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Recognition of HLA-A*11:01-B*51:01-C*14:02-DRB1*11:01-DQB1*03:13 and HLA-A*02-B*40-C*03:77-DRB1*14 haplotypes restricted to Taiwanese

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ABSTRACT

Objective: In the Tzu Chi Taiwanese Bone Marrow Donor Registry individuals carrying the human leukocyte antigen (HLA)-C*03:77 allele and -DQB1*03:13 allele were registered. Here we report the confirmed DNA sequences of C*03:77 and DQB1*03:13 and the probable HLA haplotypes deduced in association with C*03:77 (i.e., HLA-A*02-B*40-C*03:77) and DQB1*03:13 (i.e., HLA-A*11:01-B*51:01-C*14:02-DRB1*11:01-DQB1*03:13) in Taiwanese.

Materials and Methods: A sequence-based typing (SBT) method was used to confirm the low incidence alleles observed. Polymerase chain reaction was carried out to amplify exons 2 and 3 of the HLA-C locus with group-specific primer sets. For HLA-DQB1 allelic typing, six group-specific primer sets were used in the SBT procedure. Amplicons were sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (versions 3.1; Applied Biosystems, Foster City, CA, USA) in both directions according to the manufacturer's protocols.

Results: We confirmed the DNA sequence of C*03:77, which is identical to the sequence of C*03:04:01:01 in exons 2 and 3 except for the nucleotide at position 527 (A→T). The nucleotide substitution caused an amino acid replacement at residue (codon) 152 (E→V). Similarly, our SBT confirmed that the DNA sequence of DQB1*03:13 is analogous to the sequence of DQB1*03:01:01:01 in exons 2 and 3, except for the nucleotide at residue 296 (T→A). The nucleotide variation caused one amino acid exchange at residue (codon) 67 (V→D).

Conclusions: The Taiwanese ethnicity of our donors identified in this study completes the ethnicity background for C*03:77 and DQB1*03:13 alleles listed on the IMGT/HLA Database and provides a strategy for marrow donor registry search coordinators to screen for unrelated HLA matched hematopoietic stem cell donors for blood disease patients bearing C*03:77 or DQB1*03:13.

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1. Introduction

Determination of haplotype is essential for the matching of unrelated hematopoietic stem cell transplantation between donor and recipient, because matching at the haplotype level has a better likelihood of matching at other loci within the human leukocyte antigen (HLA) region than donors merely matched at the individual allelic level. It can provide useful information to transplant clinicians and donor recruitment centers to design search strategies to identify matched donors for confirmatory testing. It can also facilitate the selection of potential marrow stem cell donors in the database where HLA-A and -B typings are known, but -DRB1 typing has not been tested previously, or when allelic levels of the potential donors need to be determined. This can optimize the selection of low resolution type donors from unrelated donor searches. Furthermore, haplotype information may be utilized to consider a mismatch strategy for patients with rare alleles where the probability of locating a matched unrelated donor is less likely [1].

Determination of the HLA-A, -B, and -DRB1 haplotypes may be accomplished by HLA typing of blood-related family members and prediction from tissue typing in a large population. Alternatively, it can be achieved by deducing typing results from donors with allelic

Conflicts of interest: none.

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homozygosities in the HLA-A, -B, and -DRB1 loci [2]. In a family study, segregation of individual HLA alleles provides evidence of allelic linkages. In a population study, the determination of haplotypes involves noting whether alleles at the other two loci are consistently present and no family study is performed. Most available haplotype data are derived from studies of unrelated individuals in whom the putative haplotype is defined by statistical association analysis [3] or by allele association patterns shared in common [4–6].

In the Taiwanese population, many alleles in the HLA-C and -DQB1 loci show characteristic linkage disequilibria with HLA-B and -DRB1 alleles, respectively [3]. Examples include C*01:02 linked with B*46:01, C*03:02 with B*58:01, C*08:01 with B*15:02, DQB1*03:03 with DRB1*09:01, DQB1*03:01 with DRB1*12:02, and DQB1*06:01 with DRB1*08:03 [3]. The patterns of linkage disequilibrium provide a useful reference tool for selecting potential donors in HLA confirmatory testing prior to transplantation and might be also employed to check when specimen mix-up is suspected. Here we report the deduced probable HLA haplotypes associated with C*03:77 and DQB1*03:13 and the Taiwanese ethnicity of C*03:77 and DQB1*03:13.

2. Materials and methods

Peripheral whole blood samples from unrelated bone marrow stem cell donors who voluntarily participated in the Tzu Chi Marrow Donor Registry were collected in acid citrate dextrose anticoagulant. Formal written consents were signed by the donors before any blood collection was performed. Acid citrate dextrose whole blood was stored at −80°C prior to 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, -C, and -DRB1 loci were first performed using the Dynal Reli-sequence-specific oligonucleotide (SSO) probe HLA-A, -B, -C, and -DRB1 Typing Kits (Dynal Biotech, Bromborough, Wirral, UK) and then we proceeded to the sequence specific primer (SSP) typing method (AllSet Gold SSP HLA high-resolution kits; Dynal Biotech, Invitrogen, Milwaukee, WI, USA) to reach high resolution allelic subtypes. The sequence-based typing (SBT) method [7–10] was employed to confirm the low incidence alleles observed and in cases of anomalous results and typing ambiguities from the SSO or SSP typing protocols. Polymerase chain reaction was carried out to amplify exons 2 and 3 of the HLA-C locus with group-specific primer sets as previously described [10]. For HLA-DQB1 allelic typing, six group-specific primer sets were used in the SBT procedure for HLA-DQB1 detection as described by Dunn et al [11]. Amplicons were sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (versions 3.1; Applied Biosystems, Foster City, CA, USA) in both directions according to the manufacturer's protocols.

3. Results

Our SBT results confirmed the DNA sequence of C*03:77, which is identical to the sequence of C*03:04:01:01 in exons 2 and 3 except for the nucleotide at position 527 (A→T). The nucleotide substitution caused an amino acid replacement at residue (codon) 152 (E→V). The extended HLA typing of our volunteer bone marrow donor with C*03:77 was A*02:06, A*24:02, B*40:03, B*55:02, C*01:02, C*03:77, DRB1*09:01, DRB1*14:05, DQB1*03:03, and DQB1*05:03. Together with the HLA typing of the cell HC19619 at the IMGT/HLA Database (A*01:CNJK, A*02:AZGG, B*40:AVGG, B*53:01:01, C*03:77, C*04:CVAF, DRB1*13:02:01 and DRB1*16:02:01; www.ebi.ac.uk/cgi-bin/imgt/hla/ethnicity.cgi; [12]), we postulate that the probable HLA-A, -B, and -C haplotype in association with C*03:77 is A*02-B*40-C*03:77. Incidentally, B*40:03 is an uncommon B*40 variant in Taiwanese with a frequency of 0.11% in Minan Taiwanese and 0.22% in Taiwanese aborigines [13].

Table 1
Variation on the number of nucleotides between HLA-C*03:77, -C*03:02:01, -C*03:03:01, and -C*03:04:01:01.

HLA-	Number of nucleotides varies with HLA-			
	C*03:02:01	C*03:03:01	C*03:04:01:01	C*03:77
C*03:02:01	0	3	2	3
C*03:03:01	3	0	1	2
C*03:04:01:01	2	1	0	1
C*03:77	3	2	1[a]	0

[a] C*03:77 varies with C*03:04:01:01 with only one nucleotide.

Our SBT confirmed that the DNA sequence of DQB1*03:13 is analogous to the sequence of DQB1*03:01:01:01 in exons 2 and 3, except for the nucleotide at residue 296 (T→A). The nucleotide variation caused one amino acid exchange at residue (codon) 67 (V→D). The extended HLA typing of the cell HC12776 is A*02:01:01, A*02:07, C*01:02, C*14:02:01, DRB1*08:03:02, DRB1*11:01:01, DQB1*03:13, and DQB1*06:01 (B locus alleles not provided by the IMGT/HLA Database; www.ebi.ac.uk/cgi-bin/imgt/hla/ethnicity.cgi; [12]). We detected two Taiwanese unrelated marrow stem cell donors bearing DQB1*03:13 using our SBT protocol. The extended HLA typings of the two individuals were A*11:01, A*24:02, B*51:01, B*58:01, C*03:02, C*14:02, DRB1*11:01, DRB1*13:02, DQB1*06:09, and DQB1*03:13; and, A*11:01, B*15:01, B*51:01, C*04:01, C*14:02, DRB1*09:01, DRB1*11:01, DQB1*03:03, and DQB1*03:13. Taken together, the probable HLA haplotype in association with DQB1*03:13 in Taiwanese may be deduced as A*11:01-B*51:01-C*14:02-DRB1*11:01-DQB1*03:13.

4. Discussion

HLA-C*03:77 was first reported to the IMGT/HLA Database (cell ID HC19619) in 2010 without a determined ethnic origin. The DQB1*03:13 allele was first reported to the IMGT/HLA Database (cell ID HC12776) in 2002, again with an unknown ethnic origin (www.ebi.ac.uk/cgi-bin/imgt/hla/ethnicity.cgi; [12]). Neither HLA-C*03:77 nor HLA- DQB1*03:13 alleles and their haplotypes had been published previously. Hence, the Taiwanese ethnicity of our donors identified in this study completes the ethnic background for the C*03:77 and DQB1*03:13 alleles listed in the IMGT/HLA Database. Most importantly, the data derived from our study provides a strategy for marrow donor registry search coordinators to look for unrelated HLA-matched hematopoietic stem cell donors for blood disease patients bearing C*03:77 or DQB1*03:13. Indisputably, identification of the two rare alleles in Taiwanese contributes to a further understanding of the polymorphism and uniqueness of HLA-C and -DQB1 alleles in the Taiwanese population.

In our opinion, C*03:77 was derived from C*03:04:01:01, instead of C*03:02:01 and C*03:03:01, as a result of a nucleotide

Table 2
Variation on the number of amino acids between HLA-C*03:77, -C*03:02:01, -C*03:03:01, and -C*03:04:01:01.

HLA-	Number of amino acids varies with HLA-			
	C*03:02:01	C*03:03:01	C*03:04:01:01	C*03:77
C*03:02:01	0	3	2	3
C*03:03:01	3	0	1	2
C*03:04:01:01	2	1	0	1
C*03:77	3	2	1[a]	0

[a] C*03:77 varies with C*03:04:01:01 with only one amino acid.

Table 3
Variation on the number of nucleotides between HLA-DQB1*03:13, -DQB1*03:01:01:01, -DQB1*03:02:01, and -DQB1*03:03:02:01.

HLA-	Number of nucleotides varies with HLA-			
	DRB1*03:01:01:01	DQB1*03:02:01	DQB1*03:03:02:01	DQB1*03:13
DQB1*03:01:01:01	0	6	5	1
DQB1*03:02:01	6	0	1	7
DQB1*03:03:02:01	5	1	0	5
DQB1*03:13	1[a]	7	5	0

[a] DQB1*03:13 varies with DQB1*03:01:01:01 with only one nucleotide.

Table 4
Variation on the number of amino acids between human leukocyte antigen (HLA)-DQB1*03:13, -DQB1*03:01:01:01, -DQB1*03:02:01, and -DQB1*03:03:02:01.

HLA-	Number of amino acids varies with HLA-			
	DQB1*03:01:01:01	DQB1*03:02:01	DQB1*03:03:02:01	DQB1*03:13
DQB1*03:01:01:01	0	4	3	1
DQB1*03:02:01	4	0	1	4
DQB1*03:03:02:01	3	1	0	3
DQB1*03:13	1[a]	4	3	0

[a] DQB1*03:13 varies with DQB1*03:01:01:01 with only one amino acid.

point mutation at position 527, because C*03:77 varies from C*03:04:01:01 by one nucleotide but varies from C*03:02:01 and C*03:03:01 by three and two nucleotides, respectively (Table 1). Similarly, we assume that DQB1*03:13 was most likely derived from DQB1*03:01:01:01 (the most frequently observed DQB1*03 variant, followed by DQB1*03:03:02:01 and DQB1* 03:02:01, in Taiwanese) via a point mutation episode at nucleotide position 296 (T→A).

The frequencies of C*03:77 and DQB1*03:13 in Taiwanese are estimated to be about one in 20,000–30,000 according to our HLA typing experience. When a minor mismatched donor is intended as a stem cell donor for a patient bearing C*03:77 or DQB1*03:13, in our opinion C*03:04:01:01 or DQB1*03:01:01:01, respectively, could be considered. Our assumption is based on the nucleotide and amino acid similarities between C*03:04:01:01 (Tables 1 and 2) and C*03:77, and DQB1*03:01:01:01 and DQB1*03:13 (Tables 3 and 4).

In summary, we detected C*03:77 and DQB1*03:13 alleles in Taiwanese bone marrow stem cell donors. Our results complete the ethnic origin for the two alleles listed in the IMGT/HLA Database. Further, the probable HLA haplotypes associated with C*03:77 or DQB1*03:13 were deduced. We believe the probable haplotypes associated with C*03:77 and DQB1*03:13 that we deduced are highly likely, because the frequencies of both alleles are very low in randomized unrelated donors and are represented by at least two unrelated donors who were examined by independent HLA laboratories. Our findings suggest that both probable HLA haplotypes that we deduced from this study are restricted to Taiwanese.

Our DNA sequences of C*03:77 and DQB1*03:13 were submitted and accepted by the European Nucleotide Archive as confirmatory sequences for C*03:77 (accession number HE995410) and DQB1*03:13 (accession number HE995409) in September, 2012.

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References

[1] Kempenich JH, Setterholm M, Maiers M. Haplotype association of 90 rare alleles from the National Marrow Donor Program. Tissue Antigens 2006;67:284–9.
[2] Vorechovsky I, Kralovicoca J, Laycock MD, Webster AD, Marsh SG, Madrigal A, et al. Short tandem repeat (STR) haplotypes in HLA: an integrated 50-kb STR/linkage disequilibrium/gene map between the *RING3* and *HLA-B* genes and identification of STR haplotype diversification in the class III region. Eur J Hum Genet 2001;9:590–8.
[3] Yang KL, Chen SP, Shyr MH, Lin PY. High-resolution human leukocyte antigen (HLA) haplotypes and linkage disequilibrium of HLA-B and -C and HLA-DRB1 and -DQB1 alleles in a Taiwanese population. Hum Immunol 2009;70:269–76.
[4] Chen MJ, Yang TC, Chu CC, Shyr MH, Lin CL, Lin PY, et al. Detection of a novel HLA-B27 allele, B*2740, in Taiwanese volunteer bone marrow donors by sequence-based typing: curiosity rewarded. Int J Immunogenet 2009;36:207–11.
[5] Yang KL, Lee SK, Kao RH, Lin CL, Lin PY. Discovery of the novel HLA-DRB1*03:77 allele in a Taiwanese unrelated haematopoietic stem cell donor by a sequence-based typing method and identification of the probable HLA haplotype in association with DRB1*03:77. Int J Immunogenet 2012;39:442–4.
[6] Yang KL, Lee SK, Kao RH, Lin CL, Lin PY. Discovery of the novel HLA-DRB1*16:16 allele in a Taiwanese unrelated bone marrow stem cell donor by a sequence-based typing method and the probable HLA haplotype in association with DRB1*16:16. Int J Immunogenet 2012;39:445–7.
[7] Chen MJ, Chu CC, Lin PY, Yang KL. Sequence-based typing of a novel HLA-DRB1*04 allele, DRB1*0461, in a Taiwanese volunteer marrow donor. Int J Immunogenet 2007;34:269–72.
[8] Chen MJ, Chu CC, Shyr MH, Lin PY, Yang KL. Discovery of HLA-B*480102 in Taiwanese. Int J Immunogenet 2008;35:15–8.
[9] Chen MJ, Chu CC, Shyr MH, Lin PY, Yang KL. Identification of a novel HLA-A allele, A*1131, in a Taiwanese. Int J Immunogenet 2009;36:121–3.
[10] Chen MJ, Chu CC, Shyr MH, Lin CL, Lin PY, Yang KL. A novel HLA-B allele, B*5214, detected in a Taiwanese volunteer bone marrow donor using a sequence-based typing method. Int J Immunogenet 2010;37:39–41.
[11] Dunn PP, Day S, Williams S, Bendukidze N. HLA-DQB1 sequencing-based typing using newly identified conserved nucleotide sequences in introns 1 and 2. Tissue Antigens 2005;66:99–106.
[12] Robinson J, Mistry K, McWilliam H, Lopez R, Parham P, Marsh SG. The IMGT/HLA Database. Nucleic Acids Res 2011;39:D1171–6.
[13] Lai MJ, Wen SH, Lin YH, Shyr MH, Lin PY, Yang KL. Distributions of human leukocyte antigen-A, -B, and -DRB1 alleles and haplotypes based on 46,915 Taiwanese donors. Hum Immunol 2010;71:777–82.