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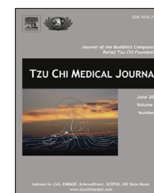

Original Article

Recognition of the deduced probable HLA haplotypes associated with HLA low incidence alleles B*13:50 (A*11:02-B*13:50-DRB1*07:01) and B*51:39 (A*02-B*51:39-DRB1*15; and A*11-B*51:39-DRB1*15) in Taiwanese unrelated hematopoietic stem cell donors

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ABSTRACT

Objectives: HLA-B*13:50 and -B*51:39 are two low incidence alleles in the HLA-B locus. The objective of this study is to report the deduced probable human leukocyte antigen (HLA) haplotypes in association with HLA-B*13:50 and -B*51:39 in Taiwanese unrelated bone marrow hematopoietic stem cell donors.

Materials and Methods: A sequence-based typing method was used to confirm the two low incidence alleles observed. 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 with group-specific primer sets. 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*13:50 is identical to B*13:01:01 in exons 2 and 3, except for a one nucleotide substitution at residue 482 (A→T), which results in a one amino acid replacement at position 137 (aspartic acid→valine). We deduced the probable HLA haplotype in association with B*13:50 in Taiwanese as A*11:02-B*13:50-DRB1*07:01. The DNA sequence of B*51:39 is identical to B*51:01:03 in exons 2 and 3 except for two nucleotide exchanges at residue 226 (A→G) and residue 228 (A→G), which result in a one amino acid substitution at position 52 (isoleucine→valine).The probable HLA haplotypes associated with B*51:39 in Taiwanese may be deduced as A*02-B*51:39-DRB1*15 and A*11-B*51:39-DRB1*15.

Conclusion: Information on the deduced HLA haplotypes in association with the low incidence B*13:50 and B*51:39 alleles that we report here is valuable for HLA testing laboratories for reference purposes and for stem cell transplantation donor search coordinators, to determine the likelihood of finding compatible donors in unrelated bone marrow donor registries for patients carrying these two uncommon HLA alleles.

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1. Introduction

The continuing discovery of new human leukocyte antigen (HLA) alleles and recognition 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 located on the short arm of chromosome 6 at 6p21.3. These loci are classified into Class I, II, and III of the MHC. The genes of HLA alleles are located in the MHC Class I and II regions. The HLA genes are characterized by their extreme allelic polymorphism and their variations and diversity among different ethnic groups and racial populations. HLA molecules have been definitely defined as transplant antigens with strong relevance in tissue transplantation, and their molecule similarity between donors and recipients is being considered as a prediction factor for graft survival and graft versus host disease. It is imperative to

Conflict of interest: none.

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precisely characterize any new and low incidence alleles encountered during routine HLA typing procedures. To facilitate successful and comprehensive unrelated bone marrow donor searches for patients in need of hematopoietic stem cell transplantation, persistent effort is needed to resolve unidentified, ambiguous or low incidence alleles in order to offer better services for HLA matching and donor selection.

HLA-B*13:50 and HLA-B*51:39 were first reported to the International ImMunoGeneTics/HLA (IMGT/HLA) database in 2011 and 2013 (Cell ID HC4927 and Cell ID HC27439) and 2005 (Cell ID HC13952), respectively [1]. Here, we report the deduced probable HLA haplotypes in association with B*13:50 and B*51:39. We further postulate that there are two plausible HLA haplotypes in association with B*51:39 in Taiwanese and that the haplotypes associated with B*13:50 and B*51:39 are restricted to Asians.

2. Materials and methods

Peripheral whole blood samples from unrelated bone marrow stem cell donors with Taiwanese ethnicity were collected in acid citrate dextrose anticoagulant. Formal written consents were signed by the donors before blood collection. The acid citrate dextrose 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

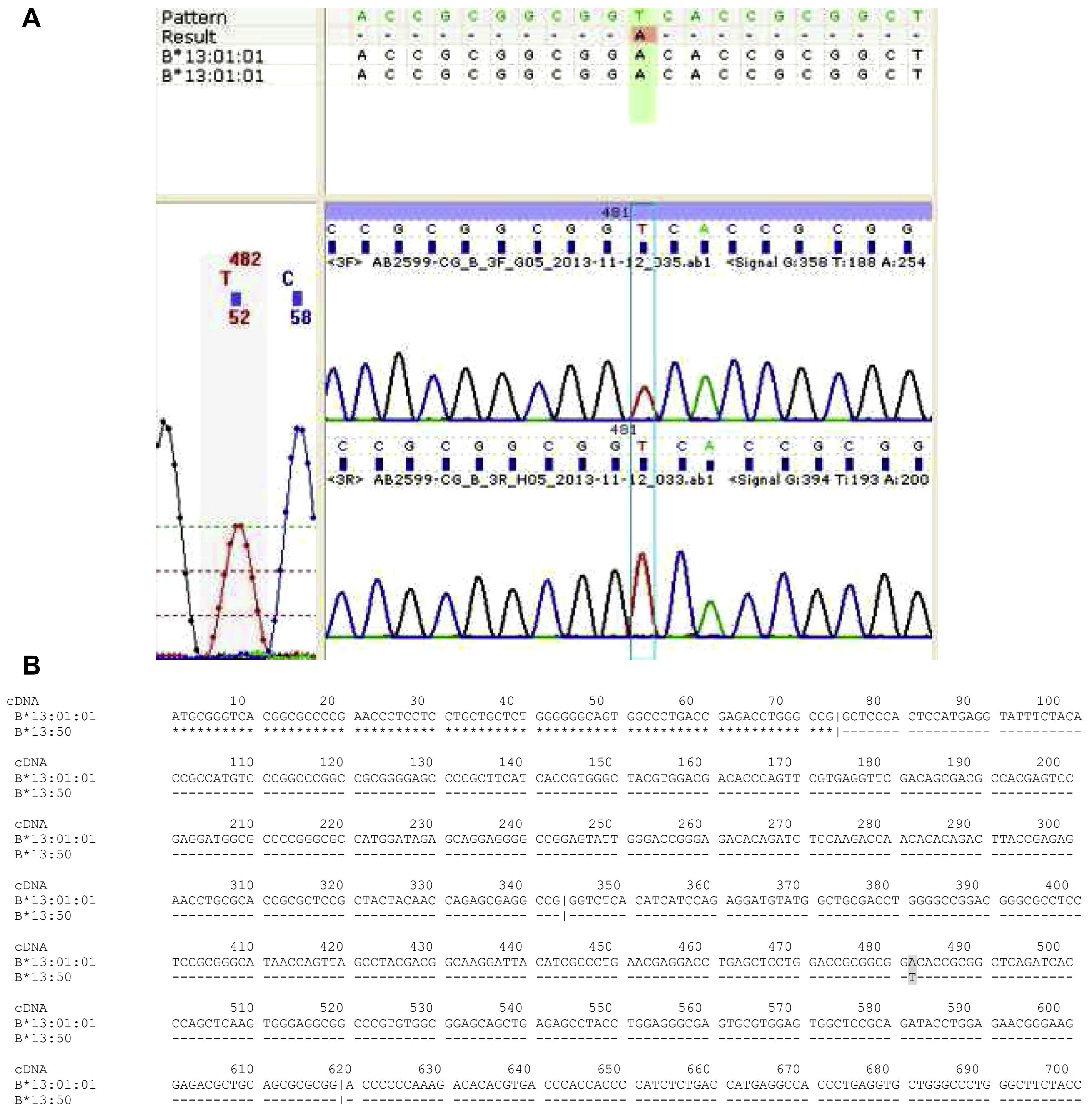

Fig. 1. (A) The raw sequence data (forward and reverse strains) show that at residue 482, the nucleotide A of B*13:01:01 is replaced by the T (in red) of B*13:50; (B) the DNA sequence of B*13:50 is identical to B*13:01:01 in exons 2 and 3, except for a one nucleotide substitution at residue 482 (A→T) (shaded).

```
 AA Pos.              10         20         30         40         50         60         70         80         90         100
  B*13:01:01    GSHSMRYFYT AMSRPGRGEP RFITVGYVDD TQFVRFDSDA TSPRMAPRAP WIEQEGPEYW DRETQISKTN TQTYRENLRT ALRYYNQSEA GSHIIQRMYG
  B*13:50       *--------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

  AA Pos.            110        120        130        140        150        160        170        180        190        200
  B*13:01:01    CDLGPDGRLL RGHNQLAYDG KDYIALNEDL SSWTAADTAA QITQLKWEAA RVAEQLRAYL EGECVEWLRR YLENGKETLQ RADPPKTHVT HHPISDHEAT
  B*13:50       ---------- ---------- ---------- ------V--- ---------- ---------- ---------- ---------- ---------- ----------

  AA Pos.            210        220        230        240        250        260        270
  B*13:01:01    LRCWALGFYP AEITLTWQRD GEDQTQDTEL VETRPAGDRT FQKWAAVVVP SGEEQRYTCH VQHEGLPKPL TLRW
  B*13:50       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----
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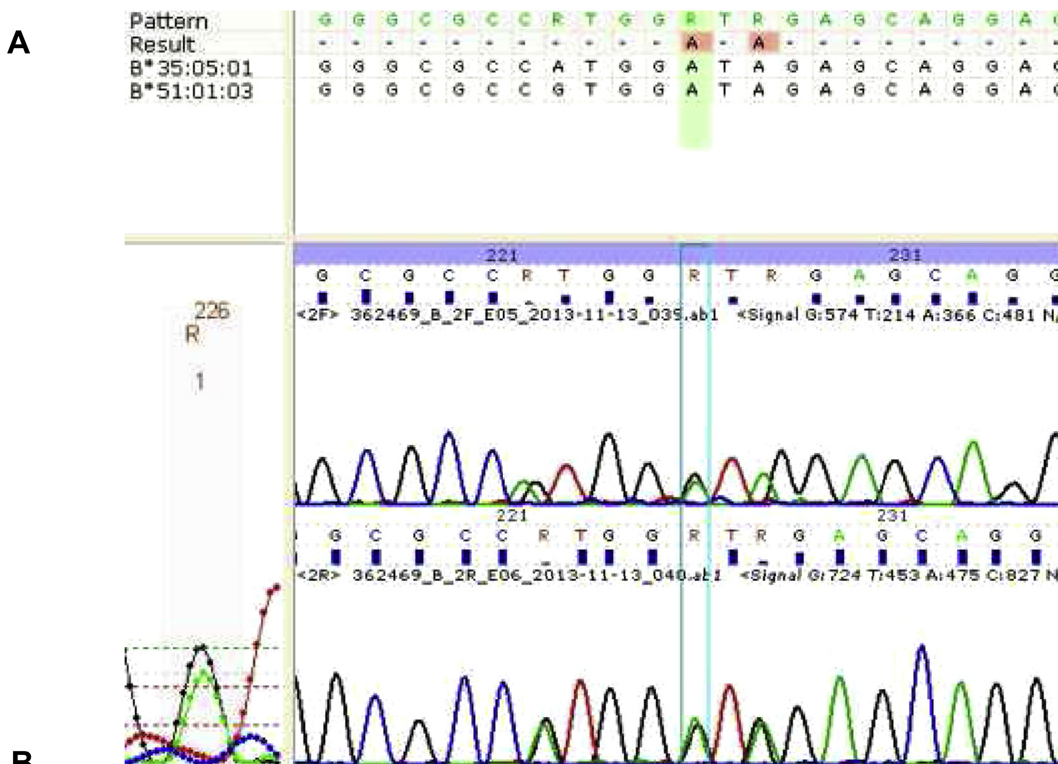
Fig. 2. The nucleotide substitution of B*13:50 from B*13:01:01 causes an amino acid replacement at residue 137 (aspartic acid→valine) (shaded).

HLA-A, -B and -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 by the BigDye

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cDNA                  80         90        100        110        120        130        140        150        160        170
 B*51:01:03      GCTCCCA CTCCATGAGG TATTTCTACA CCGCCATGTC CCGGCCCGGC CGCGGGGAGC CCCGCTTCAT TGCAGTGGGC TACGTGGACG ACACCCAGTT
 B*51:39         ------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                180        190        200        210        220        230        240        250        260        270
 B*51:01:03   CGTGAGGTTC GACAGCGACG CCGCGAGTCC GAGGACGGAG CCCCGGGCGC CGTGGATAGA GCAGGAGGGG CCGGAGTATT GGGACCGGAA CACACAGATC
 B*51:39      ---------- ---------- ---------- ---------- ---------- -----G-G-- ---------- ---------- ---------- ----------

 cDNA                280        290        300        310        320        330        340        350        360        370
 B*51:01:03   TTCAAGACCA ACACACAGAC TTACCGAGAG AACCTGCGGA TCGCGCTCCG CTACTACAAC CAGAGCGAGG CCG|GGTCTCA CACTTGGCAG ACGATGTATG
 B*51:39      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---|-------- ---------- ----------

 cDNA                380        390        400        410        420        430        440        450        460        470
 B*51:01:03   GCTGCGACGT GGGGCCGGAC GGGCGCCTCC TCCGCGGGCA TAACCAGTAC GCCTACGACG GCAAAGATTA CATCGCCCTG AACGAGGACC TGAGCTCCTG
 B*51:39      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                480        490        500        510        520        530        540        550        560        570
 B*51:01:03   GACCGCGGCG GACACCGCGG CTCAGATCAC CCAGCGCAAG TGGGAGGCGG CCCGTGAGGC GGAGCAGCTG AGAGCCTACC TGGAGGGCCT GTGCGTGGAG
 B*51:39      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

 cDNA                580        590        600        610
 B*51:01:03   TGGCTCCGCA GACACCTGGA GAACGGGAAG GAGACGCTGC AGCGCGCGG
 B*51:39      ---------- ---------- ---------- ---------- ---------
```

Fig. 3. (A) The raw sequence data (forward and reverse strains) show that at residues 226 and 228, the nucleotides A of B*51:01:03 are replaced by the R (A in green) of B*51:39; (B) the DNA sequence of B*51:39 is identical to B*51:01:03 in exons 2 and 3 except for two nucleotide exchanges at residue 226 (A→G) (shaded) and residue 228 (A→G) (shaded).

```
AA Pos.              10         20         30         40         50         60         70         80         90         100
 B*51:01:03   *SHSMRYFYT AMSRPGRGEP RFIAVGYVDD TQFVRFDSDA ASPRTEPRAP WIEQEGPEYW DRNTQIFKTN TQTYRENLRI ALRYYNQSEA GSHTWQTMYG
 B*51:39      *--------- ---------- ---------- ---------- ---------- -V-------- ---------- ---------- ---------- ----------

 AA Pos.             110        120        130        140        150        160        170        180
 B*51:01:03   CDVGPDGRLL RGHNQYAYDG KDYIALNEDL SSWTAADTAA QITQRKWEAA REAEQLRAYL EGLCVEWLRR HLENGKETLQ RA
 B*51:39      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --
```

Fig. 4. The nucleotide substitutions of B*51:39 from B*51:01:03 cause an amino acid replacement at residue 52 (isoleucine→valine) (shaded).

Table 1
HLA-A, -B, and -DRB1* alleles of donors with B*51:39 and the deduced probable HLA-A-B-DRB1 haplotype associated with B*51:39.

Donor	HLA-A*		HLA-B*		HLA-DRB1*		Deduced probable HLA-A-B-DRB1 haplotype
Donor 1	02:06	02:07	46:01	51:39	09:01	15:01	A*02-B*51:39-DRB1*15
Donor 2	02	11	15	51:39	15	15	A*02-B*51:39-DRB1*15 or A*11-B*51:39-DRB1*15
Donor 3	02:03	—	46	51:39	12	15	A*02-B*51:39-DRB1*15
Donor 4	02:06	02:07	46:01	51:39	11:01	15:01	A*02-B*51:39-DRB1*15
Donor 5	11:02	33:03	46	51:39	09:01	15:01	A*11-B*51:39-DRB1*15
Donor 6	02:06	24:02	40	51:39	12:02	15	A*02-B*51:39-DRB1*15
Donor 7	02:01	02:06	40	51:39	11	15	A*02-B*51:39-DRB1*15
Donor 8	02	02	46	51:39	11	15	A*02-B*51:39-DRB1*15
Donor 9	02	02	15	51:39	11	15	A*02-B*51:39-DRB1*15
Donor 10	02	26	39	51:39	08	15	A*02-B*51:39-DRB1*15
Donor 11	01	11	37	51:39	10	15	A*11-B*51:39-DRB1*15
Donor 12	02	11	40	51:39	15:01	15:01	A*02-B*51:39-DRB1*15 or A*11-B*51:39-DRB1*15

Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) in both directions.

3. Results

We confirmed the DNA sequence of B*13:50 was identical to B*13:01:01 in exons 2 and 3, except for a one nucleotide substitution at residue 482 (A→T) (Fig. 1), which results in a one amino acid replacement at amino acid position 137 (aspartic acid→valine) (Fig. 2). The extended HLA-A, -B and -DRB1 typing of our donor with B*13:50 was A*11:02, A*68:01, B*13:50, B*52:01, DRB1*07:01, and DRB1*09:01. Based on HLA linkage disequilibria in the Asian population [7], we deduced the probable HLA haplotype in association with B*13:50 in our Taiwanese donor as A*11:02-B*13:50-DRB1*07:01. We confirmed that the DNA sequence of B*51:39 was identical to B*51:01:03 in exons 2 and 3, except for two nucleotide exchanges at residue 226 (A→G) and residue 228 (A→G) (Fig. 3). The DNA substitutions result in a one amino acid replacement at position 52 (isoleucine→valine) (Fig. 4). In our unrelated marrow stem cell donor registry's database, we have 12 donors carrying B*51:39. The extended HLA typings of the 12 donors carrying B*51:39 are shown in Table 1. Taken together, the two HLA-A, -B and -DRB1 haplotypes associated with B*51:39 in Taiwanese donors may be deduced as A*02-B*51:39-DRB1*15 and A*11-B*51:39-DRB1*15.

4. Discussion

We confirmed the DNA sequences and amino acid sequences of two low frequency HLA alleles, B*13:50 and B*51:39, in this study. B*13:50 was initially detected in an individual (with HLA typing of A*02:07, A*11:02, B*13:50, B*46:01, DRB1*09:01, and DRB1*15:01) in Singapore [8]. Based on the known haplotype frequencies of HLA in the Chinese population and excluding the commonly observed haplotype A*02:07-B*46:01-DRB1*09:01 by linkage disequilibrium, a probable HLA haplotype in association with B*13:50 was deduced as A*11:02-B*13:50-DRB1*15:01 [8]. This haplotype differs from the B*13:50 haplotype (A*11:02-B*13:50-DRB1*07:01) that we deduced in this study. Our deduction is based on the known haplotype frequency in the Asian population [7] and by eliminating the commonly observed probable haplotype of A*68:01-B*52:01-DRB1*09:01 in our donor. B*51:39 was first detected in a donor from Singapore with HLA-A*02, A*11, B*27:04, B*51:39, DRB1*12:02, and DRB1*15:02 alleles [9]. Two probable B*51:39 -associated HLA haplotypes may be deduced from the Singapore blood donor as A*02-B*51:39-DRB1*15:02 and A*11-B*51:39-DRB1*15:02. In agreement, we deduced two identical most probable B*51:39 -associated HLA haplotypes from our unrelated bone marrow donors as shown in Table 1. Taken together from the Singapore [9] and Taiwanese individuals bearing B*51:39, we may further speculate that B*51:39 is strongly associated with DRB1*15. Since B*13:50 and B*51:39 have been detected only in donors from Singapore and Taiwan, we think that these two low incidence HLA alleles and their associated haplotypes may be restricted to the Asian population. Determining the ethnicity of B*13:50 and B*51:39 and their linked HLA haplotypes is significant, because the information may be employed in anthropological investigations 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.

It is worth mentioning that the classic and most direct method of determining HLA haplotypes is through family study, if test materials for a number of key family members are available. Alternatively, a population study may be employed if a significant number of unrelated donors is available [10]. However, the haplotypes deduced via population investigation are considered as likely or most probable. In this study, because of the 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 unrelated donors bearing the same alleles of interest. In addition, 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 the associated probable haplotypes [11–18]. According to our HLA typing practice and Allele Frequencies in World Populations (http://www.allelefrequencies.net/hla6006a.asp?hla_locus_type=Classical#), the frequencies of B*13:50 and B*51:39 in Taiwanese are extremely low, at about 1 in 20,000. Therefore, we think the probable B*13:50 and B*51:39 in the Taiwanese-associated haplotypes that we deduced in this study are highly likely. The number of HLA alleles is exponentially increasing with the recent development of DNA-based molecular typing technology. HLA diversity in ethnic groups is unique and

important. Facilitating an appropriate HLA-matched unrelated bone marrow stem cell donor for a given patient 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. Our challenge is enormous and rewarding.

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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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