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Original Article

Human leukocyte antigen-A*24:02-B*40:247-C*03:04-DRB1*16:02, a deduced probable human leukocyte antigen haplotype associated with a low-incidence human leukocyte antigen allele B*40:247 in Taiwanese individuals: A case analysis

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Abstract

Objective: HLA-B*40:247 is a low incidence allele in the HLA-B locus. The aim of this study is to confirm the ethnicity of B*40:247 and its deduced probable HLA- associated haplotype in Taiwanese individuals. **Materials and Methods:** A total of 2,329 unrelated Taiwanese individuals and 66,212 unrelated mainland Chinese individuals were tested for HLA using a sequence-based typing method. We confirmed the low incidence allele B*40:247 in Taiwanese. Polymerase chain reaction was performed to amplify exons 2, 3 and 4 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*40:247 is identical to B*40:01:01 in exons 2, 3 and 4 except for residue 853, where G of B*40:01:01 is changed to A in B*40:247 (codon 261, GTA->ATA). The nucleotide replacement causes a one amino acid change at codon 261 where V (valine) of B*40:01:01 is replaced by I (isoleucine) in B*40:247. We deduced the probable HLA haplotype associated with B*40:247 in Taiwanese to be HLA-A*24:02-B*40:247-C*03:04-DRB1*16:02. **Conclusion:** Information on the ethnicity and distribution of B*40:247 and its deduced probable HLA haplotype in association with the low incidence 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.

Keywords: *Haplotype, HLA, Sequence-based typing, Taiwanese, Transplantation*

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 HLA genes are characterized by their extreme allelic polymorphism as well as their variations and diversity in different ethnic groups and racial populations. 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. Their molecular similarity in donors and recipients is being considered a predictive factor for graft survival and graft versus host disease [1]. It is imperative to precisely characterize any unknown and low-incidence alleles encountered during routine HLA typing procedures. To provide successfully, 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*40:247, a rare frequency allele (http://www.allele-frequencies.net), was first reported to the IMGT/HLA database in 2013 (IMGT/HLA Ass No: HLA09904) without information

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on the B*40:247-associated HLA haplotype and ethnic origin of the source individual [2]. In this report, we confirm the ethnicity of B*40:247 and identify the deduced plausible HLA haplotype in association with B*40:247 based on the HLA typing of Taiwanese individuals and the donor submitted to the IMGT/HLA database [2].

Materials and methods

A total of 2329 unrelated Taiwanese individuals and 66,212 unrelated mainland Chinese participants were tested for B*40:247 in this study. Peripheral whole blood samples from donors with Taiwanese ethnicity and individuals with mainland Chinese 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 −80ºC 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, HLA-C, and HLA-DRB1 loci using a commercial polymerase chain reaction-sequencing-based typing kit (TBG, Medigen Biotechnology, Taipei, Taiwan). The amplicons were sequenced in both directions with the *BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocols.

Results

In a total of 2329 randomized unrelated Taiwanese individuals tested, two unrelated Taiwanese Hakka individuals with B*40:247 were identified. This means that the frequency of B*40:247 in the Taiwanese population is approximately 0.086%. However, in a total of 66,212 randomized mainland Chinese blood donors studied, no individual with B*40:247 was found. We confirmed that the DNA sequence of B*40:247 is identical to B*40:01:01 in exons 2, 3, and 4 except for a one nucleotide substitution at residue 853 (G->A, codon 261 GTA->ATA) in exon 4 [Figure 1]. The nucleotide substitution results a one amino acid replacement at amino acid position 261 where V (valine) of B*40:01:01 is replaced by I (isoleucine) in B*40:247 [Figure 2]. The extended HLA-A, HLA-B, HLA-C, and HLA-DRB1 typing of the donors with B*40:247 in this study was A*02:06, A*24:02, B*15:25, B*40:247, C*03:04, C*04:03, DRB1*12:02, and DRB1*16:02, and A*02:03, A*24:02, B*38:02, B*40:247, C*03:04, C*07:02, and DRB1*16:02. Based on the common HLA-A, HLA-B, HLA-C, and HLA-DRB1 alleles of these two donors and the donor with HLA typing (A*11:01, A*24:02, B*40:01, B*40:247, C*03:04, C*07:02, DRB1*12:01, and DRB1*16:02) reported to the IMGT/HLA database [2,3], we deduced the probable HLA haplotype in association with B*40:247 in Taiwanese to be A*24:02-B*40:247-C*03:04-DRB1*16:02 [Table 1]. Our observation strongly indicates Taiwanese ethnicity of the rare HLA-B allele, B*40:247.

Discussion

We confirmed the DNA sequence and amino acid sequence of the low-frequency allele HLA-B*40:247 [2]. The DNA sequence of B*40:247 is identical to B*40:01:01 in exons 2, 3, and 4 except for a one nucleotide substitution at residue 853. The nucleotide substitution results in a one amino acid replacement at amino acid position 261 in B*40:247. Whether the mutation of B*40:247 in exon 4 has any impact on antigen presentation, and immune regulation functions of its protein molecule are unclear. This warrants the future study.

B*40:247 was initially discovered in a Taiwanese individual (Genbank Accession Number KF208638; IMGT/HLA Database HWS10018882) [2] without knowledge of the probable HLA haplotype in association with the allele. In this study, we ascertained the ethnicity of B*40:247 and proposed the deduced probable B*40:247-associated HLA haplotype to be A*24:02-B*40:247-C*03:04-DRB1*16:02 based on the HLA-A, HLA-B, HLA-C, and HLA-DRB1 alleles shared in common by our randomized unrelated donors and the donor reported to the IMGT/HLA Database [Table 1]. Although we found two Hakka Taiwanese individuals carrying B*40:247, we cannot be certain B*40:247 is restricted to Hakka Taiwanese, pending further investigation. Nevertheless, we propose that the deduced probable B*40:247-associated HLA haplotype is most likely restricted to Taiwanese since B*40:247 has only been found in the Taiwanese population but not in the mainland Chinese population. Furthermore, our search on the Allele Frequency Net (http://www.allelefrequencies.net) failed to find B*40:247 in other populations. In addition, the lack of B*40:247 in the mainland Chinese population is supported by Zhou *et al*.[4], who found no individual with B*40:247 in a total of 169,995 volunteer donors tested from the China Bone Marrow Donor Registry Program. Information on the ethnicity of B*40:247 and its linked HLA haplotype can be employed in the anthropological investigation of races. In addition, bone marrow donor registries can allocate appropriate unrelated stem cell donors for patients with B*40:247. In addition, knowing the

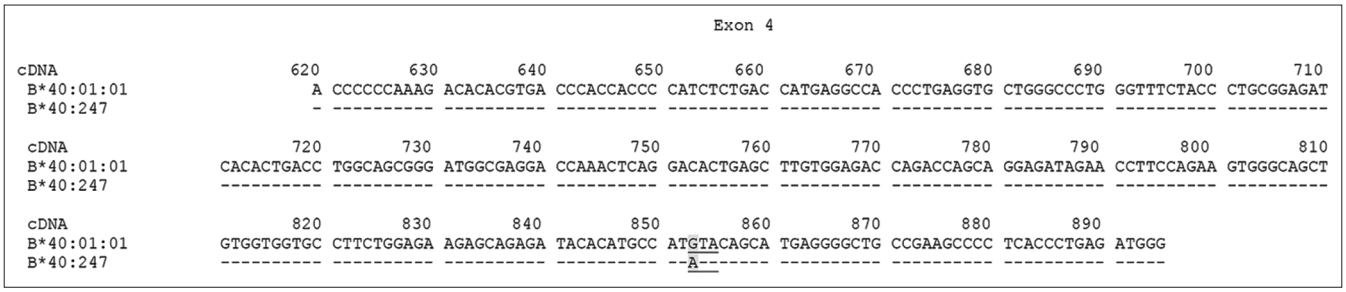

Figure 1: The DNA sequence of B*40:247 is identical to B*40:01:01 in exons 2, 3, and 4 except for a one nucleotide substitution at residue 853 (shaded) where G of B*40:01:01 is replaced by A in B*40:247 (codon 261; GTA->ATA; underlined) in exon 4 (only exon 4 is shown here). Dashes indicate nucleotide identity with B*40:01:01

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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*40:247          *--------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 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*40:247          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 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 LAVL.AVVVIG
 B*40:247          ---------- ---------- ---------- ---------- ---------- ---------- I--------- ----****** ********** ***********
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Figure 2: The nucleotide substitution of B*40:01:01 results in a one amino acid replacement at amino acid position 261 (shaded) where V (valine) of B*40:01:01 is replaced by I (isoleucine) in B*40:247. Dashes indicate amino acid identity with B*40:01:01

Table 1: The deduced plausible HLA haplotype in association with B*40:247

Donor ID	HLA-A, -B, -C, -DRB1 typing of donors	Deduced probable HLA haplotype in association with B*40:247
Donor 1	A*02:06, A*24:02, B*15:25, B*40:247, C*03:04, C*04:03, DRB1*12:02 DRB1*16:02	A*24:02-B*40:247-C*03:04-DRB1*16:02
Donor 2	A*02:03, A*24:02, B*38:02, B*40:247, C*03:04, C*07:02, DRB1*16:02	A*24:02-B*40:247-C*03:04-DRB1*16:02
Donor 3[a]	A*11:01, A*24:02, B*40:01, B*40:247, C*03:04, C*07:02, DRB1*12:01 DRB1*16:02	A*24:02-B*40:247-C*03:04-DRB1*16:02

The plausible HLA haplotype in association with B*40:247 is deduced based on HLA typing of the two Taiwanese individuals we observed and the donor[a] submitted to the IMGT/HLA Database

nucleotide and amino acid variation between B*40:247 and the prevalently observed B*40:01:01 allele may be helpful when selecting a minor HLA mismatched unrelated bone marrow stem cell donor for a patient with the rare B*40:247 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 [5]. However, the haplotypes deduced through population investigation are considered to be likely or most probable. In this study, because of the lack of availability of necessary test materials from the families of the individuals with B*40:247, 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 employed to deduce the associated probable haplotypes [6-13]. The frequency of B*40:247 in Taiwanese is extremely low at approximately 0.086%. Therefore, we think that the deduced probable B*40:247-associated HLA haplotype in Taiwanese in this study is 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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Declaration of patient consent

The authors certify that all patients provided appropriate patient consent forms. In the form, all patients gave consent for their images and other clinical information to be reported in the journal. All patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There is no conflict of interest.

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