Recognition of HLA-A*11:01-B*51:01-C*14:02-DRB1*11:01-DQB1*03:13 and HLA-A*02-B*40-C*03:77-DRB1*14 haplotypes restricted to Taiwanese

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1. Introduction

Determination of haplotype is essential for the matching of unrelated hematopoietic stem cell transplantation between donor and recipient, because matching at the haplotype level has a better likelihood of matching at other loci within the human leukocyte antigen (HLA) region than donors merely matched at the individual allelic level. It can provide useful information to transplant clinicians and donor recruitment centers to design search strategies to identify matched donors for confirmatory testing. It can also facilitate the selection of potential marrow stem cell donors in the database where HLA-A and -B typings are known, but -DRB1 typing has not been tested previously, or when allelic levels of the potential donors need to be determined. This can optimize the selection of low resolution type donors from unrelated donor searches. Furthermore, haplotype information may be utilized to consider a mismatch strategy for patients with rare alleles where the probability of locating a matched unrelated donor is less likely [1].

Determination of the HLA-A, -B, and -DRB1 haplotypes may be accomplished by HLA typing of blood-related family members and prediction from tissue typing in a large population. Alternatively, it can be achieved by deducing typing results from donors with allelic...
homzygosities in the HLA-A, -B, and -DRB1 loci [2]. In a family study, segregation of individual HLA alleles provides evidence of allelic linkages. In a population study, the determination of haplotypes involves noting whether alleles at the other two loci are consistently present and no family study is performed. Most available haplotype data are derived from studies of unrelated individuals in whom the putative haplotype is defined by statistical association analysis [3] or by allele association patterns shared in common [4–6].

In the Taiwanese population, many alleles in the HLA-C and -DQB1 loci show characteristic linkage disequilibria with HLA-B and -DRB1 alleles, respectively [3]. Examples include C*01:02 linked with B*46:01, C*03:02 with B*58:01, C*08:01 with B*15:02, DQB1*03:03 with DRB1*09:01, DQB1*03:01 with DRB1*12:02, and DQB1*06:01 with DRB1*08:03 [3]. The patterns of linkage disequilibrium provide a useful reference tool for selecting potential donors in HLA confirmatory testing prior to transplantation and might be also employed to check when specimen mix-up is suspected. Here we report the deduced probable HLA haplotypes associated with C*03:77 and DQB1*03:13 and the Taiwanese ethnicity of C*03:77 and DQB1*03:13.

2. Materials and methods

Peripheral whole blood samples from unrelated bone marrow stem cell donors who voluntarily participated in the Tzu Chi Marrow Donor Registry were collected in acid citrate dextrose anticoagulant. Formal written consents were signed by the donors before any blood collection was performed. Acid citrate dextrose whole blood was stored at −80°C prior to use. Genomic DNA was extracted using the QiAamp DNA Blood Mini Kit according to the manufacturer’s instructions (Qiagen, Hilden, Germany). Genomic DNA typing of HLA-A, -B, -C, and -DRB1 loci were performed using the Dynal Reli-sequence-specific oligonucleotide (SSO) probe HLA-A, -B, -C, and -DRB1 Typing Kits (Dynal Biotech, Bromborough, Wirral, UK) and then we proceeded to the sequence specific primer (SSP) typing method (AllSet Gold SSP HLA high-resolution kits; Dynal Biotech, Invitrogen, Milwaukee, WI, USA) to reach high resolution allelic subtypes. The sequence-based typing (SBT) method [7–10] was employed to confirm the low incidence alleles observed and in cases of anomalous results and typing ambiguities from the SSO or SSP typing protocols. Polymerase chain reaction was carried out to amplify exons 2 and 3 of the HLA-C locus with group-specific primer sets as previously described [10]. For HLA-DQB1 allelic typing, six group-specific primer sets were used in the SBT procedure for HLA-DQB1 detection as described by Dunn et al [11]. Amplicons were sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (versions 3.1; Applied Biosystems, Foster City, CA, USA) in both directions according to the manufacturer’s protocols.

3. Results

Our SBT results confirmed the DNA sequence of C*03:77, which is identical to the sequence of C*03:04:01:01 in exons 2 and 3 except for the nucleotide at position 527 (A→T). The nucleotide substitution caused an amino acid replacement at residue (codon) 152 (E→V). The extended HLA typing of our volunteer bone marrow donor with C*03:77 was A*02:06, A*24:02, B*40:03, B*55:02, C*01:02, C*03:77, DRB1*09:01, DRB1*14:05, DQB1*03:03, and DQB1*05:03. Together with the HLA typing of the cell HC19619 at the IMGT/HLA Database (A*01:CNJK, A*02:AZGC, B*40:AVGC, B*53:01:01, C*03:77, C*04:CVAF, DRB1*13:02:01 and DRB1*16:02:01; www.ebi.ac.uk/cgi-bin/imgt/hla/ethnicity.cgi; [12]), we postulate that the probable HLA-A, -B, and -C haplotype in association with C*03:77 is A*02-B*40-C*03:77. Incidentally, B*40:03 is an uncommon B*40 variant in Taiwanese with a frequency of 0.11% in Minan Taiwanese and 0.22% in Taiwanese aborigines [13].

Our SBT confirmed that the DNA sequence of DQB1*03:13 is analogous to the sequence of DQB1*03:01:01:01 in exons 2 and 3, except for the nucleotide at residue 296 (T→A). The nucleotide variation caused one amino acid exchange at residue (codon) 67 (V→D). The extended HLA typing of the cell HC12776 is A*02:01:01, A*02:07, C*01:02, C*14:02:01, DRB1*08:03:02, DRB1*11:01:01, DQB1*03:13, and DQB1*06:01 (B locus alleles not provided by the IMGT/HLA Database; www.ebi.ac.uk/cgi-bin/imgt/hla/ethnicity.cgi; [12]). We detected two Taiwanese unrelated marrow stem cell donors bearing DQB1*03:13 using our SBT protocol. The extended HLA typings of the two individuals were A*11:01, A*24:02, B*51:01, B*58:01, C*03:02, C*14:02, DRB1*11:01, DRB1*13:02, DRB1*06:09, and DQB1*03:13; and, A*11:01, B*15:01, B*51:01, C*04:01, C*14:02, DRB1*09:01, DRB1*11:01, DQB1*03:03, and DQB1*03:13. Taken together, the probable HLA haplotype in association with DQB1*03:13 in Taiwanese may be deduced as A*11:01-B*51:01-C*14:02-DRB1*11:01-DQB1*03:13.

4. Discussion

HLA-C*03:77 was first reported to the IMGT/HLA Database (cell ID HC19619) in 2010 without a determined ethnic origin. The DQB1*03:13 allele was first reported to the IMGT/HLA Database (cell ID HC12776) in 2002, again with an unknown ethnic origin (www.ebi.ac.uk/cgi-bin/imgt/hla/ethnicity.cgi; [12]). Neither HLA-C*03:77 nor HLA- DQB1*03:13 alleles and their haplotypes had been published previously. Hence, the Taiwanese ethnicity of our donors identified in this study completes the ethnic background for the C*03:77 and DQB1*03:13 alleles listed in the IMGT/HLA Database. Most importantly, the data derived from our study provides a strategy for marrow donor registry search coordinators to look for unrelated HLA-matched hematopoietic stem cell donors for blood disease patients bearing C*03:77 or DQB1*03:13. Indisputably, identification of the two rare alleles in Taiwanese contributes to a further understanding of the polymorphism and uniqueness of HLA-C and -DQB1 alleles in the Taiwanese population.

In our opinion, C*03:77 was derived from C*03:04:01:01, instead of C*03:02:01 and C*03:03:01, as a result of a nucleotide variation caused one amino acid exchange at residue (codon) 67 (V→D).

![Table 1](image)

<table>
<thead>
<tr>
<th>HLA-</th>
<th>C*03:02:01</th>
<th>C*03:03:01</th>
<th>C*03:04:01:01</th>
<th>C*03:77</th>
</tr>
</thead>
<tbody>
<tr>
<td>C*03:02:01</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>C*03:03:01</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>C*03:04:01:01</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>C*03:77</td>
<td>3</td>
<td>2</td>
<td>1*</td>
<td>0</td>
</tr>
</tbody>
</table>

* C*03:77 varies with C*03:04:01:01 with only one nucleotide.

![Table 2](image)

<table>
<thead>
<tr>
<th>HLA-</th>
<th>C*03:02:01</th>
<th>C*03:03:01</th>
<th>C*03:04:01:01</th>
<th>C*03:77</th>
</tr>
</thead>
<tbody>
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<td>2</td>
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</tr>
<tr>
<td>C*03:03:01</td>
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<td>2</td>
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<td>C*03:04:01:01</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
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<td>C*03:77</td>
<td>3</td>
<td>2</td>
<td>1*</td>
<td>0</td>
</tr>
</tbody>
</table>

* C*03:77 varies with C*03:04:01:01 with only one nucleotide.
point mutation at position 527, because C*03:77 varies from C*03:04:01:01 by one nucleotide but varies from C*03:02:01 and C*03:03:01 by three and two nucleotides, respectively (Table 1). Similarly, we assume that DQB1*03:13 was most likely derived from DQB1*03:01:01:01 (the most frequently observed DQB1*03 variant, followed by DQB1*03:02:01 and DQB1*03:01:01:01, in Taiwanese) via a point mutation episode at nucleotide position 296 (T → A).

The frequencies of C*03:77 and DQB1*03:13 in Taiwanese are estimated to be about one in 20,000–30,000 according to our HLA typing experience. When a minor mismatched donor is intended as a stem cell donor for a patient bearing C*03:77 or DQB1*03:13, in our opinion C*03:04:01:01 or DQB1*03:01:01:01, respectively, could be considered. Our assumption is based on the nucleotide and amino acid similarities between C*03:04:01:01 (Tables 1 and 2) and C*03:77, and DQB1*03:01:01:01 and DQB1*03:13 (Tables 3 and 4).

In summary, we detected C*03:77 and DQB1*03:13 alleles in Taiwanese bone marrow stem cell donors. Our results complete the ethnic origin for the two alleles listed in the IMGT/HLA Database. Nucleic Acids Res 2011;39:D1171 e6. Similarly, we assume that DQB1*03:13 was most likely derived from DQB1*03:01:01:01 (the most frequently observed DQB1*03 variant, followed by DQB1*03:02:01 and DQB1*03:01:01:01, in Taiwanese) via a point mutation episode at nucleotide position 296 (T → A).

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In summary, we detected C*03:77 and DQB1*03:13 alleles in Taiwanese bone marrow stem cell donors. Our results complete the ethnic origin for the two alleles listed in the IMGT/HLA Database. Further, the probable HLA haplotypes associated with C*03:77 or DQB1*03:13 were deduced. We believe the probable haplotypes associated with C*03:77 and DQB1*03:13 that we deduced are highly likely, because the frequencies of both alleles are very low in randomized unrelated donors and are represented by at least two unrelated donors who were examined by independent HLA laboratories. Our findings suggest that both probable HLA haplotypes that we deduced from this study are restricted to Taiwanese.

Our DNA sequences of C*03:77 and DQB1*03:13 were submitted and accepted by the European Nucleotide Archive as confirmatory sequences for C*03:77 (accession number HE995410) and DQB1*03:13 (accession number HE995409) in September, 2012.

Acknowledgments

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References