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Original Article

Recognition of the three deduced probable human leukocyte antigen haplotypes in association with HLA-A*31:30 (A*31:30-B*15-DRB1*14) and HLA-B*40:55 (A*02:07-B*40:55-DRB1*04:05 and A*26:01-B*40:55-DRB1*09:01) in a Taiwanese population



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ABSTRACT

Objectives: HLA-A*31:30 and HLA-B*40:55 are two rarely observed alleles in the HLA-A locus and HLA-B locus, respectively. The objective of this study is to report three deduced probable human leukocyte antigen (HLA) haplotypes in association with HLA-A*31:30 and HLA-B*40:55 in 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. A polymerase chain reaction was performed to amplify exons 2, 3, and 4 of the HLA-A, -B, and -C loci and exon 2 of 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 A*31:30 is identical to A*31:01:02 in exons 2, 3, and 4, except for a nucleotide substitution at residue 539 ($T \rightarrow G$) resulting in an amino acid replacement at position 156 (Leu \rightarrow Trp). We deduced the probable HLA haplotype in association with A*31:30 as A*31:30-B*15-DRB1*14. The DNA sequence of B*40:55 is identical to B*40:01:01 in exons 2, 3, and 4 except for a nucleotide exchange at residue 814 ($G \rightarrow A$) resulting in an amino acid substitution at position 248 (Val \rightarrow Met). Two probable HLA haplotypes associated with B*40:55 may be deduced as A*02:07-B*40:55-DRB1*04:05 and A*26:01-B*40:55-DRB1*09:01.

Conclusion: Information about the deduced HLA haplotypes associated with the rare A*31:30 and B*40:55 alleles that we reported here is valuable for HLA tissue typing 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 bearing these two uncommon HLA alleles. Because A*31:30 and B*40:55 have been found in Taiwanese population so far, we think the haplotypes that we reported here are most likely conserved in their population.

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1. Introduction

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

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the MHC and the genes of human leukocyte antigen (HLA) alleles are situated 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. As the HLA molecule similarity between donors and recipients is being used as a prediction factor for graft survival and graft-versus-host disease, it is imperative to characterize precisely any new allele 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, we are persistently working on resolving

Conflicts of interest: none.

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cDNA	80	90	100	110	120	130	140	150	160	170
A*31:01:02 A*31:30	GCTCCCA	CTCCATGAGG	TATTTCACCA	CATCCGTGTC	CCGGCCCGGC	CGCGGGGGAGC	CCCGCTTCAT	CGCCGTGGGC	TACGTGGACG	ACACGCAGTT
cDNA	180	190	200	210	220	230	240	250	260	270
A*31:01:02	CGTGCGGTTC	GACAGCGACG	CCGCGAGCCA	GAGGATGGAG	CCGCGGGCGC	CGTGGATAGA	GCAGGAGAGG	CCTGAGTATT	GGGACCAGGA	GACACGGAAT
A*31:30										
cDNA	280	290	300	310	320	330	340	350	360	370
A*31:01:02	GTGAAGGCCC	ACTCACAGAT	TGACCGAGTG	GACCTGGGGA	CCCTGCGCGG	CTACTACAAC	CAGAGCGAGG	CCG GTTCTCZ	A CACCATCCAC	G ATGATGTATG
A*31:30										
cDNA	380	390	400	410	420	430	440	450	460	470
A*31:01:02	GCTGCGACGT	GGGGTCGGAC	GGGCGCTTCC	TCCGCGGGTA	CCAGCAGGAC	GCCTACGACG	GCAAGGATTA	CATCGCCTTG	AACGAGGACC	TGCGCTCTTG
A*31:30										
cDNA	480	490	500	510	520	530	540	550	560	570
A*31:01:02	GACCGCGGCG	GACATGGCGG	CTCAGATCAC	CCAGCGCAAG	TGGGAGGCGG	CCCGTGTGGC	GGAGCAGTTG	AGAGCCTACC	TGGAGGGCAC	GTGCGTGGAG
A*31:30							G-			
cDNA	580	590	600	610						
A*31:01:02	TGGCTCCGCA	GATACCTGGA	GAACGGGAAG	GAGACGCTGC	AGCGCACGG					
A*31:30										

Fig. 1. Comparison of DNA sequences between HLA-A*31:30 and -A*31:01:02 in exons 2 and 3. The DNA sequence of A*31:30 is identical to A*31:01:02 in exons 2 and 3, except at residue 539 where the T of A*31:01:02 is replaced by G (shaded). cDNA = complementary DNA.

ambiguous or unidentified alleles that we find to offer better services for HLA matching and donor selection.

HLA-A*31:30 and HLA-B*40:55 were first reported to the immunogenetics (IMGT)/HLA database in 2010 and 2004, respectively [1], without an indication of probable HLA-associated haplotypes. Here we report the deduced probable HLA haplotypes in association with A*31:30 and B*40:55. We further postulate that the deduced plausible HLA haplotype in association with A*31:30 is most likely conserved in the Taiwanese population, based on its low frequency in the general population and the fact that it has so far been reported only in a Taiwanese population [1]. Similarly, the deduced probable B*40:55-associated HLA haplotype A*02:07-B*40:55-DRB1*04:05 is most likely conserved in the Taiwanese population, whereas the other deduced probable B*40:55associated haplotype, A*26:01-B*40:55-DRB1*09:01, is conserved in Japanese and Taiwanese populations.

2. Materials and methods

Peripheral whole blood samples from three unrelated bone marrow stem cell donors with Taiwanese ethnicity were collected in acid citrate dextrose (ACD) anticoagulant. Formal written consents were signed by the donors before blood collection. The ACD whole blood was stored at $-80\ ^\circ C$ until use. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). Genomic DNA typing of HLA-A, -B, and -DRB1 loci was first performed using Dynal Reli-sequence-specific oligonucleotide probe (SSO) HLA-A, -B, and -DRB1 Typing Kits (Dynal Biotech, Bromborough, Wirral, UK), followed by the sequence-specific primer (SSP) typing method (AllSet Gold SSP HLA high-resolution kits; Dynal Biotech, Invitrogen, Brown Deer, WI, USA) to reach high-resolution allelic subtypes. The sequence-based typing method [2-6] was used to confirm the low-incidence alleles observed, and in cases of anomalous results and typing ambiguities from the SSO and SSP typing protocols. Polymerase chain reaction was carried out to amplify exons 2, 3, and 4 of the HLA-C locus and exon 2 of the DQB1 locus with group-specific primer sets as previously described [7]. Amplicons were sequenced by the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) in both directions. In this study, A*31:30 from one blood donor and B*40:55 from another two blood donors were sequenced and analyzed.

3. Results

We confirmed that the DNA sequence of A*31:30 is identical to A*31:01:02 in exons 2, 3, and 4, except for a nucleotide substitution at residue 539 (T \rightarrow G) [1] (Fig. 1). The nucleotide substitution caused an amino acid replacement at residue 156 (Leu \rightarrow Trp; Fig. 2). The extended HLA typing of our donor carrying A*31:30 was A*02:07, A*31:30, B*15:01, B*46:01, DRB1*09:01, and DRB1*14:05. Together with the HLA typing of the cell (328573) with A*31:30 reported to the IMGT/HLA database by Yang et al (A*11:01, A*31:30, B*15, DRB1*14, and DRB1*16) [1], the probable HLA haplotype in association with A*31:30 may be deduced as A*31:30-B*15-DRB1*14.

Historically, we detected three unrelated bone marrow stem cell donors bearing the B*40:55 allele in our bone marrow donor registry. We confirmed that the DNA sequence of B*40:55 is identical to B*40:01:01 in exons 2, 3, and 4 except for a nucleotide substitution at position 814 ($G \rightarrow A$; Fig. 3). The nucleotide replacement resulted in an amino acid substitution at position 248 (Val \rightarrow Met; Fig. 4). The extended HLA typing of the three bone marrow donors was as follows: A*02:07, A*33:03, B*40:55, B*46:01, DRB1*04:05, DRB1*09:01; A*02:07, B*40:55, B*51:01, DRB1*04:05, DRB1*09:01; A*02:07, B*40:55, B*46:01, DRB1*04:05, DRB1*09:01. Together with the extended HLA typing of the Japanese donor (TBC 46239) bearing B*40:55 reported to the IMGT/HLA database (A*24:02, A*26:01, B*40:01, B*40:55, DRB1*09:01, DRB1*15:01) [1], the following two plausible HLA haplotypes associated with

AA Pos. A*31:01:02 A*31:30	10 GSHSMRYFTT *	20 SVSRPGRGEP	30 RFIAVGYVDD	40 TQFVRFDSDA	50 ASQRMEPRAP	60 WIEQERPEYW	70 DQETRNVKAH	80 SQIDRVDLGT	90 LRGYYNQSEA	100 GSHTIQMMYG
AA Pos. A*31:01:02 A*31:30	110 CDVGSDGRFL	120 RGYQQDAYDG	130 KDYIALNEDL	140 RSWTAADMAA	150 QITQRKWEAA	160 RVAEQLRAYL W	170 EGTCVEWLRR	180 YLENGKETLQ	RT 	

Fig. 2. Comparison of amino acid sequences between HLA-A*31:30 and -A*31:01:02 in exons 2 and 3. The amino acid sequence of A*31:30 is identical to A*31:01:02 in exons 2 and 3, except at residue 156 where the L of A*31:01:02 is replaced by W (shaded). HLA = human leukocyte antigen.

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cDNA B*40:01:01 B*40:55	80 GCTCCCA	90 CTCCATGAGG	100 TATTTCCACA	110 CCGCCATGTC	120 CCGGCCCGGC	130 CGCGGGGGAGC	140 CCCGCTTCAT	150 CACCGTGGGC	160 TACGTGGACG	170 ACACGCTGTT
CDNA B*40:01:01 B*40:55	180 CGTGAGGTTC	190 GACAGCGACG	200 CCACGAGTCC	210 GAGGAAGGAG	220 CCGCGGGCGC	230 CATGGATAGA	240 GCAGGAGGGG	250 CCGGAGTATT	260 GGGACCGGGA	270 GACACAGATC
CDNA B*40:01:01 B*40:55	280 TCCAAGACCA	290 ACACACAGAC	300 TTACCGAGAG	310 AGCCTGCGGA	320 ACCTGCGCGG	330 CTACTACAAC	340 CAGAGCGAGG	350 CCG GGTCTCZ	360 CACCCTCCAC) 370 G AGGATGTACG
cDNA B*40:01:01 B*40:55	380 GCTGCGACGT	390 GGGGCCGGAC	400 GGGCGCCTCC	410 TCCGCGGGCA	420 TAACCAGTAC	430 GCCTACGACG	440 GCAAGGATTA	450 CATCGCCCTG	460 AACGAGGACC	470 TGCGCTCCTG
cDNA B*40:01:01 B*40:55	480 GACCGCCGCG	490 GACACGGCGG	500 CTCAGATCTC	510 CCAGCGCAAG	520 TTGGAGGCGG	530 CCCGTGTGGC	540 GGAGCAGCTG	550 AGAGCCTACC	560 TGGAGGGCGA	570 GTGCGTGGAG
cDNA B*40:01:01 B*40:55	580 TGGCTCCGCA	590 GATACCTGGA	600 GAACGGGAAG	610 GACAAGCTGG	620 AGCGCGCTG 4 -	0 630 A CCCCCCAAA) 640 G ACACACGTGA) 650 A CCCACCACCO	66 C CATCTCTGAC	50 670 C CATGAGGCCA
cDNA B*40:01:01 B*40:55	680 CCCTGAGGTG	690 CTGGGCCCTG	700 GGTTTCTACC	710 CTGCGGAGAT	720 CACACTGACC	730 TGGCAGCGGG	740 ATGGCGAGGA	750 CCAAACTCAG	760 GACACTGAGC	770 TTGTGGAGAC
CDNA B*40:01:01 B*40:55	780 CAGACCAGCA	790 GGAGATAGAA	800 CCTTCCAGAA	810 GTGGGCAGCT	820 GTGGTGGTGC A	830 CTTCTGGAGA	840 AGAGCAGAGA	850 TACACATGCC	860 ATGTACAGCA	870 TGAGGGGCTG
CDNA B*40:01:01 B*40:55	880 CCGAAGCCCC	890 TCACCCTGAG	ATGGG							

Fig. 3. Comparison of DNA sequences between HLA-40:55 and -B*40:01:01 in exons 2, 3 and 4. The DNA sequence of B*40:55 is identical to B*40:01:01 in exons 2, 3 and 4 except at residue 814 where the G of B*40:01:01 is replaced by A (shaded). cDNA = complementary DNA.

AA Pos. B*40:01:01 B*40:55	10 GSHSMRYFHT *	20 AMSRPGRGEP	30 RFITVGYVDD	40 TLFVRFDSDA	50 TSPRKEPRAP	60 WIEQEGPEYW	70 DRETQISKTN	80 TQTYRESLRN	90 LRGYYNQSEA	100 GSHTLQRMYG
AA Pos. B*40:01:01 B*40:55	110 CDVGPDGRLL	120 RGHNQYAYDG	130 KDYIALNEDL	140 RSWTAADTAA	150 QISQRKLEAA	160 RVAEQLRAYL	170 EGECVEWLRR	180 YLENGKDKLE	190 RADPPKTHVT	200 HHPISDHEAT
AA Pos. B*40:01:01 B*40:55	210 LRCWALGFYP	220 AEITLTWQRD	230 GEDQTQDTEL	240 VETRPAGDRT	250 FQKWAAVVVP M	260 SGEEQRYTCH	270 VQHEGLPKPL	TLRW		

Fig. 4. Comparison of amino acid sequences between HLA-40:55 and $-B^*40:01:01$ in exons 2, 3 and 4. The amino acid sequence of $B^*40:55$ is identical to $B^*40:01:01$ in exons 2, 3 and 4 except at residue 248 where the V of $B^*40:01:01$ is replaced by M (shaded). HLA = human leukocyte antigen.

B*40:55 may be deduced: A*02:07-B*40:55-DRB1*04:05 and A*26:01-B*40:55-DRB1*09:01. We postulate that the B*40:55-associated haplotype A*02:07-B*40:55-DRB1*04:05 is most probably conserved in Taiwanese people because of its presence in Taiwanese population, whereas the other B*40:55-associated HLA haplotype, A*26:01-B*40:55-DRB1*09:01, is probably conserved in Taiwanese and Japanese population. These speculations await future verification.

4. Discussion

In this study we confirmed the DNA sequence of two lowfrequency HLA alleles, A*31:30 and B*40:55. We validated the Taiwanese ethnicity of A*31:30 and the Asian ethnicity of B*40:55. We further deduced the probable HLA haplotypes in association with A*31:30 and B*40:55 based on the common alleles shared by the blood donors carrying A*31:30 and B*40:55. We further postulated the two HLA haplotypes in association with B*40:55 in the Asian populations. Information about the ethnicity of carriers of A*31:30 and B*40:55 and their linked HLA haplotypes can be used in anthropological investigations of race in addition to allowing search coordinators from unrelated bone marrow donor registries to allocate appropriate unrelated bone marrow hematopoietic stem cell donors for their patients.

It is worth mentioning that the classical and most direct method of determining HLA haplotype is through family study if test materials from a number of key family members are available. Alternatively, population study may be used if a sufficient number of unrelated donors are available [7]. However, the haplotypes deduced through population investigation are only considered likely or most probable. In this study, because of the availability of the necessary test materials, 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 haplotypes is for rare HLA alleles, alleles shared in common by unrelated individuals may be used to deduce the probable associated haplotypes [7-16]. The frequencies of A*31:30 and B*40:55 in the Taiwanese population are extremely low at about 1 in 20,000 to 1 in 30,000 according to our HLA typing practice. Therefore, we think the probable HLA-A*31:30 and HLA-B*40:55-associated haplotypes that we postulated in this study are highly reliable.

The HLA alleles are increasing exponentially with the recent development of DNA-based molecular typing technology. By contrast, HLA diversity in every ethnic group is unique and important. Finding appropriate HLA-matched unrelated bone marrow stem cell donors for a given needy patient for successful stem cell transplantations relies on the accuracy of HLA typing results and the spirit and strength to resolve unknown, discrepant, and ambiguous genes in the HLA system.

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