Original Article

Deduced probable HLA-C*07:359-associated human leukocyte antigen haplotypes found by case analysis of Taiwanese unrelated bone marrow hematopoietic stem cell donors

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Objectives: HLA-C*07:359 is a low-incidence allele in the human leukocyte antigen (HLA)-C locus. The objective of this study is to report the ethnicity and haplotypes of HLA-C*07:359 that were found during an analysis of Taiwanese unrelated bone marrow hematopoietic stem cell donors.

Materials and methods: A sequence-based typing method was employed to confirm low-incidence alleles. Polymerase chain reaction was performed to amplify exon 2 and exon 3 of the HLA-A, HLA-B, and HLA-C loci, as well as exon 2 of the HLA-DRB1 locus, using group-specific primer sets. The amplicons were sequenced in both directions using BigDye Terminator Cycle Sequencing Ready Reaction kits, according to the manufacturer’s protocols. The potential unrelated bone marrow stem cell donors investigated here are individuals with Taiwanese ethnicity who are participating in the Tzu Chi Bone Marrow Donor Registry.

Results: The DNA sequence of C*07:359 is identical to that of C*07:02:01:01 in exons 2, 3, and 4 except at residue 862, where the G of C*07:02:01:01 is substituted by the A of C*07:359. The nucleotide exchange leads to an amino acid replacement at codon 264, where the glutamic acid of C*07:02:01:01 is replaced by the lysine of C*07:359. We deduced a probable HLA-B and HLA-C haplotype that is associated with C*07:359 in Taiwanese, namely B*39-C*07:359.

Conclusion: Information on the ethnicity of the C*07:359 allele and its deduced probable HLA haplotypes that are associated with the low-incidence C*07:359 allele reported here are of value to HLA testing laboratories for reference purposes. In addition, they can be used by stem cell transplantation donor search coordinators to determine a strategy for finding compatible donors using unrelated bone marrow donor registries when patients carry this uncommon HLA allele.

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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 gene loci 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 MHC class I and II regions. These HLA genes are characterized by their extreme allelic polymorphism, as well as their variation and diversity across different ethnic groups and racial populations. HLA molecules have been definitively defined as transplant antigens and have a strong relevance to tissue transplantation. The presence of molecular similarity between donors and recipients is considered to be a predictive factor regarding 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 and comprehensive unrelated bone marrow hematopoietic stem cell donor searches for patients in

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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 DNA sequence of C*07:359 was first submitted to IMGT/HLA database in January 2014 without any indication as to its ethnicity and haplotype [1]. A second C*07:359 submission to the IMGT/HLA database was submitted in June 2014 [1] by Tzu Chi Immunogenetics Laboratory, and the donor in this case was a Taiwanese individual. There was no report at that time of a haplotype associated with C*07:359 because of a lack of blood samples from the family of the donor with this allele.

2. Materials and methods

Potential unrelated bone marrow stem cell donors are individuals with Taiwanese ethnicity who participate in the Tzu Chi Bone Marrow Donor Registry and who have given informed consent. Peripheral whole blood samples from eight unrelated bone marrow hematopoietic stem cell donors with Taiwanese ethnicity were collected in acid citrate dextrose anticoagulant. Formal written consents were signed by the donors before blood collection. The acid citrate dextrose whole blood samples were stored at −80 ºC until use. Genomic DNA was extracted using the QIAamp DNA Blood Mini kits according to the manufacturer’s instructions.
(Qiagen, Hilden, Germany). The DNA samples were subjected to HLA genotyping for the HLA-A, HLA-B, HLA-C, and HLA-DRB1 loci using commercial polymerase chain reaction–sequencing-based typing kits (Secore A/B/C/DRB1 Locus Sequencing kits; Life Technologies, Brown Deer, WI, USA). High-resolution allelic sequencing was performed as previously described [2–7]. The following two sets of primer sequences were used: (1) B-CG: M13-BIN1-CGG (sense): TGTAACAGCGCCGACCGGGGCGGCGACGGGCG (exon 5B) (antisense): GCTCCGATGACCACTGCT and (2) B-TA: M13-BIN1-TGA (sense): TGTAAAACGACGGCCAGTCGGGGGCACGTG (exon 5B) (antisense): GCTCCGATGACCACTGCT. The amplicons were sequenced in both directions using a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA).

Deduction of the HLA allele-associated probable haplotype, which was carried out in this study, involved looking at the commonly shared HLA typing of the donors carrying C*07:359. Where applicable, haplotype deduction based on HLA allelic homozgyosity was carried out as described previously [8,9]. For example, if a donor is typed for HLA-A, HLA-B, and HLA-DRB1 as 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. On the same token, 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

In this study, the DNA and protein sequences and the Taiwanese ethnicity of C*07:359 allele were confirmed. The DNA sequence of C*07:359 is identical to that of C*07:02:01:01 (frequency 20%) in exons 2, 3, and 4 except at residue 862, where the G (glutamic acid) of C*07:02:01:01 is replaced by the K (lysine) in C*07:359 (Fig. 1A). The nucleotide exchange leads to an amino acid replacement at codon 264 where the E (glutamic acid) of C*07:02:01:01 is replaced by the K (lysine) in C*07:359 (Fig. 1B). From the eight Taiwanese unrelated potential bone marrow donors studied here (Table 1), it is apparent that C*07:359 is closely linked with B*39 in the HLA-B locus, as all the eight donors with C*07:359 were found to be carry the B*39 allele. However, when the HLA-A alleles of the donors are taken into consideration, two probable HLA-A, HLA-B, and HLA-C haplotypes may be determined in Taiwanese donors, namely A*11-B*39-C*07:359 and A*24:02-B*39-C*07:359. This indicates that B*39-C*07:359 haplotype is not strictly restricted to a particular allele in the HLA-A locus. This variable linkage phenomenon is also observed in the case of C*07:359-carrying cells (ABAL 772993) submitted to the IMGT/HLA database (1). The C*07:359 allele of the ABAL 772993 cells is linked neither to B*39 in the HLA-B locus nor to A*11 or A*24 in the HLA-A locus, which was the case with the Taiwanese studied. Therefore, the haplotype HLA-B*39-C*359 is possibly strictly restricted to individuals of Taiwanese ethnicity.

4. Discussion

In this study, the Taiwanese ethnicity of C*07:359 and the DNA and protein sequences of C*07:359 allele were confirmed. Further, C*07:359 was found to be connected closely to B*39. However, the B*39-C*07:359 haplotype is not strictly restricted to associate with a particular HLA-A allele. Therefore, two probable HLA-C*07:359-associated HLA-A, HLA-B, and HLA-C haplotypes that are present in the Taiwanese population were identified. Furthermore, in the case of the C*07:359-carrying cells (ABAL 772993) that were submitted to the IMGT/HLA database (1), this is linked neither to B*39 in the HLA-B locus nor to A*11 or A*24 in the HLA-A locus. Further investigation on ABAL 772993 is still pending and its associated HLA haplotype remains undetermined.

It is worth mentioning that the most direct and classic method to determine HLA haplotypes is through a family study if samples from appropriate 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-based investigation are considered to be likely or most probable rather than certain. In this study, because of the lack of availability of necessary test material from the family of donors with C*07:359, determination of the haplotypes was carried out by examining the HLA alleles carried in common by unrelated donors bearing the same allele of interest. By the same token, if determination of plausible HLA haplotypes involves rare or low-frequency HLA alleles, the alleles shared in common by unrelated donors can be employed to deduce associated probable haplotypes [4–7,10–13].

The frequency of C*07:359 in Taiwanese is about 0.03%, according to our HLA typing practice. Furthermore, up to the present, the Allele Frequency Net Database (http://www.allelefrequencies.net/hla6006a.asp?hla_locus_type=Classical) has yet to show the existence of the C*07:359 allele in the world’s population outside of Taiwan.

The significance of determining the ethnicity of individuals with C*07:359 and its HLA-linked haplotypes is that the information may be employed in anthropological investigation of races. In addition, it also helps search coordinators, using unrelated bone marrow donor registries, in identifying appropriate unrelated bone marrow hematopoietic stem cell donors for their patients.

The number of known HLA alleles is increasing exponentially due to recent developments in DNA-based molecular typing technology. The outstanding HLA diversity in ethnic groups is unique and important. Facilitation of the identification of an appropriate HLA-matched unrelated bone marrow stem cell donor, which will
allow successful stem cell transplantations, relies on the accuracy of HLA typing and the spirit and strength to resolve the various unknown, ambiguous, and low-incidence genes present in the HLA system. Additionally, determination of haplotype is essential for the matching of unrelated stem cell transplantation between the donor and the recipient, since matching at the haplotype level has a better likelihood of achieving a match at other loci within the HLA region than when donors are merely matched at the individual allelic level.

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