



## Original Article

## Two deduced probable HLA-A\*24:287-associated HLA haplotypes (A\*24:287-B\*40-DRB1\*15 and A\*24:287-B\*58-DRB1\*03:01) found in Taiwanese unrelated hematopoietic bone marrow stem cell donors-case analysis

Kuo-Liang Yang <sup>a, b, \*</sup>

<sup>a</sup> 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

<sup>b</sup> Department of Laboratory Medicine, Tzu Chi University, Hualien, Taiwan

## ARTICLE INFO

*Article history:*

Received 25 March 2015  
 Received in revised form  
 14 April 2015  
 Accepted 8 May 2015  
 Available online 2 July 2015

*Keywords:*

Haplotype  
 Hematopoietic stem cell  
 HLA  
 Sequence-based typing  
 Transplantation

## ABSTRACT

**Objective:** HLA-A\*24:287 is a low incidence allele in the HLA-A locus. The objective of this study is to report the ethnicity of A\*24:287 and its deduced probable human leukocyte antigen (HLA)-associated haplotypes in Taiwanese unrelated bone marrow hematopoietic stem cell donors.

**Materials and methods:** A sequence-based typing method was employed to confirm the low incidence allele A\*24:287. Polymerase chain reaction was performed to amplify exon 2 and exon 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 employing BigDye Terminator Cycle Sequencing Ready Reaction kits in both directions according to the manufacturer's protocols.

**Results:** The DNA sequence of A\*24:287 is identical to A\*24:02:01:01 in exons 2 and 3, except for residue 617 where C is changed to G (codon 182, ACG->AGG). The nucleotide exchange leads to an amino acid alteration to the protein sequence of A\*24:02:01:01 at residue 182 where the threonine of A\*24:02:01:01 is changed to the arginine of A\*24:287. We deduced the probable HLA haplotypes in association with A\*24:287 in Taiwanese to be A\*24:287-B\*40-DRB1\*15 and A\*24:287-B\*58-DRB1\*03:01.

**Conclusion:** Information on the deduced probable HLA haplotypes in association with the low incidence A\*24:287 allele that we report here are of value for HLA testing laboratories for reference purposes. In addition, they 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.

Copyright © 2015, Buddhist Compassion Relief Tzu Chi Foundation. Published by Elsevier Taiwan LLC. All rights reserved.

### 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, Class II, and Class III regions. The genes encoding the HLA alleles are located in the MHC Class I and Class II regions. 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 in tissue transplantation. The 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

Conflict of interest: none.

\* Corresponding author. Buddhist Tzu Chi Stem Cells Centre, Hualien Tzu Chi Hospital, 707, Section 3, Chung-Yang Road, Hualien, Taiwan. Tel: +886 38561825x3373; fax: +886 38567851.

E-mail address: [edward@tzuchi.com.tw](mailto:edward@tzuchi.com.tw).

<http://dx.doi.org/10.1016/j.tcmj.2015.05.006>

1016-3190/Copyright © 2015, Buddhist Compassion Relief Tzu Chi Foundation. Published by Elsevier Taiwan LLC. All rights reserved.

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-A24:287 was first submitted to GenBank (accession number LK021330) and the name HLA-A24:287 (HWS1002300) was officially assigned by the World Health Organization HLA Nomenclature Committee in June 2014 [1]. However, there was no indication of its ethnicity and its associated HLA haplotype in the report. Here, we report the Taiwanese ethnicity of A24\*287 and the deduced probable HLA haplotypes in association with A\*24:287 based on the HLA-A, -B, and -DRB1 alleles commonly shared by the HLA typing of our donors and a donor (donor ID 360391) submitted to the IMGT database [1]. We further speculate that the deduced plausible HLA haplotypes associated with A\*24:287 are 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 consents were signed by the donors before blood collection. The acid citrate dextrose whole blood samples were stored at  $-80^{\circ}\text{C}$  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 subjected 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): TGTAACGACGGCCAG

TCGGGGGCGCAGGACCCGG; P3'exon 5B (antisense): GCTCCGATGACCACAACCTGCT; and (2) B-TA: M13-BIN1-TGA (sense): TGTAACGACGGCCAGTGGCGGGGCGCAGGACCTGA; P3'exon 5B (antisense): GCTCCGATGACCACAACCTGCT. The amplicons were sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA) in both directions. The HLA allele-associated probable haplotypes postulated in this study were determined by looking at the commonly shared HLA typing of the donors carrying A\*24:287. Where applicable, haplotype deduction based on HLA allelic homozygosity is as described previously [7,8]. For example, if a donor is typed for HLA-A, -B and -DRB1 as having A\*33:03, -, B\*58:01, -, DRB1\*03:01, -, it is possible to deduce the putative haplotypes of the donor derived from the biological parents as HLA-A\*33:03-B\*58:01-DRB1\*03:01 and A\*33:03-B\*58:01-DRB1\*03:01, respectively. Also, if the typing of a donor is A\*02:01, A\*02:07, B\*46:01, -, DRB1\*09:01, -, the putative haplotypes of the donor are A\*02:01-B\*46:01-DRB1\*09:01 and A\*02:07-B\*46:01-DRB1\*09:01 [8].

## 3. Results

We confirmed that the DNA sequence of A\*24:287 is identical to A\*24:02:01:01 in exon 2 and exon 3, except for residue 617 where C is changed to G (codon 182, ACG→AGG) (Fig. 1). The nucleotide replacement causes a one amino acid alteration to the protein sequence of A\*24:02:01:01 at residue 182 where the threonine of A\*24:02:01:01 is replaced by the arginine of A\*24:287. The extended HLA-A, -B and -DRB1 typing of our donors with A\*24:287 is shown in Table 1. Together with the HLA typing (A\*02, A\*24:287, B\*40, B\*67, DRB1\*15 and DRB1\*16) of the cell (D360391) submitted to the IMGT/HLA database [1], we deduced the two probable HLA haplotypes in association with A\*24:287 in our Taiwanese donors as A\*24:287-B\*40-DRB1\*15 and A\*24:287-B\*58-DRB1\*03:01.

(A)

CDNA	80	90	100	110	120	130	140	150	160	170
A*24:02:01:01	GCTCCCA	CTCCATGAGG	TATTTCTCCA	CATCCGTGTC	CCGGCCCGGC	CGCGGGGAGC	CCCGCTTCAT	CGCCGTGGGC	TACGTGGACG	ACACGCAGTT
A*24:287	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
CDNA	180	190	200	210	220	230	240	250	260	270
A*24:02:01:01	CGTGCGGTTT	GACAGCGACG	CCGCAGGCCA	GAGGATGGAG	CCGCGGGCGC	CGTGAGTAGA	GCAGGAGGGG	CCGAGATATT	GGGACGAGGA	GACAGGGAAA
A*24:287	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
CDNA	280	290	300	310	320	330	340	350	360	370
A*24:02:01:01	GTGAAGGCC	ACTCACAGAC	TGACCGAGAG	AACCTGCGGA	TCGCGCTCCG	CTACTACAAC	CAGAGCGAGG	CCG GTTTCTCA	CACCTCCAG	ATGATGTTTTG
A*24:287	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
CDNA	380	390	400	410	420	430	440	450	460	470
A*24:02:01:01	GCTGCGACGT	GGGGTCGGAC	GGGCGCTTCC	TCCGCGGGTA	CCACCAAGTAC	GCCTACGACG	GCAAGGATTA	CATCGCCCTG	AAAGAGGACC	TGCGCTCTTG
A*24:287	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
CDNA	480	490	500	510	520	530	540	550	560	570
A*24:02:01:01	GACCGCGGGC	GACATGGCGG	CTCAGATCAC	CAAGCGCAAG	TGGGAGGCGG	CCCATGTGGC	GGAGCAGCAG	AGAGCCTACC	TGGAGGGCAC	GTGCGTGGAC
A*24:287	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
CDNA	580	590	600	610						
A*24:02:01:01	GGGCTCCGCA	GATACCTGGA	GAACGGGAAG	GAGACGCTGC	AGCGCACGG					
A*24:287	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

(B)

Amino Acid Position	10	20	30	40	50	60	70	80	90	100
A*24:02:01:01	GSLSMRVFST	SVSRPGRGEP	RFIAVGYVDD	TQVFRFSDSA	ASQRMEPRAP	WIEQEGPEYW	DEETGKVKAH	SQTDRENLR	ALRYYNQSEA	GSHTLQMMFG
A*24:287	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Amino Acid Position	110	120	130	140	150	160	170	180		
A*24:02:01:01	CDVGS DGRFL	RGYHQYAYDG	KDYIALKEDL	RSWTAADMAA	QITKRKWEAA	HVAEQQRAYL	EGTCVDGLRR	YLENGKETLQ	RT	
A*24:287	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

**Fig. 1.** (A) The DNA sequence of A\*24:287 is identical to A\*24:02:01:01 in exons 2 and 3, except at residue 617 (codon 182; underlined), where the C of A\*24:02:01:01 is changed to the G (shaded) of A\*24:287. Exon 2 and exon 3 are separated by two vertical lines between nucleotide 343 and nucleotide 344. (B) The nucleotide exchange causes a one amino acid exchange at codon 182 where the threonine of A\*24:02:01:01 is replaced by the arginine (shaded) of A\*24:287. Dashes indicate nucleotide or amino acid identity with A\*24:02:01:01.

**Table 1**  
HLA-A, -B, and -DRB1 alleles of donors with A\*24:287 and deduced probable HLA-A-B-DRB1 haplotypes associated with A\*24:287.

Donor	HLA-A*		HLA-B*		HLA-DRB1*		Deduced probable A*24:287 associated HLA haplotypes
Donor 1	24:287	11	15	40	12	15	A*24:287-B*40-DRB1*15
Donor 2	24:287	24	54	40	4:05:01	15	A*24:287-B*40-DRB1*15
Donor 3	24:287	33	58	—	03:01	—	A*24:287-B*58-DRB1*03:01
IMGT 360391	24:287	02	67	40	16	15	A*24:287-B*40-DRB1*15

#### 4. Discussion

We confirmed the DNA sequence and amino acid sequence of the HLA allele A\*24:287 in this study. A\*24:287 was initially detected in an individual from Taiwan (with HLA typing of A\*02, A\*24:287, B\*40, B\*67, DRB1\*15, and DRB1\*16) [1]. We deduced the two probable A\*24:287-associated HLA haplotypes to be A\*24:287-B\*40-DRB1\*15 and A\*24:287-B58-DRB1\*0301, based on the commonly shared HLA typing of three donors carrying A\*24:287 (1 of them with HLA-B and -DRB1 homozygosity; A\*24:287, A\*33, B\*58, and DRB1\*03:01) listed in our registry and the donor information submitted to the IMGT (D360391) (Table 1). We further speculate that individuals bearing the A\*24:287 allele are probably Taiwanese, since the donors carrying A\*24:287 in our registry and the donor (D360391) with A\*24:287 reported to the IMGT/HLA database [1] are all Taiwanese.

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 likely or most probable. In this study, because of the lack of availability of necessary test materials from the families of the donors with A\*24:287, we opted to determine the haplotype by looking at the HLA alleles carried in common by unrelated donors bearing the same alleles of interest. Also, 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 [3–6,9–12].

The frequency of A\*24:287 in Taiwanese is about one in 40,000 according to our HLA typing practice. To date, the Allele Frequency Net Database ([http://www.allelefreqencies.net/hla6006a.asp?hla\\_locus\\_type=Classical#](http://www.allelefreqencies.net/hla6006a.asp?hla_locus_type=Classical#)) has yet to show the existence of the allele in the world population. Therefore, we think the probable A\*24:287-associated HLA haplotypes in Taiwanese that we deduced in this study are highly accountable.

The significance of determining the ethnicity of individuals with A\*24:287 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.

The numbers of known HLA alleles are increasing exponentially with the recent development of DNA-based molecular typing technology. Understanding HLA diversity in ethnic groups is 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 determination to resolve unknown, ambiguous, and low incidence genes in the HLA system. Additionally, determination of haplotypes is

essential for matching in unrelated stem cell transplantation between donor and recipient, since matching at the haplotype level has a better likelihood of matching at other loci within the HLA region than that for donors merely matched at the individual allelic level.

#### Acknowledgments

We are indebted to all volunteer donors who willingly joined the Taiwan Buddhist Tzu Chi Bone Marrow Donor Registry and gave consents 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.

#### References

- [1] Robinson J, Mistry K, McWilliam H, Lopez R, Parham P, Marsh SG. The IMGT/HLA database. *Nucleic Acids Res* 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] Vorechovsky I, Kralovicova J, Laycock MD, Webster ADB, Marsh SGE, Madrigal A, et al. Short tandem repeat (STR) haplotypes in HLA: an integrated 50-kb STR/linkage disequilibrium/gene map between the *RING3* and *HLA-B* genes and identification of STR haplotype diversification in the class III region. *Eur J Hum Genet* 2001;9:590–8.
- [8] Yang KL, Lin PY. Determination of HLA-A, -B and -DRB1 haplotypes based on allelic homozygosity data in selected bone marrow donors of the Taiwanese marrow donor registry. *Int J Immunogen* 2007;34:385–92.
- [9] 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.
- [10] 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.
- [11] 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.
- [12] 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.