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Original Article

Human leukocyte antigen-A*33:03-B*58:01-DRB1*15:140, a deduced probable human leukocyte antigen haplotype in association with a human leukocyte antigen low-incidence allele DRB1*15:140 in Taiwanese individuals: A case analysis

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Abstract

Objective: Human leukocyte antigen (HLA)-DRB1*15:140 is a low-frequency allele in the HLA-DRB1 locus. The aim of this study is to confirm the ethnicity of DRB1*15:140 and to deduce a probable HLA-DRB1*15:140-associated HLA haplotype in Taiwanese individuals. **Materials and Methods:** A total of 1815 healthy unrelated Taiwanese individuals and 14,562 unrelated mainland Chinese individuals were tested for HLA using a sequence-based typing method. Polymerase chain reaction was performed to amplify exons 2 and 3 of the HLA-A and HLA-B loci and exons 1 and 2 of the HLA-DRB1 locus using group-specific primer sets. The amplicons were sequenced in both directions with the BigDye Terminator Cycle Sequencing Ready Reaction Kit according to the manufacturer's protocols. **Results:** The DNA sequence of HLA-DRB1*15:140 is identical to DRB1*15:02:01:01 in exons 1 and 2, except at residue 91 of DRB1*15:02:01:01 where a G in DRB1*15:02:01:01 is replaced by an A in DRB1*15:140 (codon 2; GAC->AAC). The nucleotide substitution in exon 1 introduces a one amino acid substitution at residue 2 where an aspartic acid (D) in DRB1*15:02:01:01 is replaced by an asparagine (N) in DRB1*15:140. We deduced the probable HLA haplotype associated with DRB1*15:140 in Taiwanese to be HLA-A*33:03-B*58:01-DRB1*15:140. **Conclusion:** Information on the ethnicity and distribution of DRB1*15:140 and its deduced probable HLA haplotype in association with the low-incidence allele is of value for HLA-testing laboratories for reference purposes and can help bone marrow donor registries find compatible donors for patients with this uncommon HLA allele.

Keywords: *DRB1*15:140, Haplotype, Human leukocyte antigen, Taiwanese, Transplantation*

Introduction

The major histocompatibility complex (MHC), including human leukocyte antigen (HLA) genes, is located in chromosome 6p21. It contains crucial genes for crucial immune responses and other genes with nonimmunological roles. The HLA genetic system, located in the MHC Class I and Class II regions, displays the highest degree of diversity of any functional genetic complex with medical impact representing a landmark for the development of preventive and predictive medicine [1].

New HLA alleles continue to be discovered, and the recognition of HLA low-incidence alleles has enriched our understanding of the complexity of the HLA system. The 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 to tissue transplantation. Their molecular similarity in donors and recipients is 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, comprehensive unrelated bone marrow

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hematopoietic stem cell donor searches for patients in need of hematopoietic stem cell transplantation, persistent effort is required to resolve unidentified, ambiguous, and low-incidence alleles to offer better HLA matching and donor selection [2].

HLA-DRB1*15:140, a rare frequency allele (http://www.allelefrequencies.net), was officially assigned by the World Health Organization HLA Nomenclature Committee in October 2016 [3]. The allele, discovered in a Taiwanese individual, was reported by Yang *et al*. without information on its associated HLA haplotype [4]. In this report, we confirm the ethnicity of DRB1*15:140 and identify the deduced plausible HLA haplotype in association with DRB1*15:140 based on HLA typing of the Taiwanese individuals we observed in our routine HLA typing practice.

Materials and Methods

A total of 1815 unrelated Taiwanese individuals and 14562 unrelated mainland Chinese individuals were tested for DRB1*15:140 in this study. Peripheral whole blood samples from donors with Taiwanese ethnicity and individuals with mainland Chinese ethnicity were collected in acid citrate dextrose (ACD) anticoagulant. Formal written consent was signed by the donors before blood collection. The ACD 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 the HLA-A, HLA-B, and HLA-DRB1 loci using a commercial polymerase chain reaction-sequencing-based typing kit (TBG, Medigen Biotechnology, Taipei, Taiwan). The amplicons were sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA) in both directions.

This study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of the Hualien Tzu Chi Hospital (IRB106-81-A).

Results

In a total of 1815 randomized Taiwanese individuals tested, four individuals with DRB1*15:140 were identified which makes the frequency of DRB1*15:140 in the Taiwanese population approximately 0.22%. However, in a total of 14,562 randomized mainland Chinese blood donors studied, no individual with DRB1*15:140 was detected.

We confirmed that the DNA sequence of HLA-DRB1*15:140 is identical to DRB1*15:02:01:01 in exons 1 and 2, except at residue 91 of DRB1*15:02:01:01 where a G in DRB1*15:02:01:01 is replaced by an A in DRB1*15:140 (codon 2; GAC->AAC) [Figure 1a]. The nucleotide substitution in exon 1 introduces a one amino acid substitution at position 2 where an aspartic acid (D) of DRB1*15:02:01:01 is replaced by an asparagine (N) in DRB1*15:140 [Figure 1b] [4].

The extended HLA-A,-B, and-DRB1 typing of the donors with DRB1*15:140 in this study was A*24:02, A*33:03, B*15:17, B*58:01, DRB1*13, DRB1*15:140; A*24:07, A*33:03, B*15:25, B*58:01, DRB1*03:01, DRB1*15:140; A*24:07, A*33:03, B*15:25, B*58:01, DRB1*03:01, DRB1*15:140; and A*11:02, A*33:03, B*58:01, DRB1*03:01, DRB1*15:140 [Table 1]. Based on the common HLA-A,-B, and-DRB1 alleles of the four donors, we deduced the most probable HLA haplotype in association with DRB1*15:140 in Taiwanese to be A*33:03-B*58:01-DRB1*15:140 [Table 1]. Our observations also strongly indicate the Taiwanese ethnicity of the rare HLA-DRB1 allele, DRB1*15:140.

Discussion

We confirmed the DNA sequence and amino acid sequence of the low-frequency HLA allele DRB1*15:140 [4] in this study. DRB1*15:140 was initially discovered in a Taiwanese individual (Genbank Accession Number KX810864; IMGT/HLA Database HWS10026909) without knowledge of the probable HLA haplotype in association with the allele [3,4]. In this study, we ascertained the ethnicity of DRB1*15:140 and proposed the deduced probable DRB1*15:140-associated HLA haplotype to be A*33:03-B*58:01-DRB1*15:140 based on the HLA-A,-B, and-DRB1 alleles shared in common by our*random donors [Table 1]. In addition, we propose that the deduced probable DRB1*15:140-associated HLA haplotype is most likely restricted to Taiwanese since DRB1*15:140 has only been found in the Taiwanese population but not in the mainland Chinese population. Furthermore, our search on the Allele Frequency Net (http://www.allelefrequencies.net) failed to find DRB1*15:140 in other populations. Information on the ethnicity of DRB1*15:140 and its linked HLA haplotype can be employed in anthropological investigation of races. In addition, bone marrow donor registries can allocate appropriate unrelated stem cell donors for patients with DRB1*15:140. In addition, knowing the nucleotide and amino acid variations between DRB1*15:140 and the prevalently observed

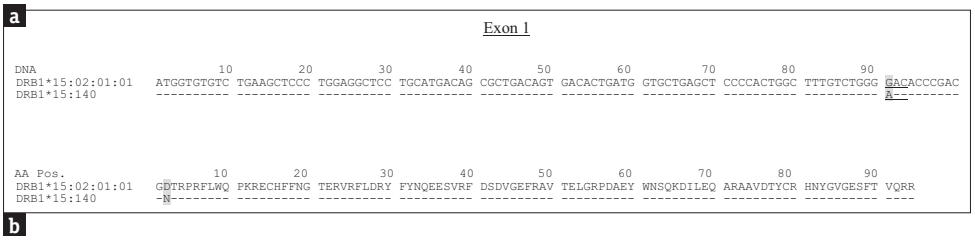

Figure 1: (a) The DNA sequence of DRB1*15:140 is identical to DRB1*15:02:01:01 in exon 1 and exon 2 (only exon 1 is shown here), except for residue 91 where a G in DRB1*15:02:01:01 is replaced by an A (shaded) (at codon 2; GAC->AAC; underlined) in DRB1*15:140. (b) The nucleotide substitution introduces a one amino acid substitution at residue 2 where an aspartic acid (D) of DRB1*15:02:01:01 is replaced by an asparagine (N) in DRB1*15:140 (shaded). Since nucleotides before codon 1 (GGG) at the 5' end of the sequence DRB1*15:140 are a noncoding sequence, the amino acid encoded by codon 2 (AAC) is the second amino acid residue of the DRB1*15:140 protein molecule. Dashes indicate nucleotide or amino acid identity with DRB1*15:02:01:01

Table 1: HLA typing of unrelated Taiwanese individuals with DRB1*15:140 and the deduced probable HLA haplotype associated with DRB1*15:140

Donor ID	HLA-A, -B and -DRB1 typing of donors	Deduced probable HLA haplotype in association with DRB1*15:140
Donor 1	A*24:02, A*33:03, B*15:17, B*58:01, DRB1*13, DRB1*15:140	A*33:03-B*58:01-DRB1*15:140
Donor 2	A*24:07, A*33:03, B*15:25, B*58:01, DRB1*03:01, DRB1*15:140	A*33:03-B*58:01-DRB1*15:140
Donor 3	A*24:07, A*33:03, B*15:25, B*58:01, DRB1*03:01, DRB1*15:140	A*33:03-B*58:01-DRB1*15:140
Donor 4	A*11:02, A*33:03, B*58:01, DRB1*03:01, DRB1*15:140	A*33:03-B*58:01-DRB1*15:140

HLA: Human leukocyte antigen

DRB1*15:02:01:01 allele may be helpful when selecting a minor HLA-mismatched unrelated bone marrow stem cell donor for a patient with the rare DRB1*15:140 allele.

It is worth mentioning that the most direct and classic method of determining HLA haplotypes 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 sufficient number of unrelated donors are available [5]. However, the haplotypes deduced through population investigation are considered to be likely or most probable. In this study, because of the lack of availability of necessary test material from the families, we opted to determine the haplotypes by looking at the HLA alleles carried in common by random unrelated donors with the same alleles of interest. By the same token, if determination of plausible HLA-associated 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 [6-13]. The frequency of DRB1*15:140 in Taiwanese is about 0.22%. Therefore, we think the deduced probable DRB1*15:140-associated HLA haplotype in Taiwanese that we deduced in this study is accurate. Nevertheless, we cannot exclude the possibility that additional DRB1*15:140-associated HLA haplotypes may be revealed in the future when more individuals are tested for DRB1*15:140.

The number of known HLA alleles is increasing with the recent development of DNA-based molecular typing technology. There is a high level of HLA diversity among ethnic groups, and knowledge of this diversity is important. Matching of bone marrow stem cell donors relies on the accuracy of HLA typing results. This is dependent on the resolution of unknown, ambiguous, and low-incidence genes in the HLA system.

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Conflicts of interest

There is no conflict of interest.

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