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Original Article

# The deduced probable human leukocyte antigen haplotype associated with human leukocyte antigen low incidence allele B*40:36 (A*02-B*40:36-DRB1*12) in Taiwanese unrelated hematopoietic bone marrow stem cell donors

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ABSTRACT

*Objective:* Human leukocyte antigen (HLA)-B*40:36 is a low incidence allele in the HLA-B locus. This study reports the ethnicity of B*40:36 and the deduced probable HLA haplotype associated with HLA-B*40:36 in Taiwanese unrelated bone marrow hematopoietic stem cell donors.
*Materials and methods:* A sequence-based typing method was employed to confirm that the low incidence allele was present. Polymerase chain reaction was performed to amplify exons 2 and 3 in the HLA-A and HLA-B loci and exon 2 in 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.
*Results:* The DNA sequence of B*40:36 is identical to B*40:01:01 in exons 2 and 3, except for a one nucleotide substitution at residue 419 (A→T), which results in a one amino acid replacement at position 116 (Y→F; tyrosine→phenylalanine). We deduced the probable HLA haplotype in an association with B*40:36 in Taiwanese to be A*02-B*40:36-DRB1*12.
*Conclusion:* Information on the deduced probable HLA haplotype in an association with the low incidence B*40:36 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 the likelihood of 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 among different ethnic groups and racial populations. HLA molecules have been definitely defined as transplant antigens and have a strong relevance to tissue transplantation. Their molecule similarity between donors and recipients is being considered to be a predictive factor for graft survival and graft versus host disease. It is imperative to characterize precisely 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, a persistent effort is needed to resolve unidentified, ambiguous or low incidence alleles in order to offer a better service in terms of HLA matching and donor selection.

Conflicts of interest: none.

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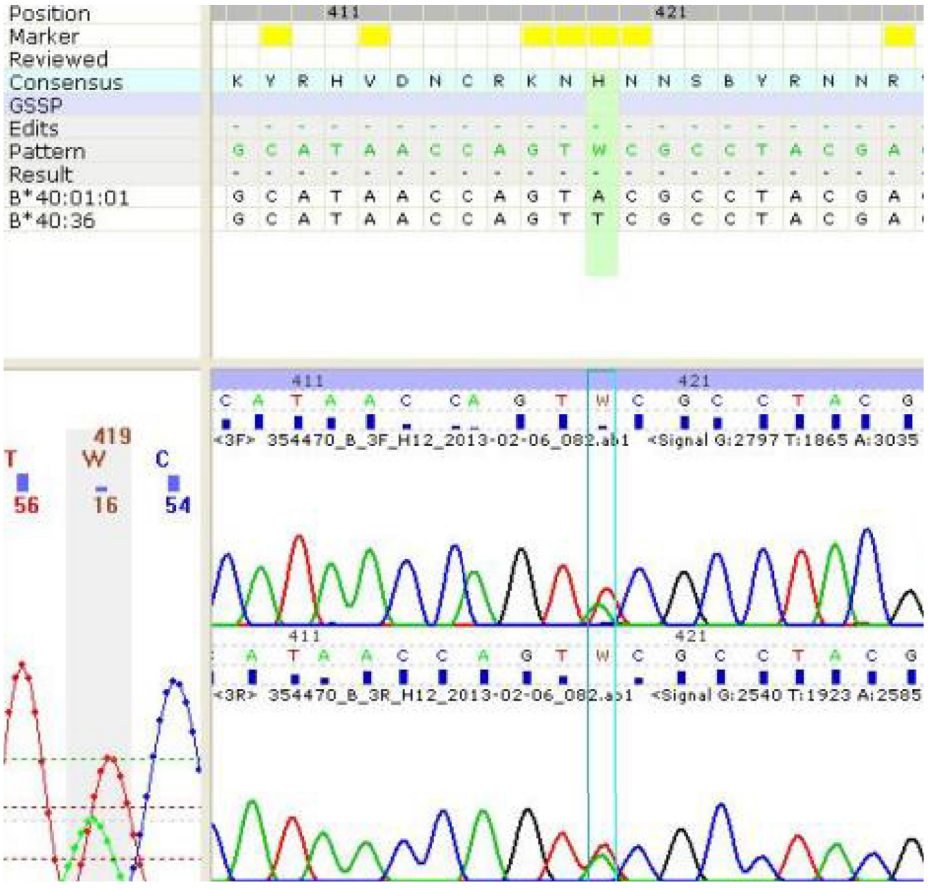
HLA-B*40:36, a rare frequency allele (http://www.allelefrequencies.net/hla6006a.asp), was first reported to the ImMunoGeneTics (IMGT)/HLA database in 2001 (HLA01482) without information on the ethnicity and its associated HLA haplotype of the source individual [1]. Here we report the Taiwanese ethnicity of B*40:36 and the deduced probable HLA haplotype in an association with B*40:36; this is based on our observation of eight Taiwanese unrelated bone marrow stem cell donors. We further speculate that the deduced plausible HLA haplotype associated with B*40:36 is restricted to Taiwanese.

## 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 anticoagulant. Formal written consent was signed by the donors prior to blood collection. The 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

**A**




**B**

```
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*40:36         ------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 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*40:36         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 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*40:36         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---|------- ---------- ----------

 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*40:36         ---------- ---------- ---------- ---------- --------T- ---------- ---------- ---------- ---------- ----------

 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*40:36         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                 580         590         600         610
 B*40:01:01      TGGCTCCGCA GATACCTGGA GAACGGGAAG GACAAGCTGG AGCGCGCTG
 B*40:36         ---------- ---------- ---------- ---------- ---------
```

**Fig. 1.** (A) The raw sequence data (forward and reverse strains) show that, at residue 419, the nucleotide A of B*40:01:01 is replaced by the nucleotide T (in red) of B*40:36. (B) The DNA sequence of B*40:36 is identical to B*40:01:01 in exons 2 and 3, except for the one nucleotide substitution at residue 419 (A→T; shaded). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

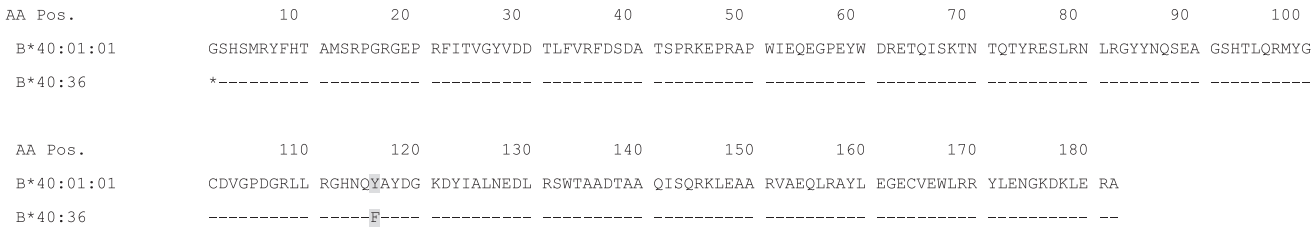



**Fig. 2.** The nucleotide substitution of B*40:36 from B*40:01:01 causes an amino acid replacement at residue 116 (tyrosine→phenylalanine; Y→F; shaded).

HLA-DRB1 loci using a commercial polymerase chain reaction-sequencing based typing kit, namely the 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): TgTAAAACgACggCCAgTCgggggCgCAg-gACCCgg; P3′exon 5B (antisense): gCTCCgATgACCACAACTgCT and 2. B-TA: M13-BIN1-TGA (sense): TgTAAAACgACggCCAgTggCgggggCg-CAggACCTgA; and P3′exon 5B (antisense): gCTCCgATgACCA-CAACTgCT. 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*40:36 was identical to B*40:01:01 in exons 2 and 3, except for a one nucleotide substitution at residue 419 (A→T; Fig. 1) which results in one amino acid replacement at position 116 (tyrosine→phenylalanine; Y→F; Fig. 2). The extended HLA-A, HLA-B, and HLA-DRB1 typing of our eight bone marrow stem cell donors with B*40:36 are shown in Table 1. Based on the commonly shared HLA alleles of the eight donors with B*40:36 in Table 1, we deduced the probable HLA haplotype in an association with B*40:36 for our Taiwanese unrelated bone marrow donors to be A*02-B*40:36-DRB1*12. Our observation also indicated the Taiwanese ethnicity of the rare HLA-B allele, B*40:36.

## 4. Discussion

We confirmed the DNA sequence and amino acid sequence of the low frequency HLA allele B*40:36 in this study. B*40:36 was initially discovered in an individual (IMGT access number HLA01482) with HLA typing of A*24, A*34, B*15:21, B*40:36 [1], but this was without information on the individual's ethnicity and the associated HLA haplotype. Two probable B*40:36 associated HLA-A-B haplotypes may be deduced from this donor and these are A*24-B*40:36 and A*34-B*40:36. In this study we have been able to deduce the probable HLA A-B-DRB1 haplotype from the eight Taiwanese unrelated bone marrow stem cell donors with A*40:36, based on their commonly shared HLA-A, HLA-B, and HLA-DRB1 alleles, to be A*02-B*40:36-DRB1*12 (Table 1). The deduced probable Taiwanese B*40:36 associated HLA haplotype differs from the deduced probable A*40:36 associated HLA haplotypes of the individual reported to the IMGT database, suggesting that B*40:36 associated HLA haplotype may vary between different ethnic groups or racial populations. Furthermore, we propose that the deduced probable A*02-B*40:36-DRB1*12 haplotype in Taiwanese is most likely to be restricted to Taiwanese.

The significance of determining the ethnicity of B*40:36 and its linked HLA haplotype is that the information may now be employed in anthropological investigation of races. In addition, it allows search coordinators using unrelated bone marrow donor registries to allocate appropriate unrelated bone marrow hematopoietic stem cell donors if their patient is carrying B*40:36. Additionally, to know the nucleotide and amino acid variation between B*40:36 and the prevalently observed B*40:01:01 allele may be helpful in hematopoietic stem cell transplantation when selecting a minor HLA mismatched unrelated bone marrow stem cell donor for a patient bearing the rare B*40:36 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 there is a significant and sufficient number of unrelated donors available [7]. However, the haplotypes deduced via population investigation are considered to be likely or most probable. In this study, due to the lack of availability of the necessary test material from the families, we opted to determine the haplotypes by looking at the HLA alleles carried in common by the unrelated donors bearing the same alleles of interest. By the same token, if determination of plausible HLA associated haplotypes is for a rare or low frequency HLA allele, the alleles shared in common by unrelated individuals may be employed to deduce the associated probable haplotypes [8–15]. The frequency of B*40:36 in Taiwanese is extremely low at about 1 in 20,000 according our HLA typing practice and the Allele Frequencies in World Population (http://www.allelefrequencies.net/hla6006a.asp?hla_locus_type=Classical#). Therefore, we think the probable B*40:36 associated HLA haplotypes in Taiwanese that we deduced in this study is accurate.

**Table 1**
The HLA-A, HLA-B, and HLA-DRB1 alleles of the donors with B*40:36 and the deduced probable HLA-A-B-DRB1 haplotype associated with B*40:36.

| Donor | HLA-A* | | HLA-B* | | HLA-DRB1* | | Deduced probable HLA-A-B-DRB1 haplotype |
|---|---|---|---|---|---|---|---|
| Donor 1 | 02:03 | 11:01 | 40:36 | 58:AD | 12:02 | 13:AB | A*02-B*40:36-DRB1*12 |
| Donor 2 | 02:03 | 33:03 | 40:36 | 58:AD | 12:02 | 13:AB | A*02-B*40:36-DRB1*12 |
| Donor 3 | 02:03 | 11:XX | 40:36 | 40:CACB | 12:XX | 08:XX | A*02-B*40:36-DRB1*12 |
| Donor 4 | 02:FFDM | 11:XX | 40:36 | 58:XX | 12:ADBG | 03:FHFN | A*02-B*40:36-DRB1*12 |
| Donor 5 | 02:SNFA | 02:SNFA | 40:36 | 40:TXZG | 12:02 | 12:02 | A*02-B*40:36-DRB1*12 |
| Donor 6 | 02:XX | 11:XX | 40:36 | 46:YGKD | 12:02 | 09:01 | A*02-B*40:36-DRB1*12 |
| Donor 7 | 02:SNFA | 11:PDVH | 40:36 | 40:XFBK | 12:02 | 08:03 | A*02-B*40:36-DRB1*12 |
| Donor 8 | 02:TSXZ | 02:XX | 40:36 | 40:ZAMB | 12:02 | 12:DUKV | A*02-B*40:36-DRB1*12 |

HLA = human leukocyte antigen.

The number of HLA alleles is ever exponentially increasing with the recent development of DNA-based molecular typing technology. By contrast, an outstanding level of the HLA diversity is found in every ethnic group and this diversity is unique and important. Facilitating an appropriate HLA-matched unrelated bone marrow stem cell donor for a given needy patient that allows successful bone marrow stem cell transplantations relies on the accuracy of HLA typing result. This is dependent on the spirit and strength needed to resolve unknown, ambiguous, and low incidence genes in the HLA system. Our challenge is enormous and most rewarding.

## Acknowledgments

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

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