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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→G) resulting in an amino acid replacement at position 156 (Leu→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→A) resulting in an amino acid substitution at position 248 (Val→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.

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 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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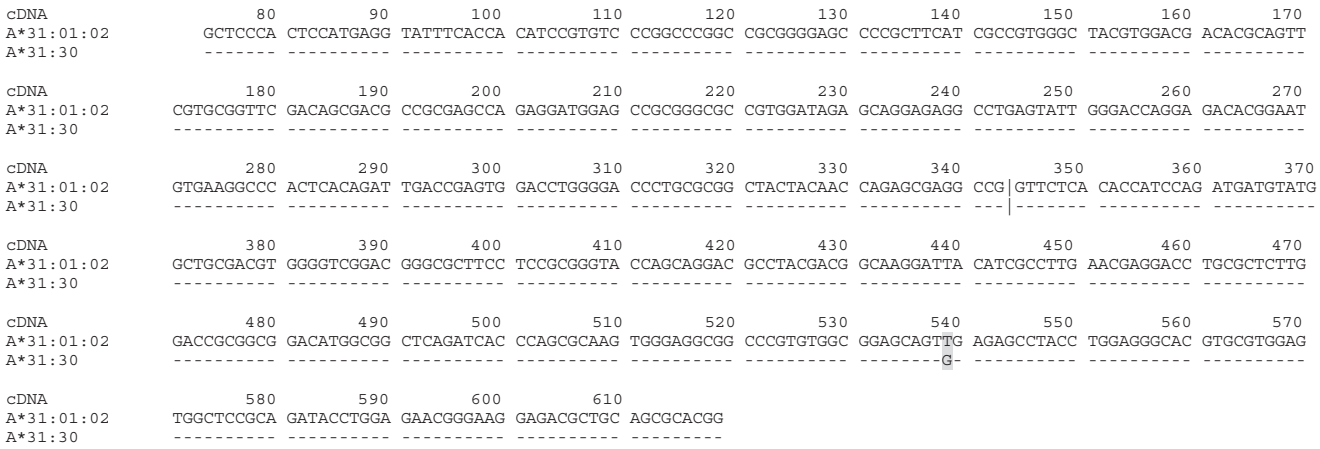

Fig. 1. Comparison of DNA sequences between HLA-A*31:30 and -A*31:01:02 in exons 2 and 3. The DNA sequence of A*31:30 is identical to A*31:01:02 in exons 2 and 3, except at residue 539 where the T of A*31:01:02 is replaced by G (shaded). cDNA = complementary DNA.

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*40:55-associated 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 °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→G) [1] (Fig. 1). The nucleotide substitution caused an amino acid replacement at residue 156 (Leu→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→A; Fig. 3). The nucleotide replacement resulted in an amino acid substitution at position 248 (Val→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*11:01; and A*24:02, A*26:01, 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

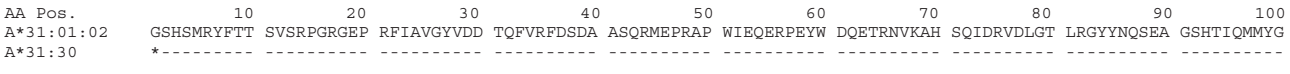

Fig. 2. Comparison of amino acid sequences between HLA-A*31:30 and -A*31:01:02 in exons 2 and 3. The amino acid sequence of A*31:30 is identical to A*31:01:02 in exons 2 and 3, except at residue 156 where the L of A*31:01:02 is replaced by W (shaded). HLA = human leukocyte antigen.

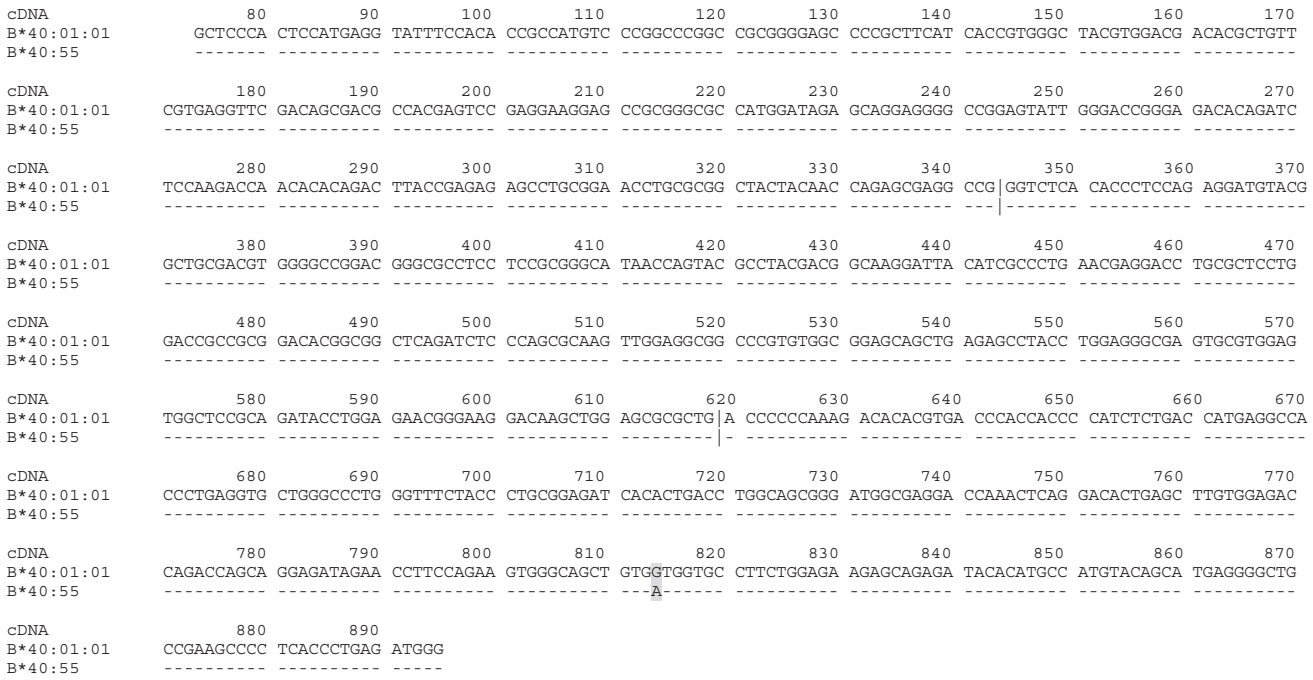

Fig. 3. Comparison of DNA sequences between HLA-40:55 and -B*40:01:01 in exons 2, 3 and 4. The DNA sequence of B*40:55 is identical to B*40:01:01 in exons 2, 3 and 4 except at residue 814 where the G of B*40:01:01 is replaced by A (shaded). cDNA = complementary DNA.

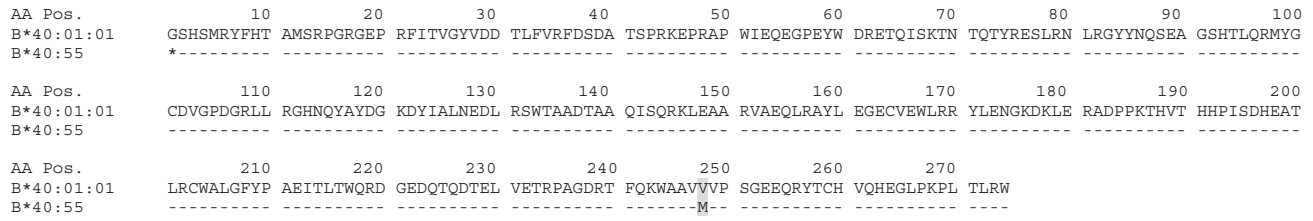

Fig. 4. Comparison of amino acid sequences between HLA-40:55 and -B*40:01:01 in exons 2, 3 and 4. The amino acid sequence of B*40:55 is identical to B*40:01:01 in exons 2, 3 and 4 except at residue 248 where the V of B*40:01:01 is replaced by M (shaded). HLA = human leukocyte antigen.

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 low-frequency 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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