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Original Article

A deduced probable HLA-DRB1*16:35-associated HLA haplotype (A*11-B*13-DRB1*16:35) found in a case analysis of two Taiwanese unrelated bone marrow hematopoietic stem cell donors

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ABSTRACT

Objective: HLA-DRB1*16:35 is a low incidence allele in the HLA-DRB1 locus. The objective of this study is to report the ethnicity of DRB1*16:35 and its deduced probable HLA associated haplotype in two Taiwanese unrelated bone marrow hematopoietic stem cell donors and to determine its variation from DRB1*16:02:01 and DRB1*16:01:01.

Materials and Methods: A sequence-based typing method was employed to confirm the low incidence allele DRB1*16:35. 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 employing BigDye Terminator Cycle Sequencing Ready Reaction kits in both directions according to the manufacturer's protocols.

Results: The DNA sequence of DRB1*16:35 is identical to DRB1*16:02:01 in exons 2, except for residue 364 where the C of DRB1*16:02:01 is replaced by the T of DRB1*16:35 (codon 93, CGG->TGG). The nucleotide exchange leads to an amino acid alteration to the protein sequence of DRB1*16:02:01 at residue 93 where the arginine (R) of DRB1*16:02:01 is changed to the tryptophan (W) of DRB1*16:35. We deduced the probable HLA haplotype in association with DRB1*16:35 in Taiwanese to be A*11-B*13-DRB1*16:35.

Conclusion: Information on the deduced probable HLA haplotype in association with the low incidence DRB1*16:35 allele that we report here is of value for HLA testing laboratories for reference purposes. In addition, it can be used by stem cell transplantation donor search coordinators to determine a strategy for finding compatible donors in unrelated bone marrow donor registries when a patient has this uncommon HLA allele.

1. Introduction

New human leukocyte antigen (HLA) alleles continue to be revealed and the recognition of HLA low incidence alleles has enriched our understanding of the complexity of the HLA system. The major histocompatibility complex (MHC) in humans consists of several loci of genes that are located on the short arm of chromosome 6 at 6p21.3. These loci are classified into MHC class I, II and III regions. The genes encoding the 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 variations and diversity in different ethnic groups and racial populations. HLA molecules have been definitely defined as transplant antigens and have a strong relevance to tissue transplantation. Their molecular similarity 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 and comprehensive unrelated bone marrow hematopoietic stem cell donor searches for patients in need of hematopoietic stem cell transplantation, persistent efforts are needed to resolve unidentified,

Conflict of interest: none.

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ambiguous or low incidence alleles to offer better HLA matching and donor selection.

The nucleotide sequence of HLA-DRB1*16:35 was first submitted to GeneBank (accession number KP711799) and the name HLA-DRB1*16:35 was officially assigned by the World Health Organization HLA Nomenclature Committee [1]. However, there was no indication of its ethnicity and its associated HLA haplotype in the report. Here we report the Taiwanese ethnicity of DRB1*16:35 and the deduced probable HLA haplotype in association with DRB1*16:35 based on the HLA-A, -B and -DRB1 alleles commonly shared by the HLA typing of our two donors and a donor (donor ID HG00010094) submitted to the IMGT database [1].

2. Materials and methods

Peripheral whole blood samples from unrelated bone marrow hematopoietic stem cell donors with Taiwanese ethnicity were collected in acid citrate dextrose (ACD) anticoagulant. Formal written consents were signed by the donors before blood collection. The ACD whole blood samples were stored at −80°C until use. Genomic DNA was extracted using QIAamp DNA Blood Mini Kits 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 commercial polymerase chain reaction sequencing- based typing kits (Secore® A/B/DRB1 Locus Sequencing kits, 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: 1. B-CG: M13-BIN1-CGG (sense): TGTAAAACGACGGCCAGTCGGGGGCGCAGGACCCGG; P3′exon 5B (anti-sense): GCTCCGATGACCACAACTGCT and 2. B-TA: M13-BIN1-TGA (sense): TGTAAAACGACGGCCAGTGGCGGGGGCGCAGGACCTGA; P3′exon 5B (anti-sense): GCTCCGATGACCACAACTGCT. The amplicons were sequenced using a 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 DRB1*16:35 is identical to DRB1*16:02:01 in exon 2, except for residue 364 where the C of DRB1*16:02:01 is replaced by the T of DRB1*16:35 (codon 93, CGG->TGG) (Fig. 1A). The nucleotide exchange causes an amino acid alteration to the protein sequence of DRB1*16:02:01 at residue 93 where the arginine (R) of DRB1*16:02:01 is changed to the tryptophan (W) of DRB1*16:35 (Fig. 1B). The HLA typing of our donors with DRB1*16:35 is A*02, A*11, B*13, B*15, DRB1*08 and DRB1*16:35, and, A*11:01, A*24:02, B*40:02, B*13:01, DRB1*14:05 and DRB1*16:35. Together with the HLA typing (A*02, A*11, B*13, B*46, DRB1*09, and DRB1*16*:35) of the cell (donor ID HG00010094) submitted to the IMGT database [1], we deduced the probable HLA haplotype in association with DRB1*16:35 in our Taiwanese donor as A*11-B*13-DRB1*16:35. In addition, we found that the DNA sequence of DRB1*16:35 varies from DRB1*16:01:01 at residues 286 (C->T) and 364 (T->C) (Fig. 1A) in exon 2. The nucleotide variations lead to two amino acid differences between DRB1*16:35 and DRB1*16:01:01 (Fig. 1B).

4. Discussion

We confirmed the DNA sequence and amino acid sequence of the HLA allele DRB1*16:35 in this study. DRB1*16:35 was reported initially to the IMGT [1] without indication of its ethnicity [1]. Here we report the Taiwanese ethnicity of the DRB1*16:35 -bearing donor in our unrelated marrow donor registry. We deduced the probable DRB1*16:35-associated HLA haplotype to be A*11-B*13-DRB1*16:35, based on the commonly shared HLA typing of three unrelated individuals carrying DRB1*16:35. Our results also confirmed that the protein sequence of DRB1*16:35 varies from DRB1*16:02:01 by one amino acid whereas it varies from DRB1*16:01:01 by two amino acids. This information is important for DRB1*16:35 patients when a minor mismatched donor is being considered as a source of hematopoietic stem cell donation for bone marrow transplantation.

It is worth mentioning that the most direct and classic method to determine HLA haplotypes is through family study if test materials from a number of key family members are available. Alternatively, population study may be employed if a significant number of unrelated donors is available [2]. However, the haplotypes deduced via population investigation are considered as likely or most probable. In this study, because of the lack of availability of necessary test materials from the family of the donor with DRB1*16:35, we opted to determine the haplotype by looking at the HLA alleles carried in common by unrelated donors bearing the same allele of interest. By the same token, when determining

(A)

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DNA                      110        120        130        140        150        160        170        180        190        200
 DRB1*16:02:01 CACGTTTCCT GTGGCAGCCT AAGAGGGAGT GTCATTTCTT CAATGGGACG GAGCGGGTGC GGTTCCTGGA CAGATACTTC TATAACCAGG AGGAGTCCGT
 DRB1*16:01:01 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
 DRB1*16:35    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 DNA                  210        220        230        240        250        260        270        280        290        300
 DRB1*16:02:01 GCGCTTCGAC AGCGACGTGG GGGAGTACCG GGCGGTGACG GAGCTGGGGC GGCCTGACGC TGAGTACTGG AACAGCCAGA AGGACCTCCT GGAAGACAGG
 DRB1*16:01:01 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -----T---- ----------
 DRB1*16:35    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 DNA                  310        320        330        340        350        360
 DRB1*16:02:01 CGCGCCGCGG TGGACACCTA CTGCAGACAC AACTACGGGG TTGGTGAGAG CTTCACAGTG CAGCGGCGAG
 DRB1*16:01:01 ---------- ---------- ---------- ---------- ---------- ---------- ----------
 DRB1*16:35    ---------- ---------- ---------- ---------- ---------- ---------- ---T------
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(B)

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 AA Pos.                10         20         30         40         50         60         70         80         90        100
 DRB1*16:02:01 GDTRPRFLWQ PKRECHFFNG TERVRFLDRY FYNQEESVRF DSDVGEYRAV TELGRPDAEY WNSQKDLLED RRAAVDTYCR HNYGVGESFT VQRRVQPKVT
 DRB1*16:01:01 ---------- ---------- ---------- ---------- ---------- ---------- ------F--- ---------- ---------- ----------
 DRB1*16:35    *****----- ---------- ---------- ---------- ---------- ---------- ------W--- ---------- ---------- --W-******
```

Fig. 1. (A) The DNA sequence of *DRB1*16:35* is identical to *DRB1*16:02:01* in exon 2, except at residue 364 (codon 93; underlined), where the C of *DRB1*16:02:01* is changed to the T (shaded) of *DRB1*16:35*. *DRB1*16:01:01* varies from *DRB1*16:35* at residues 286 (T->C) and 364 (C->T). (B) The nucleotide exchange of *DRB1*16:02:01* causes an amino acid exchange at codon 93 where the arginine (R) of *DRB1*16:02:01* is replaced by the tryptophan (W) (shaded) of *DRB1*16:35*. The nucleotide variations between *DRB1*16:01:01* and *DRB1*16:35* lead to two amino acid differences between *DRB1*16:35* and *DRB1*16:01:01* (underlined). Dashes indicate nucleotide or amino acid identity with *DRB*16*02:01*.

plausible HLA haplotypes for rare or low frequency HLA alleles, the alleles shared in common by unrelated individuals may be employed to deduce associated probable haplotypes [3–10].

The frequency of DRB1*16:35 in Taiwanese is about 1 in 40,000 according our HLA typing practice. To date, the Allele Frequency Net Database (http://www.allelefrequencies.net/hla6006a.asp?hla_locus_type=Classical#) has yet to show the existence of the allele in the world population. Therefore, we think the probable DRB1*16:35-associated HLA haplotype in Taiwanese that we deduced in this study is highly accountable.

The significance of determining the ethnicity of individuals with DRB1*16:35 and its HLA linked haplotypes is that the information may be employed in anthropological investigation of races in addition to allowing search coordinators in unrelated bone marrow donor registries to allocate appropriate unrelated bone marrow hematopoietic stem cell donors for their patients. Evolution plays an essential force in the generation of polymorphism on HLA genotypes in humans. Polymorphism may promote recognition of variation between an individual and its environmental pathogens which may then allow the individual to mount an immunological defense strategy against infection. However, when compatibility becomes an issue in transplantation, HLA determination for polymorphism is essential in order to avoid graft rejection or graft-versus -host disease after tissue or organ transplantation.

The numbers of known HLA alleles are increasing exponentially with the recent development of DNA-based molecular typing technology. The outstanding HLA diversity in ethnic groups is unique and important. Facilitating an appropriate HLA-matched unrelated bone marrow stem cell donor for successful stem cell transplantations relies on the accuracy of HLA typing and the spirit and strength to resolve unknown, ambiguous and low incidence genes in the HLA system.

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