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Original Article

HLA-A*33-B*58:45-DRB1*03, a deduced probable human leukocyte antigen haplotype associated with a human leukocyte antigen low-incidence allele B*58:45 in Taiwanese unrelated hematopoietic bone marrow stem cell donors

Kuo-Liang Yang [a, b, *], Reuy-Ho Kao [a], Chin-Lon Lin [a], Py-Yu Lin [a]

[a] Laboratory of Immunogenetics, Tzu Chi Cord Blood Bank and Buddhist Tzu Chi Marrow Donor Registry, Buddhist Tzu Chi Stem Cells Centre, Buddhist Tzu Chi General Hospital, Hualien, Taiwan
[b] Department of Laboratory Medicine, Tzu Chi University, Hualien, Taiwan

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ABSTRACT

Objective: HLA-B*58:45 is a low-incidence allele in the human leukocyte antigen-B (HLA) locus. The aim of this study is to confirm the ethnicity of B*58:45 and its deduced probable HLA-associated haplotype in Taiwanese unrelated bone marrow hematopoietic stem cell donors.
Materials and methods: A total of 40,000 healthy unrelated volunteer bone marrow stem cell donors (aged, 20–45 years) were tested using a sequence-based typing method. We confirmed the low-incidence allele B*58:45 in Taiwanese donors. Polymerase chain reaction was performed to amplify exon 2 and exon 3 of the HLA-A and HLA-B loci and exon 2 of the HLA-DRB1 locus using group-specific primer sets. The amplicons were sequenced in both directions with the BigDye Terminator Cycle Sequencing Ready Reaction Kit according to the manufacturer's protocols.
Results: The DNA sequence of B*58:45 is identical to B*58:01:01 in exon 2 and exon 3 except for residue 595, where G is changed to A (codon 175, GGG→AGG). The nucleotide replacement causes an amino acid change at codon 175 where G (glycine) of B*58:01:01 is replaced by R (arginine). We deduced the probable HLA haplotype associated with B*58:45 in Taiwanese donors to be A*33-B*58:45-DRB1*03.
Conclusion: Information on this deduced probable HLA haplotype in association with the low-incidence B*58:45 allele is of value for HLA testing laboratories for reference purposes and can help bone marrow donor registries find compatible donors for patients with this uncommon HLA allele.

1. Introduction

The human leukocyte antigen (HLA) genes are characterized by their extreme allelic polymorphism and by their variations and diversity in different ethnic groups and racial populations. New HLA alleles continue to be discovered and the recognition of HLA low-incidence alleles has enriched our understanding of the complexity of the HLA system. The genes encoding the HLA alleles are located in the major histocompatibility complex class I and II regions. HLA molecules have been definitely defined as transplant antigens and have a strong relevance to tissue transplantation. The similarity of these molecules in donors and recipients is being considered a predictive factor for graft survival and graft versus host disease. It is imperative to precisely characterize any unknown and low-incidence alleles encountered during routine HLA typing procedures. To facilitate successful, comprehensive unrelated bone marrow hematopoietic stem cell donor searches for patients in need of hematopoietic stem cell transplantation, persistent effort is needed to resolve unidentified, ambiguous, and low-incidence alleles to offer better HLA matching and donor selection.

HLA-B*58:45, a rare-frequency allele (http://www.allelefrequencies.net/hla6006a.asp), was first reported to the

Conflicts of interest: None.

* Corresponding author. Laboratory of Immunogenetics, Tzu Chi Cord Blood Bank and Buddhist Tzu Chi Marrow Donor Registry, Buddhist Tzu Chi Stem Cells Centre, Buddhist Tzu Chi General Hospital, 707, Section 3, Chung-Yang Road, Hualien, Taiwan. Tel.: +886 3 8561825x3373; fax: +886 3 8567851.
E-mail address: edward@tzuchi.com.tw (K.-L. Yang).

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IMGT/HLA database in 2013 (HWS10018426) without information on the associated HLA haplotype of the source individual [1]. The discovery of B*58:45 from an unknown HLA-B locus allele was the initial step in the identification of this previously unknown HLA allele. We report here the deduced plausible HLA haplotype in association with B*58:45, based on our observation of four Taiwanese unrelated bone marrow stem cell donors. Clinically, recognizing the Taiwanese ethnicity of B*58:45 can help bone marrow donor registries allocate stem cell donors for patients carrying B*58:45.

2. Materials and methods

We tested 40,000 healthy unrelated volunteer bone marrow stem cell donors (aged, 20–45 years) in this study. Peripheral whole-blood samples from donors with Taiwanese ethnicity were collected in acid citrate dextrose (ACD) anticoagulant. Formal written consent was signed by the donors before blood collection. The ACD whole-blood samples were 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). The DNA material was subjected to HLA genotyping for the HLA-A, HLA-B, and HLA-DRB1 loci using a commercial polymerase chain reaction sequencing-based typing kit (SeCore A/B/DRB1 locus sequencing kit; Life Technologies, Brown Deer, WI, USA). High-resolution allelic sequencing was performed as previously described [2–6]. The two sets of primer sequences used were as follows: (1) B-CG: M13-BIN1-CGG (sense): TGTAAAACGACGGCCAGTCGGGGGGCGCAGGACCCGG; P3′ exon 5B (antisense): GCTCCGATGACCACAACTGCT and (2) B-TA: M13-BIN1-TGA (sense): TGTAAAACGACGGCCAGTGGCGGGGGCGCAGGACCTGA; P3′ exon 5B (antisense): GCTCCGATGACCACAACTGCT. The amplicons were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) in both directions.

3. Results

Using a sequence-based typing method in 40,000 unrelated volunteer bone marrow stem cell donors, we detected four donors bearing the low-incidence B*58:45 allele. We confirmed that the DNA sequence of B*58:45 is identical to B*58:01:01 in exons 2 and 3, except for substitution of one nucleotide at residue 595 (G→A, codon 175 GGG→AGG; Fig. 1), which results in replacement of one amino acid at the amino acid position 175 (G→R; Fig. 2). The extended HLA-A, HLA-B, and HLA-DRB1 typing of the four unrelated bone marrow hematopoietic stem cell donors in our registry is shown in Table 1. Based on the common HLA-A, HLA-B, and HLA-DRB1 alleles of these donors, we deduced the probable HLA haplotype in association with B*58:45 in Taiwanese donors to be A*33-B*58:45-DRB1*03. Our observations also indicated the Taiwanese ethnicity of the rare HLA-B allele, B*58:45.

4. Discussion

We confirmed the DNA sequence and amino acid sequence of the low-frequency HLA allele B*58:45 in this study. B*58:45 was initially discovered in a Taiwanese individual (Genbank access number HF933202; IMGT HWS10018426) with HLA typing of A*02, A*33, B*46:01, B*58:45, DRB1*03, and DRB1*09:01 [1], without knowledge of the probable HLA haplotype in association with the allele. In this study, we propose that the deduced probable B*58:46-associated HLA haplotype to be A*33-B*58:45-DRB1*03 based on the HLA-A, HLA-B, and HLA-DRB1 alleles shared in common by the four unrelated bone marrow hematopoietic stem cell donors in our

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cDNA                 80         90        100        110        120        130        140        150        160        170
 B*58:01:01      GCTCCCA CTCCATGAGG TATTTCTACA CCGCCATGTC CCGGCCCGGC CGCGGGGAGC CCCGCTTCAT CGCAGTGGGC TACGTGGACG ACACCCAGTT
 B*58:45          ------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                180        190        200        210        220        230        240        250        260        270
 B*58:01:01    CGTGAGGTTC GACAGCGACG CCGCGAGTCC GAGGACGGAG CCCCGGGCGC CATGGATAGA GCAGGAGGGG CCGGAGTATT GGGACGGGGA GACACGGAAC
 B*58:45        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                280        290        300        310        320        330        340        350        360        370
 B*58:01:01    ATGAAGGCCT CCGCGCAGAC TTACCGAGAG AACCTGCGGA TCGCGCTCCG CTACTACAAC CAGAGCGAGG CCG|GGTCTCA CATCATCCAG AGGATGTATG
 B*58:45        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---|------- ---------- ----------

 cDNA                380        390        400        410        420        430        440        450        460        470
 B*58:01:01    GCTGCGACCT GGGGCCCGAC GGGCGCCTCC TCCGCGGGCA TGACCAGTCC GCCTACGACG GCAAGGATTA CATCGCCCTG AACGAGGACC TGAGCTCCTG
 B*58:45        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                480        490        500        510        520        530        540        550        560        570
 B*58:01:01    GACCGCGGCG GACACCGCGG CTCAGATCAC CCAGCGCAAG TGGGAGGCGG CCCGTGTGGC GGAGCAGCTG AGAGCCTACC TGGAGGGCCT GTGCGTGGAG
 B*58:45        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                580        590        600        610
 B*58:01:01    TGGCTCCGCA GATACCTGGA GAACGGGAAG GAGACGCTGC AGCGCGCGG
 B*58:45        ---------- ---------- ----A----- ---------- ---------
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Fig. 1. The raw sequence data (forward and reverse strains) show that the nucleotide G of B*58:01:01 is replaced by the nucleotide A (shaded) of B*58:45 at residue 595. cDNA = complementary DNA.

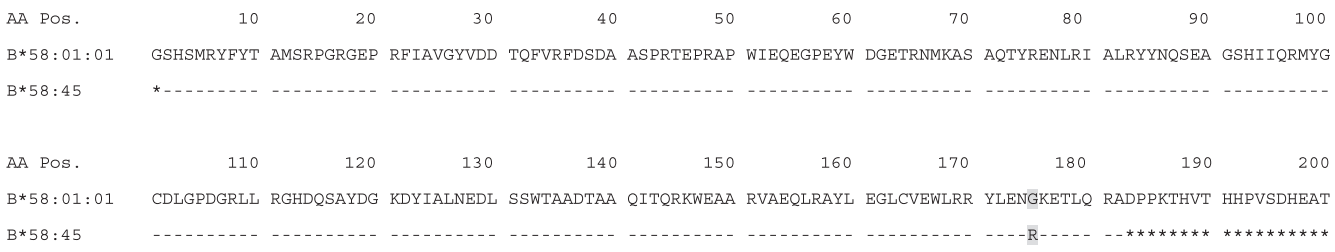

Fig. 2. The nucleotide substitution of B*58:45 from B*58:01:01 causes an amino acid replacement at residue 175 where glycine (G) of B*58:01:01 is changed to arginine (R) of B*58:45 (shaded). AA = amino acid.

Table 1
The HLA-A, HLA-B, and HLA-DRB1 alleles of the donors with B*58:45 and the deduced probable HLA-A-B-DRB1 haplotype associated with B*58:45.

Donor	HLA-A*	HLA-B*	HLA-DRB1*	Deduced probable B*58:45-associated HLA-A-B-DRB1 haplotype
346583	11 33	40 58:45	03 03	A*33-B*58:45-DRB1*03
346597	24 33	46:01 58:45	03 09:01	A*33-B*58:45-DRB1*03
370495	02 33	40 58:45	03 15	A*33-B*58:45-DRB1*03
371466	24 33	81 58:45	03 12	A*33-B*58:45-DRB1*03

HLA = human leukocyte antigen.

registry (Table 1). Furthermore, we propose that the deduced probable B*58:45-associated HLA haplotype is most likely restricted to Taiwanese individuals because, as far as we know, B*58:45 has only been reported in Taiwanese individuals [7].

Information on the ethnicity of B*58:45 and its linked HLA haplotype can be used in anthropological investigation of races. In addition, bone marrow donor registries can allocate appropriate unrelated stem cell donors for patients with B*58:45. Knowing the nucleotide and amino acid variation between B*58:45 and the prevalently observed B*58:01:01 allele may also be helpful when selecting a minor HLA-mismatched unrelated bone marrow stem cell donor for a patient with the rare B*58:45 allele.

It is worth mentioning that the most direct and classic method of determining HLA haplotypes is through a family study if test materials from a number of key family members are available. Alternatively, a population study may be employed if a sufficient number of unrelated donors are available [8]. However, the haplotypes deduced via population investigation are considered to be likely or most probable. In this study, because of the lack of availability of necessary test material from the families, we opted to determine the haplotypes by looking at the HLA alleles carried in common by unrelated donors with the same alleles of interest. By the same token, if determination of plausible HLA-associated haplotypes is for rare- or low-frequency HLA alleles, the alleles shared in common by unrelated individuals may be used to deduce the associated probable haplotypes [9–16]. The frequency of B*58:45 in Taiwanese individuals is extremely low at about one in 10,000 according our HLA typing practice and the allele frequency reported worldwide [7]. Therefore, we think the deduced probable B*58:45-associated HLA haplotypes in Taiwanese individuals that we deduced in this study are accurate.

The number of known HLA alleles is increasing with the recent development of DNA-based molecular typing technology. There is a high level of HLA diversity among ethnic groups and knowledge of this diversity is important. Matching of bone marrow stem cell donors relies on the accuracy of HLA typing results. This is dependent on the resolution of unknown, ambiguous, and low-incidence genes in the HLA system.

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