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

Kuo-Liang Yang a, b, *

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

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
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 subject 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): TGTAACCCGACCGCATTGGGCCCCGACGCGCGG; P3’ exon 5B (anti-sense): GCTCGATGACCAACTGTGCT and 2. B-TA: M13-BIN1-TGA (sense): TGTAACCCGACCGCATTGGGCCCCGACGCGCGG; P3’ exon 5B (anti-sense): GCTCGATGACCAACTGTGCT. 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 (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)

| DNA | 110 | 120 | 130 | 140 | 150 | 160 | 170 | 180 | 190 | 200 |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| DRB1*16:02:01 | CAACCTTTCCC GTGGCCAGCT AGAGGAGAC GTCACTTCTT CATGCGGAG CCAGGGCTG CTGTTGCTG ACGATATCT TCYTAACCAG AGGAGCTCTT |
| DRB1*16:16:01:01 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| DRB1*16:35 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |

| DNA | 210 | 220 | 230 | 240 | 250 | 260 | 270 | 280 | 290 | 300 |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| DRB1*16:02:01 | GCTCGATGACCA ACTGCTGCT ACGGCGCGCC GGGTGCAGGC CTGTTGACGG TGGTACCTGG ACGGCGCGG AGGAGCTCTT GGGAGCAAG |
| DRB1*16:16:01:01 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| DRB1*16:35 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |

| DNA | 310 | 320 | 330 | 340 | 350 | 360 |
|-----|-----|-----|-----|-----|-----|-----|
| DRB1*16:02:01 | GGGAGGCGGG TGGGCAACCC CTGGGACGC CAACTCGGAG TGGTGCAGGC CTTGACAGC CCAGGCGAG |
| DRB1*16:16:01:01 | ----- | ----- | ----- | ----- | ----- | ----- |
| DRB1*16:35 | ----- | ----- | ----- | ----- | ----- | ----- |

(B)

| AA Pos | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
|-------|----|----|----|----|----|----|----|----|----|----|
| DRB1*16:02:01 | GUTPHPLMG FKESECHFYNG TELKGFLSMT FYQHESSVVF DSDVOYERAV TELGRYPAELY HDSQKQLED SSAADVTYCR IMVGVKQLST VQHQVQPKVI |
| DRB1*16:16:01:01 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |
| DRB1*16:35 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- |

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 (C>T) and 364 (T>C). (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 DRB1*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?hlalocus_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.

Acknowledgements

We are indebted to all volunteer donors who willingly joined the Taiwan Buddhist Tzu Chi Bone Marrow Donor Registry and gave consent for our research project. Their unselfishness and efforts to help needy patients are most respected. We would like to give sincere gratitude to Dharma Master Cheng Yen, founder of the Buddhist Compassion Relief Tzu Chi Foundation, for continuing support and kind encouragement both intellectually and spiritually. Furthermore, the generosity and camaraderie of our colleagues are also greatly and deeply appreciated.

References

[1] Robinson J, Mistry K, McWilliam H, Lopez R, Parham P, Marsh SG. The IMGT/HLA database. Nucleic Acids Research 2011;39:D1171–6.
[2] Yang KL, Chen SP, Shyr MH, Lin PY. High-resolution human leukocyte antigen (HLA) haplotypes and linkage disequilibrium of HLA-B and -C and HLA-DRB1 and – DQB1 alleles in a Taiwanese population. Hum Immunol 2009;70:269–76.
[3] Yang KL, Lee SK, Lin CC, Jiang S, Chiu HM, Lin S, et al. Oriental HLA-A*11:90 detected in a Taiwanese cord blood sample and the haplotype in association with A*11:90 allele. Int J Immunogen 2011;38:543–6.
[4] Yang KL, Lee SK, Kao RH, Lin CL, Lin PY. Discovery of the novel HLA-DRB1*03:77 allele in a Taiwanese unrelated hematopoietic stem cell donor by a sequence-based typing method and identification of the probable HLA haplotype in association with DRB1*03:77. Int J Immunogen 2012;39:442–4.
[5] Yang KL, Lee SK, Kao RH, Lin CL, Lin PY. Discovery of the novel HLA-DRB1*16:16 allele in a Taiwanese unrelated bone marrow stem cell donor by a sequence-based typing method and the probable haplotype associated with DRB1*16:16. Int J Immunogen 2012;39:445–7.
[6] Yang KL, Lee SK, Kao RH, Lin CL, Lin PY. Discovery of the novel HLA-DRB1*10:04 allele in a Taiwanese volunteer bone marrow donor and identification of the probable HLA-A, -B, -C and -DRB1 haplotype in association with DRB1*10:04. Int J Immunogen 2012;39:448–50.
[7] Yang KL, Lee SK, Lin PY. Discovery of a novel HLA-B*51 variant, B*51:112, in a Taiwanese bone marrow donor and identification of the plausible HLA haplotype in association with B*51:112. Int J Immunogen 2012;39:451–3.
[8] Yang KL, Lee SK, Lin PY. Identification of the novel HLA allele, HLA-B*40:159, in a Taiwanese hematopoietic stem cell donor and the probable haplotype in an association with B*40:159. Int J Immunogen 2012;39:520–3.
[9] Yang KL, Lee SK, Kao RH, Lin CL, Lin PY. Recognition of HLA-A*24:137 allele in a Taiwanese unrelated bone marrow stem cell donor and the plausible HLA haplotype associated with A*24:137. Int J Immunogen 2012;39:530–1.
[10] Yang KL, Lin PY. Two conserved HLA haplotypes [HLA-A*11:127N-B*54:01-DRB1*04:05 and HLA-A*11:01-B*40:221-C*03:04-DRB1*14:54-DQB1*05:02] observed in the Taiwan population. Tzu Chi Med J 2013;25:218–20.