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Original Article

Recognition of the three deduced probable HLA haplotypes that are associated with HLA-C*16:04:01 (A*33:03-B*44:02-C*16:04:01-DRB1*11:04:01 and A*24-B*44:02-C*16:04:01-DRB1*11:04) and HLA-B*15:109 (A*11-B*15:109-DRB1*04) in Taiwanese unrelated hematopoietic stem cell donors

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ABSTRACT

Objective: HLA-C*16:04:01 and HLA-B*15:109 are two uncommon alleles at the HLA-C locus and HLA-B locus, respectively. The objective of this study is to report the deduced probable human leukocyte antigen (HLA) haplotypes associated with HLA-C*16:04:01 and HLA-B*15:109 among Taiwanese unrelated bone marrow hematopoietic stem cell donors.
Materials and methods: A sequence-based typing method was employed to confirm the two low incidence alleles observed. Polymerase chain reaction was performed to amplify exons 2 and 3 of the HLA-A, and HLA-B loci with group-specific primer sets. Amplicons were sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction Kit in both directions according to the manufacturer's protocols.
Results: The DNA sequence of C*16:04:01 is identical to that of C*16:01:01 in exons 2 and 3, except for two nucleotide substitutions at residues 538 (C->T) and 539 (A->G), which results in a single amino acid replacement at position 156 (glutamine->tryptophan). We deduced two probable HLA haplotypes that are found in association with C*16:04:01 as A*33:03-B*44:02-C*16:04:01-DRB1*11:04 and A*24-B*44:02-C*16:04:01-DRB1*11:04. The DNA sequence of B*15:109 is identical to B*15:27:01 in exons 2 and 3 except for one nucleotide substitution at residue 200 (C->T), which results in a single amino acid replacement at position 43 (proline->leucine). A probable HLA haplotype associated with B*15:109 was deduced to be A*11-B*15:109-DRB1*04.
Conclusion: Information on the deduced HLA haplotypes that are found in association with the rare C*16:04:01 and B*15:109 alleles that we report here is useful for reference purposes at HLA testing laboratories and will help stem cell transplantation donor search coordinators when they are determining the likelihood of finding a compatible donor for patients bearing these two uncommon HLA alleles from unrelated bone marrow donor registries.

1. Introduction

The major histocompatibility complex (MHC) in humans consists of several gene loci situated on the short arm of chromosome 6 at 6p21.3. These loci are classified into three MHC classes; I, II, and III. The genes encoding the human leukocyte antigen (HLA) alleles are located in the MHC class I and II regions. The HLA genes are characterized by their extreme allelic polymorphism as well as their high variation and diversity among different ethnic groups and

Conflicts of interest: none.

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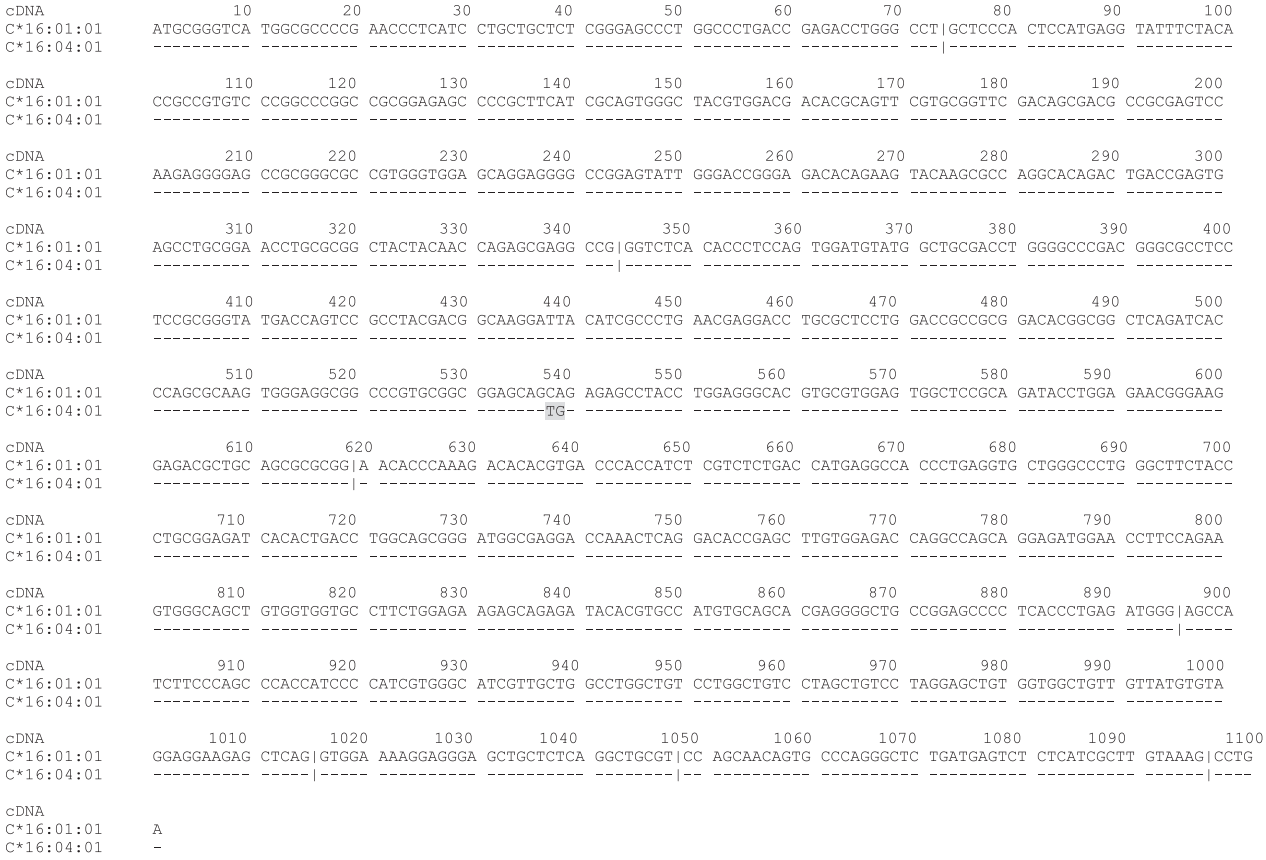

Fig. 1. The DNA sequence of C*16:04:01 is identical to C*16:01:01 in exons 2 and 3, except for two nucleotide substitutions at residues 538 (C->T) and 539 (A->G) (shaded).

racial populations. HLA protein molecules have been definitely defined to be transplant antigens and have strong relevance when tissue transplantation is carried out. The similarity of these protein molecules between donors and recipients is being considered to be an important factor when predicting graft survival and graft versus host disease (GVHD). It is very important to precisely characterize any new allele encountered during routine HLA typing procedures. To facilitate successful and comprehensive unrelated bone marrow donor searches for patients in need of hematopoietic stem cell transplantation, we are persistently working on resolving unidentified or ambiguous alleles that pass through our hands in order to offer better HLA matching and donor selection as part of our service.

HLA-C*16:04:01 and HLA-1*15:109 were first reported to the IMGT/HLA database in 1997 (HC 11164; HC 11181) and 2006 (HC14070), respectively [1]. Here, we report the deduced probable HLA haplotypes that are found in association with C*16:04:01 and B*15:109. We further postulate that there are two plausible HLA haplotypes found in association with C*16:04:01 in Taiwanese and that the haplotype in association with B*15:109 is likely to be restricted to Oriental populations, due to the fact that it has so far been reported only in Singapore [1] and in Taiwan.

2. Materials and methods

Peripheral whole blood samples from the unrelated bone marrow stem cell donors with Taiwanese ethnicity were collected in acid citrate dextrose (ACD) anticoagulant. Formal written consent was signed by the donors prior to blood collection. The ACD whole blood was stored at −80°C until use. Genomic DNA was extracted using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA was then subjected to HLA genotyping for HLA-A, HLA-B, and HLA-DRB1 loci using a commercial PCR-SBT kit and a SeCore A/B/DRB1 Locus Sequencing kit (Life Technologies, Brown Deer, WI, USA). High resolution allelic sequencing was performed as previously described [2–6]. Amplicons were sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) in both directions.

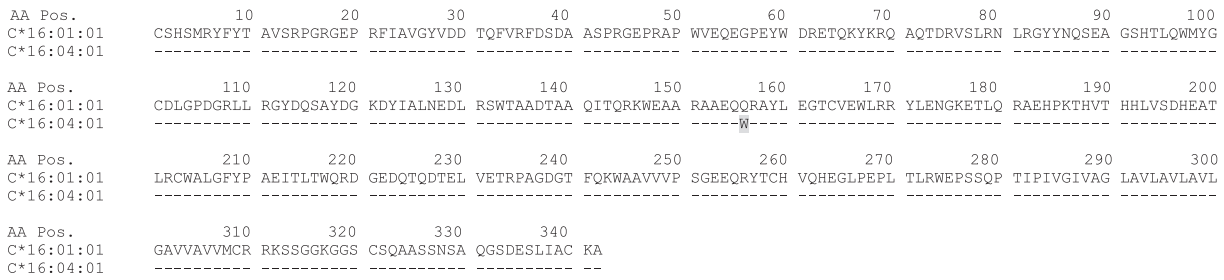

Fig. 2. The nucleotide substitutions of C*16:04:01 cause an amino acid replacement at residue 156 (glutamine->tryptophan) (shaded).

Table 1
The HLA-A, HLA-B, HLA-C, and HLA-DRB1* alleles of bone marrow donors with C*16:04:01.

HLA-	HLA-A*		HLA-B*		HLA-C*		HLA-DRB1*	
Donor 1	33:03	11:01	44:02	15:02	16:04:01	08:01	11:04	12:02
Donor 2	33:03	11:02	44:02	40:01	16:04:01	04:01	11:04	04:04
Donor 3	33:03	11:01	44:02	15:02	16:04:01	08:01	11:04	12:02
Donor 4	33:03	11:01	44:02	40:01	16:04:01	03:04	11:04	04:03
Donor 5	24:02	02:01	44:02	40:02	16:04:01	07:02	11:04	09:01
Donor 6	24	02	44:02	40:02	16:04:01	03:03	11:04	14:54

3. Results

We confirmed the DNA sequence of C*16:04:01 to be identical to C*16:01:01 in exons 2 and 3, except for the presence of two nucleotide substitutions at residues 538 (C->T) and 539 (A->G) (Fig. 1). The nucleotide substitutions result in an amino acid replacement at residue 156 (glutamine>tryptophan) (Fig. 2). The extended HLA typings of our donors who are carrying C*16:04:01 are shown in Table 1. Taken all together, two HLA-A, HLA-B, HLA-C, and HLA-DRB1 haplotypes may be deduced and these are A*33:03-B*44:02-C*16:04:01-DRB1*11:04 and A*24-B*44:02-C*16:04:01-DRB1*11:04. Because the haplotype A*24-B*44:02-C*16:04:01-DRB1*11:04 was initially found in an Italian individual [7], we assume this C*16:04:01 associated HLA haplotype is a European and Oriental haplotype, whereas the haplotype A*33:03-B*44:02-C*16:04:01-DRB1*11:04 is an Asian haplotype. In addition, it is apparent that C*16:04:01 is in linkage with B*44:02 and DRB1*11:04, because all donors bearing C*16:04:01 also carry B*44:02 and DRB1*11:04 (Table 1).

We confirmed the DNA sequence of B*15:109 to be identical to B*15:27:01 in exons 2 and 3 except for a single nucleotide substitution at residue 200 (C->T) (Fig. 3). The nucleotide substitution results in one amino acid replacement at position 43 (proline>-leucine) (Fig. 4). Extended HLA typing of our donor who carried B*15:109 is: A*11:XEWG, A*11:PDVH, B*15:109, B*40:NPHZ, DRB1*04:HAJB, and DRB1*08:ANM. When this is taken together with the extended HLA typing of the Singapore donor with B*15:109 that has been reported to the IMGT/HLA database (HC 14070) (A*02ANDC, A*11:XSH, B*15:109, B*54:01, DRB1*04:ASEJ and DRB1*15:GEP), the probable HLA haplotype found in association with B*15:109 may be postulated to be A*11-B*15:109-DRB1*04. We speculate that the haplotype A*11-B*15:109-DRB1*04 is most probably limited to Asian populations, because B*15:109 has been only found in Asian individuals. Our speculation remains to be verified in the future.

4. Discussion

We have confirmed in this study the DNA sequences and amino acid sequences of the two low frequency HLA alleles, C*16:04:01 and B*15:109. C*16:04:01 was initially detected in a family from south Italy and subsequently found in three unrelated Mediterranean Caucasoid donors [7]. C*16:04: 01 could possibly result from a gene conversion-like recombination event involving C*16:01 as recipient and any one of the Trp 156-positive alleles (C*02, C*06 and C*12) as donor [7]. We deduced two most probable C*16:04:01 associated HLA haplotypes from our unrelated bone marrow donors, one of which, (A*24-B*44:02-C*16:04:01-DRB1*11:04), is a European and Asian haplotype, and was also identified in an Italian individual as reported by Grundschober et al [7]. We further deduced another C*16:04:01 associated HLA haplotype (A*33:03-B*44:02-C*16:04:01-DRB1*11:04) based on our Taiwanese population. We presume the second C*16:04:01 associated HLA haplotype is most likely restricted to the Taiwanese population. Furthermore, we verified a probable HLA haplotype that is found in association with B*15:109. We think B*15:109, like its associated probable HLA haplotype, is likely to be restricted to Oriental populations.

The significance of determining the ethnicity of C*16:04:01 and B*15:109 and their linked HLA haplotypes is that such information may be applied in anthropological investigations of races; in addition, it also allows search coordinators from unrelated bone marrow donor registries to allocate appropriate unrelated bone marrow hematopoietic stem cell donors to the appropriate patients.

It is worth mentioning that the most direct and classical method of determining a HLA haplotype is through family studies if the test materials from a number of key family members are available. Alternatively, population study may be employed if a significant and sufficient number of unrelated donors are available [8]. However, the haplotypes deduced via population investigations are generally considered to be likely or most probable haplotypes rather than confirmed haplotypes. In this study, due to the type and availability of the necessary test material, we were unable to perform family studies on the donors with C*16:04:01 and B*15:109. Therefore, we have opted to determine the haplotypes by examining the HLA alleles carried in common by the unrelated donors bearing the same alleles of interest. By the same token, if determination of plausible HLA haplotypes is carried out for rare frequency HLA alleles, the alleles shared in common by unrelated individuals may be also employed to deduce the associated probable haplotypes [9–16]. Thus, we took the opportunity to employ six unrelated bone marrow stem cell donors with C*16:04:01 and the one donor with B*15:109 in our Registry's database together

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cDNA                    80         90        100        110        120        130        140        150        160        170
 B*15:27:01        GCTCCCA CTCCATGAGG TATTTCTACA CCGCCATGTC CCGGCCCGGC CGCGGGGAGC CCCGCTTCAT CGCAGTGGGC TACGTGGACG ACACCCAGTT
 B*15:109          ------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                   180        190        200        210        220        230        240        250        260        270
 B*15:27:01        CGTGAGGTTC GACAGCGACG CCGCGAGTCC GAGGATGGCG CCCCGGGCGC CATGGATAGA GCAGGAGGGG CCGGAGTATT GGGACCGGGA GACACAGATC
 B*15:109          ---------- ---------- ---------T ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                   280        290        300        310        320        330        340        350        360        370
 B*15:27:01        TCCAAGACCA ACACACAGAC TTACCGAGAG AGCCTGCGGA ACCTGCGCGG CTACTACAAC CAGAGCGAGG CCG|GGTCTCA CACCCTCCAG AGGATGTTTG
 B*15:109          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---|------- ---------- ----------

 cDNA                   380        390        400        410        420        430        440        450        460        470
 B*15:27:01        GCTGCGACGT GGGGCCGGAC GGGCGCCTCC TCCGCGGGCA TGACCAGTCC GCCTACGACG GCAAGGATTA CATCGCCCTG AACGAGGACC TGAGCTCCTG
 B*15:109          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                   480        490        500        510        520        530        540        550        560        570
 B*15:27:01        GACCGCGGCG GACACGGCGG CTCAGATCAC CCAGCGCAAG TGGGAGGCGG CCCGTGAGGC GGAGCAGTGG AGAGCCTACC TGGAGGGCCT GTGCGTGGAG
 B*15:109          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                   580        590        600        610
 B*15:27:01        TGGCTCCGCA GATACCTGGA GAACGGGAAG GAGACGCTGC AGCGCGCGG
 B*15:109          ---------- ---------- ---------- ---------- ---------
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Fig. 3. The DNA sequence of B*15:109 is identical to B*15:27:01 in exons 2 and 3 except for one nucleotide exchange at residue 200 (C->T) (shaded).

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AA Pos.              10        20        30        40        50        60        70        80        90        100
 B*15:27:01  GSHSMRYFYT AMSRPGRGEP RFIAVGYVDD TQFVRFDSDA ASPRMAPRAP WIEQEGPEYW DRETQISKTN TQTYRESLRN LRGYYNQSEA GSHTLQRMFG
 B*15:109    *--------- ---------- ---------- ---------- --L------- ---------- ---------- ---------- ---------- ----------

 AA Pos.              110       120       130       140       150       160       170       180
 B*15:27:01  CDVGPDGRLL RGHDQSAYDG KDYIALNEDL SSWTAADTAA QITQRKWEAA REAEQWRAYL EGLCVEWLRR YLENGKETLQ RA
 B*15:109    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --
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Fig. 4. The nucleotide substitution of B*15:27:01 causes an amino acid replacement at residue 43 (proline->leucine) (shaded).

with the Singapore donor with B*15:109 as reported to the IMGT/HLA database (HC 14070) in order to determine the most probable HLA haplotypes that are found in association with C*16:04:01 and B*15:109 alleles, respectively. The frequencies of C*16:04:01 and B*15:109 in Taiwanese are extremely rare and occur at about 1 in 20,000 according to our HLA typing dataset. Therefore, we think the probable HLA-C*16:04:01 and HLA-B*15:109 associated haplotypes that we have deduced in this study are highly likely to be correct.

The HLA alleles are exponentially increasing in number because of recent improvements and developments in DNA-based molecular typing technology. By contrast, one important and outstanding fact is that the HLA diversity of every ethnic group is unique and important. Such information is important to securing safe outcomes for stem cell transplantation by avoiding graft rejection; furthermore, it also helps us understand graft-versus-host disease. Therefore, in addition to accurate determination of HLA rare alleles, haplotype matching between donors and recipients is of significant benefit to patients. Based on the above, our endeavor, which is aimed at resolving rare HLA alleles and determining their associated HLA haplotypes, will be persisted with and continued into the future.

Acknowledgments

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