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Original Article

Deduced probable HLA haplotypes associated with HLA-C*04:82 found by case analysis of Taiwanese individuals

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ABSTRACT

Objective: HLA-C*04:82 is a low incidence HLA-C allele. The aim here is to report the ethnicity of C*04:82 and its associated HLA haplotypes among Taiwanese individuals.
Materials and Methods: A sequence-based typing method was used to confirm this low incidence allele. Polymerase chain reaction was performed to amplify exons 2 and 3 of the HLA-A, HLA-B, and HLA-C loci and exon 2 of the HLA-DRB1 locus using group-specific primer sets. The amplicons were sequenced in both directions using Terminator Cycle Sequencing Ready Reaction kits and the manufacturer's protocols. The potential unrelated bone marrow stem cell donors in this study were randomized individuals with Taiwanese ethnicity who participated in the Tzu Chi Bone Marrow Donor Registry. The family members in the family part of the study were volunteer blood donors.
Results: The DNA sequence of C*04:82 was identical to C*04:01:01:01 in exons 2, 3, and 4. It differed from C*04:01:01:01 in exon 5 where a segment of nucleotides (CTAGCTGTC) was inserted between residues 969 and 970 of C*04:01:01:01. The insertion of these nucleotides caused a 35 amino acid alteration to the protein sequence of C*04:01:01:01. Three probable HLA haplotypes that were associated with C*04:82 among Taiwanese individuals were deduced. Confirmation of the DNA and protein sequences of C*04:82 and its Taiwanese ethnicity were established in this study.
Conclusion: The ethnicity of the C*04:82 allele and the deduced probable HLA haplotypes associated with the low-incidence C*04:82 allele are of value for reference purposes for HLA testing laboratories. In addition, they can be used by search coordinators to aid the creation of a strategy for finding compatible stem cell donors for patients who carry this uncommon HLA allele.
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1. Introduction

New HLA alleles continue to be revealed and 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 of genes that are located on the short arm of chromosome 6 at 6p21.3. These loci are classified into MHC class I, class II and class III regions. The genes encoding the HLA alleles are located in the MHC Class I and Class II regions. The HLA genes are characterized by their extreme allelic polymorphism as well as their variations and diversity across different ethnic groups. HLA molecules have been definitively defined as transplant antigens and have a strong relevance to tissue transplantation. The molecular similarity of these molecules between donors and recipients is considered to be 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. To facilitate successful and comprehensive unrelated bone marrow hematopoietic stem cell donor searches, persistent efforts are needed to resolve unidentified, ambiguous, or low-incidence alleles in order to offer better HLA matching and donor selection.

The nucleotide sequence of HLA-C*04:82 was first submitted to GenBank and the IMGT/HLA database (Accession No. FN550110;

Conflicts of interest: none.

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Cell ID LHLAS 000678) in September 2009, and the name HLA-C*04:82 officially assigned by the World Health Organization HLA Nomenclature Committee [1,2]. The donor is reported to be a Chinese individual. In 2013, a second individual (Cell ID D10044420) with C*04:82 was reported to the IMGT/HLA database by a laboratory in China [1]. For both these cases, however, there was no indication of C*04:82 associated HLA haplotypes. Here, we report the Taiwanese ethnicity of C*04:82 and the deduced probable HLA haplotypes associated with C*04:82, based on one family study and the HLA-A, HLA-B, HLA-C, and HLA-DRB1 alleles commonly shared across our randomized unrelated bone marrow stem cell donors and the two donors whose data were submitted to the IMGT/HLA database [1]. We further speculate that the deduced plausible HLA haplotypes associated with C*04:82 are restricted to individuals who are members of the Chinese and Taiwanese ethnic groups.

2. Materials and methods

Peripheral whole blood samples from a family consisting of five members and a range of unrelated bone marrow hematopoietic stem cell donors with Taiwanese ethnicity were collected in acid citrate dextrose anticoagulant. Formal written consent was individually given by the donors before blood collection. The acid citrate dextrose whole blood samples were stored at −80°C until use. Peripheral blood genomic DNA was extracted using QIAamp DNA Blood Mini kits (Qiagen, Hilden, Germany). The DNA obtained was 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 [3–8]. Two sets of primer sequences used were: (1) B-CG: M13-BIN1-CGG (sense): TGTAAAACGACGGCCAGTCGGGGGCGCAGGACCCGG; P3' exon 5B (anti-sense): GCTCCGATGACCACAACTGCT; and (2) B-TA: M13-BIN1-TGA (sense): TGTAAAACGACGGCCAGTGGCGGGGGCG-CAGGACCTGA; P3'exon 5B (anti-sense): GCTCCGATGACCA-CAACTGCT. The amplicons were then sequenced in both directions using a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA).

Determination of the deduced C*04:82 associated probable HLA haplotypes in this study involved looking at the commonly shared HLA typing of the donors carrying C*04:82 in one family under study and in the randomized unrelated donors who form the Tzu Chi Bone Marrow Donor Registry. Where applicable, haplotype deduction based on HLA allelic homozygosity, as described previously, was used [9,10]. For example, if a donor was typed for HLA-A, HLA-B and HLA-DRB1 as having *A*33:03*, –, *B*58:01*, –, *DRB1*03:01*, –, it was possible to deduce the putative haplotypes of the donor derived from the biological parents to be HLA-*A*33:03-B*58:01-DRB1*03:01* and *A*33:03-B*58:01-DRB1*03:01*, respectively. Similarly, if the typing of a donor was *A*02:01*, *A*02:07*, *B*46:01*, –, *DRB1*09:01*, –, then 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* [10].

3. Results

The frequency of HLA-C*04:82 was found to be about 0.048% based on the individuals analyzed in the present study, and in this study the oriental ethnicity of C*04:82 was identified. In addition, the report of Li et al [2] was confirmed, whereby the DNA sequence of C*04:82 was identical to that of C*04:01:01:01 in exons 2, 3 and 4 but differed from that of C*04:01:01:01 in exon 5 where a segment of nucleotides (CTAGCTGTC) was inserted between residues 969 and 970 of C*04:01:01:01 (Fig. 1). This nucleotide insertion led to the addition of three amino acids to the protein sequence of C*04:01:01:01, stretching from residue 301 to residue 303 (Fig. 2). Table 1 shows a family study in which C*04:82 was detected in the father and in two of his four children. In this family, C*04:82 was consistently associated with A*24:02, B*40:01 and DRB1*04:03 forming the haplotype A*24:02-B*40:01-C*04:82-DRB1*04:03. However, among the unrelated bone marrow (Table 2), all donors with C*04:82 were found to carry the B*40:01 allele, which is in full agreement with the family study (Table 1). However, when HLA-A and HLA-DRB1 alleles were taken into consideration, C*04:82 was found to be not restricted to A*24:02 and DRB1*04:03, respectively. Therefore, based on the studies of the family and the randomized unrelated donors, we are able to deduce three probable HLA-A and HLA-B haplotypes that were associated with C*04:82 in Taiwanese individuals: A*24:02-B*40:01-C*04:82; A*02:01-B*40:01-C*04:82; and A11- B*40:01-C*04:82 (Tables 1 and 2). Furthermore, when the randomized unrelated bone marrow stem cell donors were studied in detail, the linkage between C*04:82 and DRB1 alleles was not consistent. While it is clear that A*24:02 or DRB1*04:03 were found in some donors with C*04:82, however, other HLA-A or DRB1 allele types were also observed to coexist with C*04:82 (Table 2). Therefore, the HLA-A, HLA-B and HLA-DRB1 haplotypes that were associated with C*04:82 in the Chinese and Taiwanese populations can be reported as polymorphic based on the present results.

4. Discussion

In this study, the Taiwanese ethnicity of C*04:82 was determined. Furthermore, based on the family study, C*04:82 was found to be linked with A*24:02, B*40:01, and DRB1*04:03 to give the haplotype A*24:02-B*40:01-C*04:82-DRB1*04:03 (Table 1). Similarly, in the study of randomized potential unrelated bone marrow stem cell donors (Table 2), C*04:82 was also found to be associated with B*40:01. This observation indicates that there is a strong linkage between C*04:82 and B*40:01. However, it was also observed that when the HLA-A and HLA-DRB1 loci were examined, C*04:82 was not restricted to an association with only one particular allele. Its association with the alleles in the HLA-A or HLA-DRB1 locus indicates haplotype polymorphism, which is in contrast to its association with B*40:01 allele in the HLA-B locus.

It is worth mentioning that the most direct and classic method to determine HLA haplotype is through family studies if suitable test material from a number of key family members is available.

```
cDNA                    900        910        920        930        940        950        960                970        980        990
C*04:01:01:01           AGCCG TCTTCCCAGC CCACCATCCC CATCGTGGGC ATCGTTGCTG GCCTGGCTGT CCTGGCTGTC CTAGCTGTC.........C TAGGAGCTAT GGTGGCTGTT
C*04:82                 ----- ---------- ---------- ---------- ---------- ---------- ---------- ---------CTAGCTGTC- ---------- ----------

cDNA                     1000       1010
C*04:01:01:01       GTGATGTGTA GGAGGAAGAG CTCAG
C*04:82             ---------- ---------- -----
```

Fig. 1. The DNA sequence of C*04:82 was identical to C*04:01:01:01 in exons 2, 3 and 4 but differed from C*04:01:01:01 in exon 5 where a segment of nucleotides (CTAGCTGTC) (shaded) was inserted between residues 969 and 970 of C*04:01:01:01. Dashes indicate nucleotide identity with C*04:01:01:01.

```
AA Pos.                 10         20         30         40         50         60         70         80         90        100
C*04:01:01:01:01  GSHSMRYFST SVSWPGRGEP RFIAVGYVDD TQFVRFDSDA ASPRGEPREP WVEQEGPEYW DRETQKYKRQ AQADRVNLRK LRGYYNQSED GSHTLQRMFG
C*04:82           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

AA Pos.                110        120        130        140        150        160        170        180        190        200
C*04:01:01:01:01  CDLGPDGRLL RGYNQFAYDG KDYIALNEDL RSWTAADTAA QITQRKWEAA REAEQRRAYL EGTCVEWLRR YLENGKETLQ RAEHPKTHVT HHPVSDHEAT
C*04:82           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

AA Pos.                210        220        230        240        250        260        270        280        290        300
C*04:01:01:01:01  LRCWALGFYP AEITLTWQWD GEDQTQDTEL VETRPAGDGT FQKWAAVVVP SGEEQRYTCH VQHEGLPEPL TLRWKPSSQP TIPIVGIVAG LAVLAVLAVL
C*04:82           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

AA Pos.                310        320        330        340
C*04:01:01:01:01  GAMVAVVMCR RKSSGGKGGS CSQAASSNSA QGSDESLIAC KA
C*04:82           AVLG-M-AVV MCRRKSS--K GGSCSQAA-S NSAQG-DESL I-
```

Fig. 2. The nucleotide insertion described in Fig. 1 led to a three amino acid insertion into the protein sequence of C*04:01:01:01 from residue 301 to 303. This resulted in the protein sequence of C*04:82 being changed greatly compared to C*04:01:01:01; there being 35 amino acid differences (shaded) in comparison with C*04:01:01:01. Dashes indicate amino acid identity with C*04:01:01:01.

Table 1
Family study.[a]

Donor ID	HLA-A*		HLA-B*		HLA-C*		HLA-DRB1*	
Father	24:02	–	40:01	–	03:04	04:82	04:03	04:05
Child 1	02:03	24:02	40:01	52:01	04:82	07:02	04:03	14:04
Child 2	02:03	24:02	40:01	52:01	04:82	07:02	04:03	14:04
Child 3	02:03	24:02	40:01	52:01	03:04	07:02	04:05	14:04
Child 4	11:01	24:02	40:01	–	03:04	–	04:05	15:01
Mother[b]	02:03	11:01	40:01	52:01	03:04	07:02	15:01	14:04

[a] In the family study, the father and Child 1 and 2 had the C*04:82 allele, which was linked with A*24:02, B*40:01, and DRB1*04:03 (shaded) to form the haplotype A*24:02-B*40:01-C*04:82-DRB1*04:03, which was associated with C*04:82.
[b] Haplotype deduced based on the family study.

The significance of determining the ethnicity of individuals with C*04:82 and its HLA linked haplotypes is that this information may now be used in anthropological investigation of races, and to help search coordinators working at unrelated bone marrow donor registries with the allocation of appropriate unrelated bone marrow hematopoietic stem cell donors to their patients in need of a transplant.

The number of known HLA alleles is increasing exponentially due to recent developments in DNA-based molecular typing technology. The outstanding HLA diversity across ethnic groups is both unique and important. Facilitating an appropriate HLA match for a given unrelated bone marrow stem cell donor allows successful

Table 2
Stem cell donors study.[a]

Donor ID	HLA-A*		HLA-B*		HLA-C*		HLA-DRB1*		HLA-DQB1*	
160205	24:02	33:03	40:01	58:01	03:02	04:82	04:03	12:02	03:02	05:02
261462	02:01	11:01	40:01	48:01	03:04	04:82	15:01	15:02		
281984	02:01	24:02	35:01	40:01	04:82	08:01	09:01	12:01	03:01	03:03
271591	11:01	24:02	40:01	51:01	04:82	14:02	04:03	11:01	03:01	03:02
239852	02:01	24:02	40:01	48:01	03:03	04:82	09:01	09:01		
323581	02:01	02:07	40:01	51:01	04:82	15:02	04:03	14:05		
310562	11:02	24:02	40:01	54:01	01:02	04:82	09:01	14:05		
342662	11:01	11:01	15:02	40:01	04:82	08:01	04:03	12:02		
362832	24:02	33:03	40:01	58:01	03:02	04:82	04:03	12:02	03:01	03:02
273635	02:01	24:02	40:01	40:02	04:82	15:02	04:03	04:05	03:02	04:01
057910	02:01	33:03	40:01	58:01	03:02	04:82	09:01	13:02		
337441	02:07	24:02	40:01	40:55	04:82	15:02	04:03	04:05	03:02	04:01
D10044420	24:02	31:01	40:01	44:03	04:82	07:06	07:01	09:01		
LHLAS000678	02:01	33:03	40:01	58:01	03:02	04:82	04:03	07:01		

[a] Deduced probable HLA-A and HLA-B haplotypes associated with C*04:82 based on the profiles of randomized unrelated donors who were Taiwanese or Chinese as reported by the IMGT/HLA database (underlined). In all cases, C*04:82 was linked with B*40:01 (shaded). However, when the HLA-A and HLA-DRB1 alleles were taken into consideration, C*04:82 was not restricted to association with only one particular HLA-A or HLA-DRB1 allele. Only a few individuals display the A*24:02-B*40:01-C*04:82-DRB1*04:03 haplotype (underlined).

Alternatively, a population study may be used if a significant number of unrelated donors are available [4]. However, the haplotypes deduced via a population investigation are considered to be either likely or most probable.

Based on the analyses of the present family study (Table 1) and the unrelated potential bone marrow stem cell donors who were investigated (Table 2), at least three probable C*04:82 associated HLA-A, HLA-B, and HLA-C haplotypes may be deduced, that is, A*02:01-B*40:01-C*04:82; A11-B*40:01-C*04:82; and A*24:02-B*40:01-C*04:82. However, in order to determine the HLA-A, HLA-B, HLA-C, and HLA-DRB1 haplotypes that are associated with C*04:82, a more extensive investigation that includes a greater number of individuals bearing C*04:82 is essential.

stem cell transplantation and relies on the accuracy of HLA typing and the spirit and strength to resolve the unknown, ambiguous, and low-incidence genes still present in the HLA system. Additionally, determination of haplotype is essential when matching for unrelated stem cell transplantation between donor and recipient, since matching at the haplotype level has a better likelihood of matching at other loci within the HLA region than when donors are merely matched at the individual allelic level.

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