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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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ABSTRACT

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.

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,

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C*03:02 with B*58:01, and C*08:01 with B*15:02, although C*01:02, C*03:02, and C*08:01 have also been found to be associated with 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/imgt/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 manufacturer'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 exchange from E to V at residue 152. In exons 2 (data not shown) and 3, the DNA sequence of C*03:56 is identical to the sequence of C*03:03:01 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*03:03:01 in exon 2 (data not shown). In exon 3, the sequence of C*03:86 is identical to C*03:03:01 from the nucleotide at position 344 to position 352, whereas the rest of the sequence in exon 3 (from position 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*02:07, A*33:03, B*46:01, B*58:01, C*01:02, C*03:36, DRB1*01:01, and DRB1*15:01, and A*11:01, A*33:03, B*40:01, B*58:01, C*07:02, 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/imgt/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 population [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,

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cDNA            350        360        370        380        390        400        410        420        430        440
C*01:02:01  GGTCTCA CACCCTCCAG TGGATGTGTG GCTGCGACCT GGGGCCCGAC GGGCGCCTCC TCCGCGGGTA TGACCAGTAC GCCTACGACG GCAAGGATTA
C*03:02:01  ------- --T------- A------A-- --------G- ---------- ---------- ---------- --------C- ---------- ----------
C*03:03:01  ------- --T-A----- A------A-- --------G- ---------- ---------- ---------- --------- ---------- ---------
C*03:36     ------- --T------- A------A-- --------G- ---------- ---------- ---------- --------C- ---------- ----------
C*03:56     ------- --T-A----- A------A-- --------G- ---------- ---------- ---------- --------- ---------- ---T------
C*03:86     ------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

cDNA             450        460        470        480        490        500        510        520        530        540
C*01:02:01  CATCGCCCTG AACGAGGACC TGCGCTCCTG GACCGCCGCG GACACCGCGG CTCAGATCAC CCAGCGCAAG TGGGAGGCGG CCCGTGAGGC GGAGCAGCGG
C*03:02:01  ---------- --------T- ---------- ---------- -----G---- ---------- ---------- ---------- ------T--- ---------T-
C*03:03:01  ---------- --------T- ---------- ---------- -----G---- ---------- ---------- ---------- ---------- ---------T-
C*03:36     ---------- --------T- ---------- ---------- -----G---- ---------- ---------- ---------- ------T--- ---------T-
C*03:56     ---------- --------T- ---------- ---------- -----G---- ---------- ---------- ---------- ---------- ---------T-
C*03:86     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

cDNA             550        560        570        580        590        600        610        619
C*01:02:01  AGAGCCTACC TGGAGGGCAC GTGCGTGGAG TGGCTCCGCA GATACCTGGA GAACGGGAAG GAGACGCTGC AGCGCGCGG
C*03:02:01  ---------- --------CT ---------- ---------- --------A- ---T------ ---------- ---------
C*03:03:01  ---------- --------CT ---------- ---------- --------A- ---T------ ---------- ---------
C*03:36     ---------- --------CT ---------- ---------- --------A- ---T------ ---------- ---------
C*03:56     ---------- --------CT ---------- ---------- --------A- ---T------ ---------- ---------
C*03:86     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------
```

In exon 3, from nucleotide 344 to nucleotide 619, the DNA sequence of C*03:86 is identical to the sequence of C*01:02:01 (shaded). In exon 3, the sequence of C*03:36 is identical to C*03:02:01 except at the position 527 (A->T; shaded). The DNA sequence of C*03:56 is identical to C*03:03:01 except at the residue 434 of exon 3 (A->T; shaded).

Fig. 1. Comparison of exon 3 nucleotide sequences between C*01:02:01, C*03:02:01, C*03:03:01, C*03:36, C*03:56, and C*03:86. cDNA = complementary DNA.

Table 1
HLA haplotype of donors with HLA-C*03:36 (A*33:03-B*58:01-C*03:36-DRB1*01:01).

Donor	HLA-A*	HLA-B*	HLA-C*	HLA-DRB1*
1	02:07	46:01	01:02	15:01
	33:03	58:01	03:36	01:01
2	11:01	40:01	07:02	12:02
	33:03	58:01	03:36	01:01
HC18734	02:03	38:02	07:02	16:02
	33:03	58:VE	03:36	01:01
HC14742	02:01	39:01	07:02	13:01
	33:03	58:01	03:36	01:01
HC23497	33	58CNSX	03:02	03:01
	33	58CNSX	03:36	01:01

HLA = human leukocyte antigen.

C*03:04, C*03:56, DRB1*01:01, DRB1*12:02, and DQB1*03:01. Together with the HLA information about the cell carrying C*03:56 listed on the IMGT/HLA database, the probable HLA haplotype of the Taiwanese unrelated donor bearing C*03:56 may be determined as A*02:01-B*48:01-C*03:56-DRB1*12:02 (Table 2).

The extended HLA typing of the two unrelated Taiwanese donors carrying C*03:86 was A*02, B*35, B*46:01, C*01:02, C*03:86, DRB1*04, and DRB1*09:01, and A*02:06, A*24:02, B*35:01, B*58:01, C*03:02, C*03:86, DRB1*04:05, and DRB1*09:01. 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, -C, and -DRB1 haplotype associated with C*03:86 in the two Taiwanese unrelated donors may be deciphered as A*02-B*35-C*03:86-DRB1*09:01 (Table 3).

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*03:86 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 a DNA sequence from C*01:02:01 consisting of at least the sequence from residue 353 to residue 619 (Fig. 1). Our theory is based on the observation that the sequence of C*03:86 is identical to the sequence of C*01:02:01 from position 353 to position 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*03:04, 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.

HLA-A and -B antigens have long been regarded as the major players in the field of transplantation, whereas the HLA-C antigen has only recently emerged as an equally important HLA. HLA-C antigen mismatching has been associated with increased grade III–IV acute graft versus host disease, treatment-related mortality, and low disease-free survival [13]. Information about the haplotypes associated with the unusual HLA-C*03:36, -C*03:56, and -C*03:86 that we deduced in this study may help in finding strategies for appropriate donors in local and international registries for HLA confirmatory typing purposes, as well as in facilitating consideration of a minor mismatch option when an HLA fully matched donor is unavailable.

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 and C*01:02:01 by eight and five amino acids, respectively.

Table 2
HLA haplotype of donors with HLA-C*03:56 (A*02:01-B*48:01-C*03:56-DRB1*12:02).

Donor	HLA-A*	HLA-B*	HLA-C*	HLA-DRB1*
1	02:01	13:01	03:04	01:01
	02:01	48:01	03:56	12:02
HC18244	24:CWTG	51:CSSF	14:02	13:02
	02:CWTF	48:HCP	03:56	12:CVT

HLA = human leukocyte antigen.

Table 3
HLA haplotype of donors with HLA-C*03:86 (A*02-B*35-C*03:86-DRB1*09:01).

Donor	HLA-A*	HLA-B*	HLA-C*	HLA-DRB1*
1	02	46	01:02	04
	02	35	03:86	09:01
2	24:02	58:01	03:02	04:05
	02:06	35:01	03:86	09:01
HC21208	02	15:DAFZ	04:FEAS	11:01
	02	35:DHXM	03:86	09:01

HLA = human leukocyte antigen.

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