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Original Article

Three deduced probable human-leukocyte-antigen haplotypes associated with HLA-DQB1*03:26 and -DRB1*14:141 from Taiwanese unrelated bone-marrow hematopoietic-stem-cell donors: Two case analyses

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ABSTRACT

Objective: HLA-DQB1*03:26 and -DRB1*14:141 are two low-incidence alleles found at the HLA-DQB1 and HLA-DRB1 loci. The objective of this study was to report the ethnicity of DQB1*03:26 and DRB1*14:141, and their deduced probable HLA-associated haplotypes among Taiwanese unrelated bone-marrow hematopoietic-stem-cell donors.

Materials and Methods: A sequence-based typing method was employed to confirm these two low-incidence alleles. Polymerase chain reaction was carried out to amplify exon 2 and exon 3 of the HLA-A and HLA-B loci, and exon 2 of the HLA-DRB1 and HLA-DQB1 loci using group-specific primer sets. The amplicons were then sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction Kit in both directions according to the manufacturer's protocols.

Results: The DNA sequence of DQB1*03:26 is most similar to DQB1*03:03:02:01 and DQB1*03:11 in exon 2. It differs from DQB1*03:03:02:01 at residue 136 where the A of DQB1*03:03:02:01 is replaced by the C of DQB1*03:26. This nucleotide exchange leads to an amino-acid alteration in the protein sequence of DQB1*03:03:02:01 at residue 14 where the methionine (M) of DQB1*03:03:02:01 is changed to the leucine (L) of DQB1*03:26. We deduced the probable HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 haplotypes associated with DQB1*03:26 in Taiwanese to be A*02:01-B*40:01-C*03:04-DRB1*08:03-DQB1*03:26 or A*02:01-B*40:01:C*03:04-DRB1*09:01-DQB1*03:26). DQB1*03:26 differs from DQB1*03:11 at residue 266 where the C of DQB1*03:11 is substituted by the A of DQB1*03:26. This nucleotide exchange leads to an amino-acid alteration to the protein sequence of DQB1*03:11 at residue 57 where the amino acid alanine (A) of DQB1*03:11 is replaced by the amino acid, aspartic acid (D), of DQB1*03:26. We also confirmed the DNA and protein sequences of RB1*14:141 and its ethnicity. The probable HLA-A, HLA-B, and HLA-DRB1 haplotypes associated with DRB1*14:141 in Taiwanese may be deduced to be A*02:03-B*15:25-DRB1*14:141.

Conclusion: Information on the ethnicity of the DQB1*03:26 and DRB1*14:141 alleles, and the deduced probable HLA haplotypes associated with the low-incidence alleles that we report here is of value to HLA testing laboratories for reference purposes. In addition, they can be used by stem-cell-transplantation-donor-search coordinators in order to determine a strategy for finding compatible donors in unrelated bone-marrow-donor registries when patients carry these uncommon HLA alleles.

Conflict of interest: none.

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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 made up 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 across various ethnic groups and racial populations. The HLA molecules have been definitively defined as transplant antigens and have a strong relevance to tissue transplantation. Molecular similarity between donors and recipients is considered 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, persistent efforts are needed to resolve unidentified, ambiguous, or low-incidence alleles in order to offer better HLA matching and improved donor selection.

The nucleotide sequence of HLA-DQB1*03:26 was first submitted to GenBank (accession number FN550110) and the name HLA-DQB1*03:26 officially assigned by the World Health Organization HLA Nomenclature Committee in September 2009 [1]. However, there was no indication of its ethnicity and its HLA-associated haplotype in the report. Here, we report Taiwanese ethnicity for DQB1*03:26 and the deduced probable HLA haplotypes in association with DQB1*03:26 based on the HLA-A, HLA-B, and HLA-DRB1 alleles commonly shared by the HLA typing of our donor and a donor (donor ID 2009-CAP-DL03) submitted to the IMGT database [1]. We further speculate that the deduced plausible HLA haplotypes associated with DQB1*03:26 are restricted to the Taiwanese.

The DNA sequence of DRB1*14:141 was first discovered in three Chinese individuals by three independent laboratories in China, and the sequences were submitted to the IMGT in 2013 [1]. Routine HLA sequence-based typing revealed that the sequences of DRB1*14:141 mismatch any known DRB1 alleles within the exon 2. Extraordinarily, the entire sequences of their exon 2 were characterized as DRB3*02:02:01:01/02, while exon 3 was characterized as DRB1*14-like alleles [2]. In this study, we confirmed the ethnicity of DRB1*14:141 and postulate the deduced probable HLA haplotype in association with DRB1*14:141 as A*02:03-B*15:25-DRB1*14:141. The haplotype, presumably, is restricted to the Chinese.

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. A formal written consent from each donor was obtained before blood collection. The acid-citrate-dextrose whole-blood samples were stored at −80°C until use. Genomic DNA was extracted using a QIAamp DNA Blood Mini Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). The DNA was subjected to HLA genotyping for the HLA-A, HLA-B, and HLA-DRB1 loci using a commercial polymerase-chain-reaction-sequencing-based typing kit (SeCore A/B/DRB1 Locus Sequencing Kits, Life Technologies, Brown Deer, WI, USA). High-resolution allelic sequencing was performed as described previously [3–7]. The two sets of primer sequences used were (1) B-CG: M13-BIN1-CGG (sense): TGTAAAACGACGGCCAGTCGGGGGCGCAGGACCCGG; P3′ exon 5B (antisense): GCTCCGATGACCACAACTGCT, and (2) B-TA: M13-BIN1-TGA (sense): TGTAAAACGACGGCCAGTGGCGGGGGCGCAGGACCTGA; P3′exon 5B (antisense): GCTCCGATGACCACAACTGCT. The amplicons were sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) in both directions. The approach used in this study to determine the HLA allele-associated probable haplotype was to examine the commonly shared HLA types of the donors carrying DQB1*03:26 or DRB1*14:141. Where applicable, haplotype deduction based on HLA allelic homozygosity as described previously was employed [8,9]. For example, if a donor is typed for HLA-A, HLA-B, and HLA-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. In the same way, 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* [9].

3. Results

We confirmed that the DNA sequence of DQB1*03:26 is most similar to DQB1*03:03:02:01 and DQB1*03:11 in exon 2. It differs from DQB1*03:03:02:01 at residue 136 where the A of DQB1*03:03:02:01 is replaced by the C of DQB1*03:26 (Fig. 1A), and it differs from DQB1*03:11 at residue 266 where the C of DQB1*03:11 is substituted by the A of DQB1*03:26 (Fig. 1A). The nucleotide replacement in DQB1*03:03:02:01 results in one amino-acid alteration in the protein sequence at residue 14 where the methionine (M) of DRB1*03:03:02:01 is substituted by the leucine (L) of DRB1*03:26 (Fig. 1B). Similarly, the nucleotide replacement of DQB1*03:11 causes one amino-acid alteration to the protein sequence of DQB1*03:11 at residue 57 where the amino acid, alanine (A), of DQB1*03:11 is replaced by the amino acid, aspartic acid (D), of DQB1*03:26 (Fig. 1B). The extended HLA typing of our bone-marrow donor with DQB1*03:26 is A*02:01, A*11:01, B*40:01, C*03:04, C*07:02 DR1*08:03, DRB1*09:01, DQB1*03:26, and DQB1*06:01. Combined together with the HLA typing of the 2009-CAP-DL03 cell [1] (A*02:01, B*13:01, B*40:01, C*03:04, C*08:01, DRB1*08:03, DRB1*09:01, DQB1*03:26, and DQB1*06:01), we postulate that the probable DQB1*03:26-associated HLA haplotype may be deduced to be A*02:01-B*40:01:C*03:04-DRB1*08:03-DQB1*03:26 or A*02:01-B*40:01:C*03:04-DRB1*09:01-DQB1*03:26.

We confirmed the DNA sequence of DRB1*14:141, which is thought to be derived from a recombination event, where its entire exon 2 is identical to DRB3*02:02:01:01/02, while its exon 3 is similar to DRB1*14 like [2]. The extended HLA typing of our bone-marrow stem-cell donor bearing DRB1*14:141 is A*02:03, A*11:01, B*15:25, B*35:01, C*03:03, C*04:03, DRB1*14:141, DRB1*15:01, DQB1*05:03, and DQB1*06:02. Together with the HLA typings of the three DRB1*14:141 carrying donors submitted to the IMGT (A*02:03, B*15:25, B*40:02, DB1*4:05, DRB1*14:141; A*02:03, A*30:01, B*13:02, B*15:25, C*04:03, C*06:02, DRB1*07:01, DRB1*14:141; and A*02:01:01:01, A*02:03:01, B*13:01:01, B*15:25:01, DRB1*09:01:02, DRB1*14:141) [1], we deduced a probable HLA haplotype associated with DRB1*14:141 as A*02:03-B*15:25-DRB1*14:141. We suggest DRB1*14:141 and its associated HLA haplotype are most likely restricted to the Chinese.

4. Discussion

In this study, the Taiwanese ethnicity of DQB1*03:26 is identified together with the Chinese ethnicity of DRB1*14:141 allele; furthermore, the DNA sequences of DQB1*03:26 and DRB1*14:141 alleles are confirmed, and the probable HLA haplotypes in

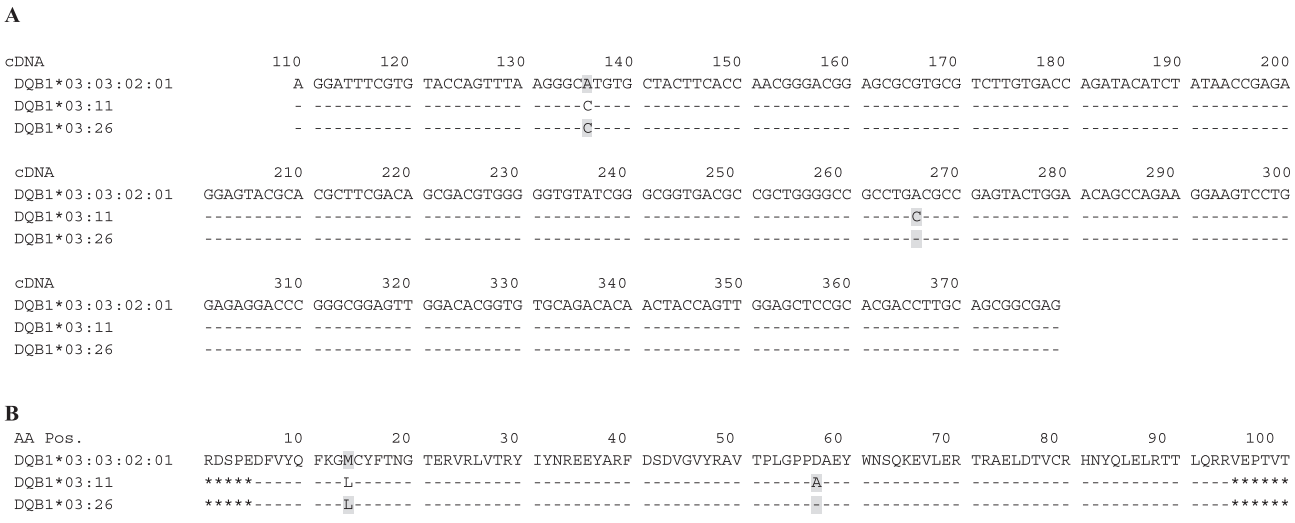

Fig. 1. (A) The DNA sequence of DQB1*03:26 is most similar to DQB1*03:03:02:01 in exon 2. It differs from DQB1*03:03:02:01 at residue 136 where the A of DQB1*03:03:02:01 is replaced by the C of DQB1*03:26 (shaded). DQB1*03:26 differs from DQB1*03:11 at residue 266 where the C of DQB1*03:11 is substituted by the A of DQB1*03:26 (shaded). (B) For DQB1*03:03:02:01, the nucleotide exchange leads to an amino-acid alteration to the protein sequence of DQB1*03:03:02:01 at residue 14 where the methionine (M) of DQB1*03:03:02:01 (shaded) is changed to the leucine (L) of DQB1*03:26 (shaded). For DQB1*03:11, the nucleotide exchange leads to an amino-acid alteration to the protein sequence of DQB1*03:11 at residue 57 where the amino acid, alanine (A), of DQB1*03:11(shaded) is replaced by the amino acid, aspartic acid (D), of DQB1*03:26 (shaded). Dashes indicate nucleotide or amino-acid identity with DQB1*03:03:02:01.

association with DQB1*03:26 and DRB1*14:141 are determined. It is worth mentioning that the most direct and classic method to determine HLA haplotype 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 a significant number of unrelated donors are available [3]. However, the haplotypes deduced via population investigation are considered either likely or most probable. In this study, because of the lack of availability of necessary test materials from the family of the donors with DQB1*03:26 and DRB1*14:141, the determination of the haplotypes was carried out by examining the HLA alleles carried in common by unrelated donors bearing the same alleles of interest. By the same token, if the determination of plausible HLA haplotypes involves rare or low-frequency HLA alleles, the alleles shared in common by unrelated individuals may be employed to deduce associated probable haplotypes [4–7,10–13].

The frequency of DQB1*03:26 and DRB1*14:141 in Taiwanese is about one in 40,000 according to our HLA typing practice. To date, the Allele Frequency Net Database (http://www.allelefrequencies.net/hla6006a.asp?hla_locus_type=Classical#) has yet to show the existence of the alleles in the world population. Therefore, it seems likely that the probable DQB1*03:26 and DRB1*14:141-associated HLA haplotypes in Taiwanese deduced in this study are highly credible.

The significance of determining the ethnicity of individuals with DQB1*03:26 and DRB1*14:141, and their HLA-linked haplotypes is that the information may be employed in the anthropological investigation of races in addition to allowing search coordinators at unrelated bone-marrow-donor registries to identify appropriate unrelated bone-marrow hematopoietic-stem-cell donors for their patients.

The number of known HLA alleles is increasing exponentially with the recent development of DNA-based molecular-typing technology. The outstanding HLA diversity in ethnic groups is unique and important. Facilitating the identification of an appropriate HLA-matched unrelated bone-marrow stem-cell donor 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. Additionally, the determination of haplotype is essential for the matching of unrelated stem-cell transplantation between a donor and a recipient, since matching at the haplotype level has a greater likelihood of producing a match at other loci within the HLA region than donors merely matched at the individual allelic level.

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