Original Article

Probable HLA haplotypes in association with the uncommon HLA-C*03:36, -C*03:56, and -C*03:86 alleles in a Taiwanese population

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Objective: HLA-C*03:36, -C*03:56 and -C*03:86 are three human leukocyte antigen type C (HLA-C) locus alleles that are rarely observed in the Taiwanese population. The objective of this study is to report the three plausible deduced HLA haplotypes in association with C*03:36, C*03:56, and C*03:86 in Taiwanese unrelated bone marrow stem cell donors.

Materials and Methods: The sequence-based typing method was used to confirm the low-incidence alleles observed. Polymerase chain reaction was carried out to amplify exons 2 and 3 of the HLA-C locus with group-specific primer sets. Amplicons were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit in both directions according to the manufacturer's protocol.

Results: We confirmed the DNA sequences of C*03:36, C*03:56, and C*03:86 and deduced the three plausible HLA haplotypes in association with C*03:36 (A*33:03-B*58:01-C*03:36-DRB1*01:01), C*03:56 (A*02:01-B*48:01-C*03:56-DRB1*12:02), and C*03:86 (A*02-B*35-C*03:86-DRB1*09:01) in unrelated Taiwanese bone marrow stem cell donors.

Conclusion: The three uncommon HLA-C haplotypes that we provide here are valuable for HLA tissue-typing laboratories for reference purposes and for stem cell transplantation donor search coordinators to determine the likelihood of finding compatible donors in unrelated bone marrow donor registries for patients bearing these three uncommon HLA-C locus alleles.

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1. Introduction

With the advent of molecular typing methodology, the numbers of alleles being unveiled at various human leukocyte antigen (HLA) loci are steadily increasing [1]. Results of many studies have shown that HLA allelic polymorphism among different ethnic groups and racial populations is widely observed and the patterns of linkage disequilibrium among various alleles differ significantly among human populations [2–5]. Clinically speaking, HLA matching has a great impact on the success of organ and tissue transplants. Therefore, persistent efforts to reveal de novo alleles in population worldwide have continued to meet stringent compatibility requirements for bone marrow stem cell donors and recipients.

Similarly, determination of haplotypes is essential for matching unrelated hematopoietic stem cell transplantation donors and recipients, because matching at the haplotype level has a better likelihood of matching at other loci within the HLA region than matching only at the individual allelic level.

The population of Taiwan consists of four major groups, namely, the Min Nan, Hakka, aborigines, and Chinese mainlanders [6]. Thus, the database of our hematopoietic stem cell registry comprises volunteer donors with HLA alleles and haplotypes that have unique characteristics. We have discovered new alleles, rare frequency alleles, and Taiwanese conserved alleles and haplotypes in our routine HLA typing studies [4,5,7].

The HLA-C genes contain eight exons. Polymorphism in exons 2 and 3 encodes proteins that are responsible for peptide-binding specificity. DNA sequence typing for this polymorphism is routinely performed for bone marrow stem cells and kidney transplantation. Many alleles in the HLA-C locus show characteristic linkage disequilibrium with HLA-B alleles in the Taiwanese population [5]. In particular, C*01:02 is linked with B*46:01,
selecting potential donors in HLA con-
linkage disequilibrium as such provide a useful reference tool for
B*55:02, B*40:01, and B*40:06, respectively [5]. The patterns of
linkage disequilibrium as such provide a useful reference tool for
selecting potential donors in HLA confirmatory testing and may
also be used when specimen mix-up is suspected.

HLA-C*03:36, -C*03:56, and -C*03:86 were first reported to the
international ImMunoGeneTics project/HLA (IMGT/HLA) database
in 2007, 2009, and 2010, respectively (http://www.ebi.ac.uk/cgi-
bin/immt/hla/ethnicity.cgi). In this article, we describe the HLA
haplotypes in association with HLA-C*03:36, -C*03:56, and
-C*03:86 alleles found in five Taiwanese individuals and propose a
likelihood-based approach on how the alleles maybe derived.

2. Materials and methods

Peripheral whole blood samples from bone marrow donors
with Min Nan Taiwanese ethnicity were collected in acid citrate
dextrose (ACD) anticoagulant. Formal written consents were
signed by the donors before any blood collection. ACD whole
blood was stored at −80 °C until use. Genomic DNA was extracted
using the QIAamp DNA Blood Mini Kit according to the manu-
facturer’s instructions (Qiagen, Hilden, Germany). Genomic DNA
typing of HLA-A, -B, -C, and -DRB1 loci were first performed using
the Dynal Reli sequence-specific oligonucleotide (SSO) probe HLA-
A, -B, -C, and -DRB1 Typing Kits (Dynal Biotech, Bromborough,
Wirral, UK), followed by the sequence-specific primer (SSP) typing
method (AllSet Gold SSP HLA high-resolution kits; Dynal Biotech,
Invitrogen, Brown Deer, WI, USA) to reach high-resolution allelic
subtypes. The sequence-based typing (SBT) method [8] was used
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 [8]. Amplicons were sequenced
by the BigDye Terminator Cycle Sequencing Ready
Reaction Kit (Applied Biosystems, Foster City, CA, USA) in both
directions.

3. Results

We confirmed that the DNA sequence of C*03:36 is analogous to
the sequence of C*03:02:01 in exons 2 (data not shown) and 3,
except for the nucleotide at position 527, in which A is replaced by T
(Fig. 1). The nucleotide replacement caused an amino-acid ex-
change from E to V at residue 152. In exons 2 (data not shown)
and 3, the DNA sequence of C*03:36 is identical to the sequence
of C*03:01 except for the nucleotide at residue 434, in which A is
replaced by T (Fig. 1). The nucleotide substitution generated an
amino-acid replacement from K to M at codon 121. Similarly, the
DNA sequence of C*03:86 is identical to the sequence of C*03:01
in exon 2 (data not shown). In exon 3, the sequence of C*03:86 is
identical to C*03:01 from the nucleotide at position 344 to poin-
tion 352, whereas the rest of the sequence in exon 3 (from posi-
tion 353 to position 619) is identical to the sequence of C*01:02:01
(Fig. 1).

The extended HLA typing of the two unrelated Taiwanese bone
marrow stem cell donors carrying C*03:36 was A*02:02, A*33:03,
B*46:01, B*58:01, C*01:02, C*03:36, DRB1*01:01, and DRB1*12:02.
Together with the typing information about the three HLA
haplotypes reported to the IMGT/HLA database (http://www.ebi.ac.uk/cgi-bin/immt/hla/ethnicity.cgi), the
plausible HLA-A, -B, and -DRB1 haplotype in association with
C*03:36 in Taiwanese population may be deduced as A*33:03-
B*58:01-C*03:36-DRB1*01:01 (Table 1). Because B*58:01 is
frequently observed to associate with C*03:02 in Taiwanese pop-
ulation [5], we think the haplotype A*33:03-B*58:01-C*03:36-
DRB1*01:01 may have been derived from A*33:03-B*58:01-
C*03:02-DRB1*01:01.

The extended HLA typing of the unrelated Taiwanese bone
marrow donor carrying C*03:56 was A*02:01, B*13:01, B*48:01,
C*03 variant alleles detected in Taiwanese population are C*03:04, C*15 (3.34%), C*12 (3.03%), C*05 (1.78%), and C*17 (1.78%). The HLA-A*08 (21.36%), C*08 (10.32%), C*04 (6.77%), C*06 (5.52%), C*14 (3.56%), C*15 (3.34%), C*12 (3.03%), C*05 (1.78%), and C*17 (1.78%) are also common in this population.

The extended HLA typing of the two unrelated Taiwanese donors carrying C*03:36 was A*02, B*35, C*03:36, DRB1*12:02, and A*02:02, B*35:01, C*05:01, C*07:02, C*09:01, and DRB1*04:05. Taking into the account the HLA typing reported to the IMGT/HLA database and the HLA databases that we established previously [4,5,9,10], the plausible HLA-A, -B, and -DRB1 haplotypes associated with C*03:36 in the two Taiwanese unrelated donors may be deciphered as A*02-B*35-DRB1*04:05 (Table 2).

We believe the probable HLA haplotypes associated with C*03:36, C*03:56, and C*03:86 that we deduced are highly likely because the frequencies of the alleles are very low in randomized unrelated donors and are represented in at least two unrelated donors that were investigated by independent HLA laboratories.

4. Discussion

C*03:36 and C*03:56 were probably derived from C*03:02:01 and C*03:03:01, respectively, as a result of a nucleotide substitution. We speculate the allele C*03:36 was most likely derived from a DNA recombination event, similar to the formations of HLA-B*35:87 [11] and HLA-B*44:150 [12], between alleles C*03:03:01 and C*01:02:01. In the hypothesized recombination episode, C*03:03:01 received a segment of DNA sequence from C*01:02:01 consisting of at least the sequence from residue 353 to residue 619 (Fig. 1).

Over 100 HLA-C*03 alleles are presently listed in the IMGT/HLA database. In Taiwanese populations, C*03 is the most commonly detected HLA-C allele (23.31%), followed by C*01 (22.06%), C*07 (21.36%), C*08 (10.32%), C*04 (6.77%), C*06 (5.52%), C*14 (3.56%), C*15 (3.34%), C*12 (3.03%), C*05 (1.78%), and C*17 (1.78%). The HLA-C*03 variant alleles detected in Taiwanese population are C*03:02, and C*03:03 with a frequency of 11.39%, 8.90%, and 3.03%, respectively [5]. We estimated the frequency of C*03:36, C*03:56, and C*03:86 in Taiwanese population to be approximately 1 in 20,000 to 1 in 30,000 based on our SBT experience from unrelated volunteer bone marrow donors.

We think C*03:02:01 may be considered when searching for minor mismatched unrelated bone marrow hematopoietic stem cells for transplantation in patients with C*03:36, because of the nucleotide and amino acid similarities between C*03:36 and C*03:02:01. Similarly, for patients with C*03:56, C*03:03:01 may be considered if a minor mismatched unrelated donor is intended as the stem cell source. By contrast, a minor mismatched donor substitute for a C*03:86 donor is rather difficult to accommodate because C*03:86 varies from C*03:03:01 by eight nucleotide and amino acid differences, respectively.

5. Acknowledgments

Special thanks are due to all volunteer donors who consented to our research, and actively participated in the Taiwan Tzu Chi Marrow Donor Registry for their great love and effort in assisting needy patients. We are grateful to Dharma Master Cheng Yen for continuing spiritual support and guidance. We appreciate the friendship and encouragement of our colleagues. It is their camaraderie that drives us forward when we are confronted with obstacles and frustrations.

References

[5] Yang KL, Chen SP, Shyr MH, Lin PY. High-resolution human leukocyte antigen (HLA) haplotypes and linkage disequilibrium of HLA-B and -C HLA-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>HLA haplotype of donors with HLA-C<em>03:36 (A</em>02:03-B<em>58:01-C</em>03:36-DRB1*01:01).</th>
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<tr>
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</tr>
<tr>
<td>1</td>
<td>02:07</td>
</tr>
<tr>
<td>2</td>
<td>33:03</td>
</tr>
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HLA – human leukocyte antigen.

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<th>Table 2</th>
<th>HLA haplotype of donors with HLA-C<em>03:56 (A</em>02:01-B<em>35:01-C</em>03:56-DRB1*12:02).</th>
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<tbody>
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<td>Donor</td>
<td>HLA-A*</td>
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<tr>
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<tr>
<td>1</td>
<td>02:01</td>
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<td>HC1244</td>
<td>24:CWTF</td>
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</table>

HLA – human leukocyte antigen.
DRB1 and -DQB1 alleles in a Taiwanese population. Hum Immunol 2009;70:269–76.


