>DB69*</td>
<td>EEDV (35-40)</td>
<td>AAGCGACGTCTCTC</td>
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<tr>
<td>DB110</td>
<td>VLEGA (66-71)</td>
<td>GAAGTCCGTGAGGGGCC</td>
<td>3x/1x</td>
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<td>DB79</td>
<td>ALL (39-44)</td>
<td>CTCGGACACGGCGTC</td>
<td>3x/1x</td>
</tr>
<tr>
<td>DB107</td>
<td>QRV (56-61)</td>
<td>CTGCTGGCGAGTC</td>
<td>3x/1x</td>
</tr>
<tr>
<td>UG82</td>
<td>TRHIY (27-34)</td>
<td>ACCAGACATCTACAAC</td>
<td>3x/1x</td>
</tr>
<tr>
<td>DB78*</td>
<td>QRS (56-61)</td>
<td>CAGTACTCGGCCG</td>
<td>3x/1x</td>
</tr>
<tr>
<td>RAP93</td>
<td>GTERVR (20-25)</td>
<td>CGCGACCGCCTCGGTCCC</td>
<td>0.5x@55°/0.1x@55°</td>
</tr>
<tr>
<td>ET0604</td>
<td>KGMCY (12-16)</td>
<td>TAAGGGCATGCTACTCTCAC</td>
<td>2x/0.5x@55°</td>
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<tr>
<td>ET0605</td>
<td>KGLCY (12-16)</td>
<td>TAAGGGCTGCTACTCTCAC</td>
<td>2x/0.5x@55°</td>
</tr>
</tbody>
</table>

*Denotes sequence from the non-coding strand.

The hybridization solution contains 0.5% SDS and SSPE at the concentration indicated above.

Hybridization temperature is 50°C or unless indicated otherwise. Wash solution contains 0.1% SDS and SSPE concentration as noted above. The wash temperature is at 50°C unless otherwise noted. All washes are for 10 minutes, except for probes DB110, DB78, ET0604 and ET0605 which wash for 15 minutes.

X = Inosine which is inserted to help destabilize the mismatch.

clones were sequenced (21) for each individual. HLA class II haplotype and pedigree data for this family are shown in Fig. 1.

The identification of the new DQB1 allele *0609 brings the total number of DQB1 alleles to 24, including three alleles with silent polymorphisms.
The sequence of the DQB1*0609 allele differs by only one base substitution from DQB1*0604 (at codon 30) and from *0605 (at codon 14). Our previous DQB1 typing system (18, 19) which lacked the SSO probes ET0604 and ET0605, was incapable of distinguishing *0609 from *0605. DQB1*0609 may have evolved from DQB1*0604 by a single (C→T) first position transition at codon 30, creating an amino acid change from (polar) histidine to (polar) tyrosine (Fig. 2). The alternative hypothesis, that DQB1*0609 evolved from *0605 by a single (C→A) first position transversion at codon 14, changing (hydrophobic) leucine to (hydrophobic) methionine, is less likely because (a) transversions are notably less common than transitions, and (b) the associated DRB1, DQA1 haplotype (DRB1*1302-DQA1*0102-DQB1*0609) is coupled to DQB1*0604 in a variety of populations (22). It is conceivable that DQB1*0609 evolved from *0609, rather than vice-versa. DQB1*0609 is found in African, Caucasian and Asian populations (22), suggesting that it is not a very recently generated allele.

As population studies to determine the frequency of DQB1*0609 could prove helpful in resolving this issue, we retyped African-American and Caucasian American samples previously typed as DQB1*0605, using the probes described herein. In this analysis, 4/5 of the African-American DQB1*0605 alleles retyped as DQB1*0609. In the African-American DQB1*0609 samples, 3/4 had the haplotype DRB1*1302-DQA1*0102-DQB1*0609, and 1/4 had the haplotype DRB1*1301-DQA1*0103-DQB1*0609; the one DQB1*0605 haplotype was DRB1*1302-DQA1*0102-DQB1*0609. In the Caucasian American samples 10/10 previously typed DQB1*0605 samples retyped as DQB1*0609; 8/10 of these samples had the haplotype DRB1*1302-DQB1*0609, and 1/10 had the haplotype DRB1*1301-DQB1*0609, and 1/10 had the haplotype DRB1*1305-DQB1*0609. Finding the DQB1*0609 allele at higher frequencies than DQB1*0605, as well as on three different haplotypes in the African Americans, Ashkenazi Jews and Caucasian Americans suggests that DQB1*0609 may predate both DQB1*0605 and DQB1*0604. Fig. 3 illustrates two pathways by which DQB1*0604, DQB1*0605 and DQB1*0609 could have been generated.

The finding of two novel DR/DQB1*0609

Figure 2. New DQB1 sequence: DQB1*0609 in comparison with *0604 and *0605.
Figure 3. Two possible pathways in which DQB1*0604, *0605 and *0609 may have been generated. Although pathway I is likely because DQB1*0604 is frequent in many different ethnic groups (22), and would therefore be quite old, pathway II is also plausible because DQB1*0609 was found on three different haplotypes in African Americans, Caucasian Americans and Ashkenazi Jews in our study. The time necessary to generate three different haplotypes suggests that DQB1*0609 is quite old, and perhaps predates DQB1*0604. Accumulating data on the frequency of DQB1*0609, and determining associated haplotypes in linkage disequilibrium with DQB1*0609 and DQB1*0604, will help to resolve this issue.

haplotypes in these and other groups (12) indicates that the *0609 allele may represent a significant proportion of haplotypes previously typed as *0605. Using a DBQ1 typing system that examines the dimorphic codon 14 region should eliminate such ambiguities. As more data become available, the age of the new allele can be roughly gauged using the associated DPB1 allele. In the Ashkenazi family, DQB1*0609 is coupled to DPB1*0301. If the DQB1*0609 alleles were found exclusively in linkage with DPB1*0301, the allele would necessarily be younger, barring strong selection for linkage disequilibrium, than if recombination between DPB1 and DQB1 generated many different DQB1-DPB1 haplotypes in the populations studied.

Our current DQB1 SSOP typing system (Table 1) generated probe patterns which revealed the presence and sequence of the new DQB1*06 allele in 6 members of this Ashkenazi family, including one daughter and a grandson whose DQB1*0609 alleles were not sequenced. These results indicate that SSOP typing systems are robust and easily augmented by the addition of new probes for previously untyped polymorphisms (e.g. codon 14) to allow for the identification of novel alleles (e.g. DQB1*0609). Our DQB1 typing system, which amplifies exon 2 with primers DB130 and GH30, easily allows for detection of allelic diversity within this region using the non-radioactive SSO probes. As more alleles are identified, complete typing may require more SSO probes. However, using the reverse dot-blot format (14), which involves a single
hybridization reaction with an immobilized array of probes, sequence-specific probes can be added as new alleles are discovered. With this system, the complexity of the test does not increase, as with other typing methods, with the addition of new probes.

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References