Tzu Chi Medical Journal 27 (2015) 15–17

Contents lists available at ScienceDirect

Tzu Chi Medical Journal

journal homepage: www.tzuchimedjnl.com

Original Article

Deduced probable HLA-B*40:01:35-associated HLA haplotype (A*24-B*40:01:35-DRB1*11) found in a Taiwanese unrelated hematopoietic bone marrow stem cell donor

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ARTICLE INFO

Article history:
Received 11 December 2014
Received in revised form
29 December 2014
Accepted 7 January 2015
Available online 5 March 2015

Keywords:
Haplotype
Hematopoietic stem cell
HLA
Sequence-based typing
Transplantation

ABSTRACT

Objective: Human leukocyte antigen (HLA)-B*40:01:35 is a low incidence allele in the HLA-B locus. The objective of this study is to report the ethnicity of B*40:01:35 and its deduced probable HLA associated haplotype in a Taiwanese unrelated bone marrow hematopoietic stem cell donor.
Materials and methods: A sequence-based typing method was employed to confirm the low incidence allele B*40:01:35. Polymerase chain reaction was performed to amplify exons 2 and 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 using the BigDye Terminator Cycle Sequencing Ready Reaction kit in both directions according to the manufacturer's protocols.
Results: The DNA sequence of B*40:01:35 is identical to B*40:01:01 in exons 2 and 3, except for residue 324 where C is changed to T (codon 84, TAC→TAT). The nucleotide exchange does not cause amino acid alteration to the protein sequence of B*40:01:01 due to the silent mutation. We deduced the probable HLA haplotype in association with B*40:01:35 in Taiwanese to be A*24-B*40:01:35-DRB1*11.
Conclusion: Information on the deduced probable HLA haplotype in association with the low incidence B*40:01:35 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 discovered 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 between 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.

HLA-B40:01:35, a rare frequency allele (http://www.allelefrequencies.net/hla6006a.asp), was first reported to the

Conflicts of interest: none.

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http://dx.doi.org/10.1016/j.tcmj.2015.01.002

IMGT/HLA database in 2014 (HC32533) without information on the ethnicity and its associated HLA haplotype of the source individual [1]. Here, we report the Taiwanese ethnicity of B*40:01:35 and the deduced probable HLA haplotype in association with B*40:01;35 based on the HLA-A, -B, and -DRB1 alleles commonly shared by the HLA typing of our donor and a donor (donor ID D10057233) submitted to the IMGT database [1]. We further speculate that the deduced plausible HLA haplotype associated with B*40:01:35 is restricted to Asians.

2. Materials and methods

A peripheral whole blood sample from an unrelated bone marrow hematopoietic stem cell donor with Taiwanese ethnicity was collected in acid citrate dextrose anticoagulant. Formal written consent was signed by the donor before blood collection. The acid citrate dextrose whole blood sample 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). 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: (1) B-CG: M13-BIN1-CGG (sense): TgTAAAACgACggCCAgTCgggggCgCAggACCCgg; P3′exon 5B (antisense): gCTCCgATgACCACAACTgCT; and (2) B-TA: M13-BIN1-TGA (sense): TgTAAAACgACggCCAgTggCgggggCgCAggACCTgA; P3′exon 5B (antisense): 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 B*40:01:35 was identical to B*40:01:01 in exons 2 and 3, except for residue 324, where C was changed to T (codon 84; TAC->TAT) (Fig. 1). The nucleotide exchange did not cause amino acid alteration to the protein sequence of B*40:01:01 due to the silent mutation. The extended HLA-A, -B, and -DRB1 typing of our donor with B*40:01:35 was A*24, B*40:01:35, B*46, DRB1*08, and DRB1*11. Together with the HLA typing (A*11:01, A*24:02, B*40:01:35, B*40:01, DRB1*04:05, and DRB1*11:01) of the cell (D10057233) submitted to the IMGT/HLA database [1], we deduced the probable HLA haplotype in association with B*40:01:35 in our Taiwanese donor as A*24-B*40:01:35-DRB1*11.

4. Discussion

We confirmed the DNA sequence and amino acid sequence of the low frequency HLA allele, B*40:01:35 in this study. B*40:01:35

A.

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cDNA                    80         90         100        110        120        130        140        150        160        170
 B*40:01:01          GCTCCCA CTCCATGAGG TATTTCCACA CCGCCATGTC CCGGCCCGGC CGCGGGGAGC CCCGCTTCAT CACCGTGGGC TACGTGGACG ACACGCTGTT
 B*40:01:35          ------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                   180        190        200        210        220        230        240        250        260        270
 B*40:01:01        CGTGAGGTTC GACAGCGACG CCACGAGTCC GAGGAAGGAG CCGCGGGCGC CATGGATAGA GCAGGAGGGG CCGGAGTATT GGGACCGGGA GACACAGATC
 B*40:01:35        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                   280        290        300        310        320        330        340        350        360        370
 B*40:01:01        TCCAAGACCA ACACACAGAC TTACCGAGAG AGCCTGCGGA ACCTGCGCGG CTACTACAAC CAGAGCGAGG CCG|GGTCTCA CACCCTCCAG AGGATGTACG
 B*40:01:35        ---------- ---------- ---------- ---------- ---------- ---T------ ---------- ---|------- ---------- ----------

 cDNA                   380        390        400        410        420        430        440        450        460        470
 B*40:01:01        GCTGCGACGT GGGGCCGGAC GGGCGCCTCC TCCGCGGGCA TAACCAGTAC GCCTACGACG GCAAGGATTA CATCGCCCTG AACGAGGACC TGCGCTCCTG
 B*40:01:35        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                   480        490        500        510        520        530        540        550        560        570
 B*40:01:01        GACCGCCGCG GACACGGCGG CTCAGATCTC CCAGCGCAAG TTGGAGGCGG CCCGTGTGGC GGAGCAGCTG AGAGCCTACC TGGAGGGCGA GTGCGTGGAG
 B*40:01:35        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                   580        590        600        610
 B*40:01:01        TGGCTCCGCA GATACCTGGA GAACGGGAAG GACAAGCTGG AGCGCGCTG
 B*40:01:35        ---------- ---------- ---------- ---------- ---------
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B.

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AA Pos.                 10         20         30         40         50         60         70         80         90         100
 B*40:01:01        GSHSMRYFHT AMSRPGRGEP RFITVGYVDD TLFVRFDSDA TSPRKEPRAP WIEQEGPEYW DRETQISKTN TQTYRESLRN LRGYYNQSEA GSHTLQRMYG
 B*40:01:35        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----Y------ ----------

 AA Pos.                110        120        130        140        150        160        170        180        190        200
 B*40:01:01        CDVGPDGRLL RGHNQYAYDG KDYIALNEDL RSWTAADTAA QISQRKLEAA RVAEQLRAYL EGECVEWLRR YLENGKDKLE RADPPKTHVT HHPISDHEAT
 B*40:01:35        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 AA Pos.                210        220        230        240        250        260        270        280        290        300
 B*40:01:01        LRCWALGFYP AEITLTWQRD GEDQTQDTEL VETRPAGDRT FQKWAAVVVP SGEEQRYTCH VQHEGLPKPL TLRWEPSSQS TVPIVGIVAG LAVLAVVVIG
 B*40:01:35        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 AA Pos.                310        320        330
 B*40:01:01        AVVAAVMCRR KSSGGKGGSY SQAACSDSAQ GSDVSLTA
 B*40:01:35        ---------- ---******* ********** ********
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Fig. 1. (A) The DNA sequence of B*40:01:35 is identical to B*40:01:01 in exons 2 and 3, except for residue 324 at codon 84 (underlined), where C is changed to T (shaded); (B) the nucleotide exchange does not cause amino acid (tyrosine; Y) alteration to the sequence of B*40:01:01 due to the silent mutation of codon 84 TAC→TAT (shaded). Dashes indicate nucleotide identity with B*40:01:01.

was initially detected in an individual from China (with HLA typing of A*11:01, A*24:02, B*40:01:35, B*40:01, DRB1*04:05, and DRB1*11:01) [1]. We deduced the probable B*40:01:35-associated HLA haplotype to be A*24-B*40:01:35-DRB1*11, based on the commonly shared HLA typing of our donor and the donor's typing submitted to the IMGT/HLA database. We further postulate that individuals bearing the B*40:01:35 allele are probably Asian, since the donor carrying B*40:01:35 we detected is Taiwanese and the other donor with B*40:01:35 reported to the IMGT/HLA database [1] is from China.

It is worth mentioning that the most direct and classic method to determine HLA haplotype is through family study if test materials from a number of key family members are available. Alternatively, a 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, we opted to determine the haplotype by looking at the HLA alleles carried in common by unrelated donors bearing the same alleles of interest. By the same token, if determination of plausible HLA haplotypes is 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 B*40:01:35 in Taiwanese is extremely low, about 1 in 20,000, according our HLA typing practice and the Allele Frequency Net Database (http://www.allelefrequencies.net/hla6006a.asp?hla_locus_type=Classical#). Therefore, we think the probable B*40:01:35-associated HLA haplotype in Taiwanese that we deduced in this study is highly accountable.

The significance of determining the ethnicity of individuals with a rare allele and its HLA linked-haplotypes is that the information may be used 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. In this particular instance, we should be aware that the nucleotide variation of B*40:01:35 from B*40:01:01 does not differ from the protein sequence of B*40:01:35 from B*40:01:01 in exons 2 and 3. Therefore, for patients with B*40:01:35 needing a donor for hematopoietic stem cell transplantation, a donor with B*40:01:01 may be considered in the absence of a donor with B*40:01:35.

The number of known HLA alleles is 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. Due to the silent mutation, B*40:01:01 and B*40:01:35 share an identical amino acid sequence of their mature proteins. Donors with B*40:01:01 or B*40:01:35 may be considered for hematopoietic stem cell transplant patients bearing the B*40:01:35 allele. Therefore, in the absence of a donor with the haplotype A*24-B*40:01:35-DRB1*11, a donor with A*24-B*40:01-DRB1*11 may be considered as a substitution for a transplant patient with the A*24-B*40:01:35-DRB1*11 haplotype. In addition, according to our previous study [2], A*24-B*40:01-DRB1*11 is a fairly common haplotype in Taiwanese.

Acknowledgments

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 thanks 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.

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