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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 Taiwanese population

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ABSTRACT

Objective: HLA-A*11:127N and HLA-B*40:221 are two rarely observed alleles in the HLA-A locus and in HLA-B locus, respectively. The objective of this study is to report the two plausible deduced HLA haplotypes in association with HLA-A*11:127N and HLA-B*40:221 in unrelated Taiwanese bone marrow stem cell donors.
Materials and Methods: A sequence-based typing method was used to confirm the low incidence alleles observed. Polymerase chain reaction was carried out to amplify exons 2, 3, and 4 of the HLA-A, -B, and -C loci 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 protocol.
Results: We deduced the two probable HLA haplotypes in association with A*11:127N and B*40:221 as 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, respectively.
Conclusion: Information on the two deduced HLA haplotypes associated with the rare A*11:127N and B*40:221 alleles that we report 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*11:127N and B*40:221 have so far been found only in the Taiwanese population, we think the haplotypes that we report here are most likely conserved in Taiwanese.

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 HLA (human leukocyte antigen) alleles are situated in the MHC class I and II regions. The recognized immunological functions contributed by HLA molecules in antigen presentation and MHC restriction clearly demonstrate the vital role of these molecules in the self-defense mechanism against microbial agents and immune regulations. Because HLA molecule similarity between donors and recipients is being utilized as a prediction factor for graft survival and graft versus host disease, it is imperative to precisely characterize any new allele encountered during routine HLA typing procedures. The HLA genes are characterized by their extreme allelic polymorphism and their variations and diversity among different ethnic groups and racial populations. The numbers of alleles at various HLA loci being unveiled are steadily increasing as human DNA is examined for HLA genes.

The population composition in Taiwan consists of Chinese mainlanders, Hakka, Minnan, aborigines [1], and other minor ethnicities. Thus, the database of the Taiwanese bone marrow donor registry consists of HLA alleles and haplotypes with unique characteristics. From time to time, new alleles, rare frequency alleles, and Taiwanese conserved haplotypes are identified in our routine HLA typing practice [2–5].

HLA-A*11:127N was first reported to the IMGT/HLA database [6] without an indication of a probable HLA haplotype in association with A*11:127N, whereas HLA-B*40:221 was first reported by Yang

Conflict of interest: none.

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et al [7]. However, the plausible HLA haplotype associated with B*40:221 deduced by Yang et al [7] was based on one individual only. Here we report the deduced HLA haplotype in association with A*11:127N and confirm the deduced probable HLA haplotype bearing B*40:221 reported by Yang et al [7]. We further postulate that these deduced HLA haplotypes in association with A*11:127N and B*40:221 are most likely conserved in Taiwanese, based on their scarce frequencies in the general population and the fact that they have so far been reported only in the Taiwanese population.

2. Materials and methods

Peripheral whole blood samples from two bone marrow donors with Taiwanese ethnicity were collected in an acid citrate dextrose anticoagulant. Formal written consent was obtained from each donor prior to blood collection. Acid citrate dextrose 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 the Dynal Reli-sequence-specific oligonucleotide probe HLA-A, -B, and -DRB1 Typing Kits (Dynal Biotech Ltd., Bromborough, Wirral, UK), followed by the sequence-specific primer (SSP) typing method (*AllSet* Gold SSP HLA high-resolution kits, Dynal Biotech Ltd; Invitrogen, Brown Deer, WI, USA) to reach high-resolution allelic subtypes. The sequence-based typing method [2,3] was used to confirm the low incidence alleles observed, and in cases of anomalous results and typing ambiguities from the sequence-specific oligonucleotide (SSO) or SSP typing protocols. Polymerase chain reaction was carried out to amplify exons 2 and 3 of the HLA-C locus and exon 2 of the DQB1 locus with group-specific primer sets as previously described [5]. Amplicons were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) in both directions. In this study, A*11:127N from one blood donor and B*40:221 from another donor were sequenced and analyzed.

3. Results

We confirmed the DNA sequence of HLA-A*11:127N as previously reported [6]. In exons 2 and 3, the DNA sequence of A*11:127N was identical to the sequence of A*11:01:01 except for a nucleotide substitution at residue 585 in exon 3, where A was substituted for C (at codon 171; TAC->TAA). The nucleotide replacement produced a stop codon (TAA) at codon 171 (Fig. 1). The extended HLA typings of the Taiwanese donor with A*11:127N reported to the IMGT/HLA database were A*02:06, A*11:127N, B*15:02, B*54:01, DRB1*04:05, and DRB1*12:02 [6], whereas the extended HLA typings of our Taiwanese donor carrying A*11:127N were A*02:07, A*11:127N, B*46:01, B*54:01, DRB1*04:05, and DRB1*09:01. With the HLA alleles that the two randomized unrelated Taiwanese donors shared in common, we deduced the most probable HLA-A, -B, and -DRB1 haplotype in association with A*11:127N as HLA-A*11:127N-B*54:01-DRB1*04:05. The other deduced HLA haplotype carried by our donor was A*02:07-B*46:01-DRB1*09:01, which is one of the most prevalent haplotypes in the Taiwanese population [5].

We also confirmed the DNA sequence of B*40:221, which was homologous to the sequence of B*40:01:01 in exons 2, 3, and 4, except for the nucleotide at codon 265 where GGG was replaced by AGG, as reported by Yang et al [7] (Fig. 2). The extended HLA typings of the Taiwanese donor bearing B*40:221 reported by Yang et al [7] were A*02:06, A*11:01, B*35:01, B*40:221, C*03:03, C*03:04, DRB1*14:54, DRB1*15:01, DQB1*05:02, and DQB1*06:02 [7], whereas the extended HLA typings of our Taiwanese donor with B*40:221 were A*02:06, A*11:01, B*39:01, B*40:221, C*03:04, C*07:02, DRB1*08:03, DRB1*14:54, DQB1*05:02, and DQB1*06:01. Considering the two randomized unrelated Taiwanese donors' HLA alleles that were shared in common, we assumed the most probable HLA haplotype in association with B*40:221 was HLA-A*11:01-B*40:221-C*03:04-DRB1*14:54-DQB1*05:02, as previously reported by Yang et al [7]. The other deduced HLA haplotype of our donor was a commonly observed HLA haplotype in the Taiwanese population: A*02:06-B*39:01-C*07:02-DRB1*08:03-DQB1*06:01 [5].

4. Discussion

The HLA system is the most polymorphic genetic system in humans. The number of recognized HLA alleles is markedly increasing with recent developments in DNA-based molecular HLA typing methodology [8]. A*11:127N was first discovered in a

Exon 3

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AA Codon                    95                 100                105                110                115
A*11:01:01     GT TCT CAC ACC ATC CAG ATA ATG TAT GGC TGC GAC GTG GGG CCG GAC GGG CGC TTC CTC CGC GGG TAC CGG CAG
A*11:02:01     -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
A*11:127N1     -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon                   120                125                130                135                140
A*11:01:01     GAC GCC TAC GAC GGC AAG GAT TAC ATC GCC CTG AAC GAG GAC CTG CGC TCT TGG ACC GCG GCG GAC ATG GCA GCT
A*11:02:01     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
A*11:127N      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon                   145                150                155                160                165
A*11:01:01     CAG ATC ACC AAG CGC AAG TGG GAG GCG GCC CAT GCG GCG GAG CAG CAG AGA GCC TAC CTG GAG GGC CGG TGC GTG
A*11:02:01     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
A*11:127N      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon                   170                175                180
A*11:01:01     GAG TGG CTC CGC AGA TAC CTG GAG AAC GGG AAG GAG ACG CTG CAG CGC ACG G
A*11:02:01     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -
A*11:127N      --- --- --- --- --- --A --- --- --- --- --- --- --- --- --- --- --- -
```

Fig. 1. Comparison of DNA sequences between A*11:01:01, A*11:02:01 and A*11:127N in exon 3. A*11:127N differs from A*11:01:01 and A*11:02:01 by one nucleotide at position 585 (C->A) (underlined). The nucleotide substitution produces a stop codon (TAA) at codon 171 (shaded).

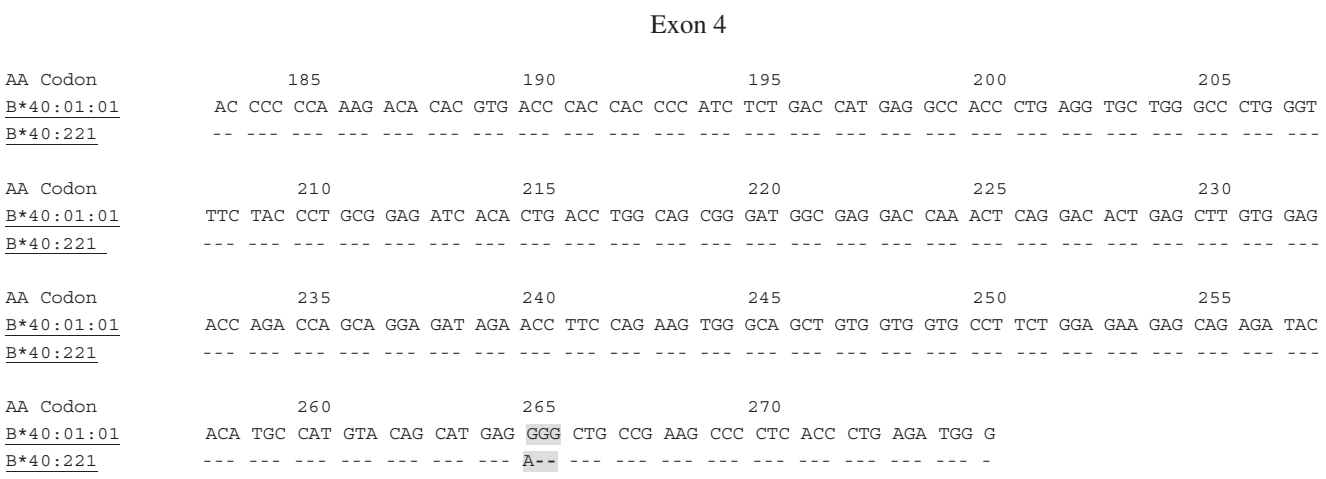

Fig. 2. Comparison of DNA sequences between B*40:01:01 and B*40:221 in exon 4. B*40:221 differs from B*40:01:01 by one nucleotide at codon 265 (GGG->AGG) (shaded).

Taiwanese individual [6]. We found a second Taiwanese individual bearing the same allele, thus confirming the ethnicity of the allele. We validated the DNA sequence of A*11:127N reported to the IMGT/HLA database [6]. Furthermore, our finding enabled us to deduce the probable HLA haplotype in association with A*11:127N based on the HLA alleles commonly shared by the two randomized unrelated Taiwanese individuals. Although no family study was performed because specimens from family members were not available, we are certain of the accuracy of the deduced probable A*11:127N-associated HLA haplotype, because the other deduced HLA haplotype (A*02:07-B46:01-DRB1*09:01) carried by our donor is one of the most prevalent HLA haplotypes in the Taiwanese ethnic group [5].

Yang et al [7] first discovered and reported the novel HLA-B*40:221 in a Taiwanese marrow stem cell donor. We report here a second individual, unrelated to the first donor, bearing the same allele. The second individual is also Taiwanese, hence confirming the ethnicity of the B*40:221 allele. We also confirmed that the second donor's DNA sequence in exons 2, 3, and 4 was identical to that of the first donor with B*40:221 (Fig. 2). Based on the established Taiwanese HLA haplotype database [5], Yang et al [7] deduced the first donor's B*40:221 associated HLA haplotype as HLA-A*11:01-B*40:221-C*03:04-DRB1*14:54-DQB1*05:02. From the second individual's extended HLA typing, the previous assumption of the B*40:221 associated HLA haplotype is fully supported. Although a family study to consolidate the B*40:221-associated HLA haplotype was not possible, we think that the haplotype deduced is not compromised considering that the individuals bearing B*40:221 were unrelated, and the incidence of B*40:221 in the general population is extremely rare.

In this study, we confirmed the DNA sequence of two low frequency HLA alleles, A*11:127N and B*40:221. We further validated the Taiwanese ethnicity for the alleles. Because both alleles have so far been found only in unrelated Taiwanese donors, we think the HLA haplotypes associated with the two alleles are likely conserved in the Taiwanese population. According to our HLA typing experience in randomized unrelated blood donors in Taiwan, we estimate the frequencies of A*11:127N and B*40:221 to be about one in 20,000 individuals to one in 30,000 individuals, respectively. The information obtained in this investigation may be helpful for hematopoietic stem cell transplant centers searching for unrelated stem cell donors and, if necessary, opting for a minor mismatched donor when a full matched donor is not readily available.

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