



## Original Article

# The deduced probable HLA-C\*03:187-associated human leukocyte antigen haplotype (A\*24:02-B\*35:01-C\*03:187-DRB1\*11:01) revealed in Taiwanese unrelated hematopoietic bone marrow stem cell donors

Kuo-Liang Yang<sup>a,b\*</sup>, Py-Yu Lin<sup>a</sup>

<sup>a</sup>Laboratory of Immunogenetics, Tzu Chi Cord Blood Bank and Buddhist Tzu Chi Bone Marrow Donor Registry, Buddhist Tzu Chi Stem Cells Centre, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan, <sup>b</sup>Department of Laboratory Medicine and Biotechnology, Tzu Chi University, Hualien, Taiwan

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## ABSTRACT

**Objective:** HLA-C\*03:187 is a rare frequency allele in the human leukocyte antigen (HLA)-C locus. The purpose of this investigation is to indicate the ethnicity of C\*03:187 and its deduced plausible HLA haplotype in association in Taiwanese unrelated bone marrow stem cell donors. **Materials and Methods:** A DNA sequence-based typing procedure was used to verify the rare frequency allele C\*03:187. Employing group-specific primer sets' polymerase chain reaction was carried out to amplify exons 2 and 3 of HLA-A locus, HLA-B locus, exon 1 to exon 7 of the HLA-C locus, and exon 2 of the HLA-DRB1 locus. The amplified gene products were sequenced employing the BigDye® Terminator Cycle Sequencing Ready Reaction kits in both directions according to the manufacturer's instructions. **Results:** The DNA sequence of C\*03:187 is identical to C\*03:03:01:01 from exon 1 to exon 7, except for codon 152 of exon 3 where GAG of C\*03:03:01:01 is replaced by GTG in C\*03:187. The nucleotide replacement causes one amino acid change to the protein sequence of C\*03:03:01:01 at position 152 where glutamic acid (E) is changed to a valine (V) in C\*03:187. The deduced plausible HLA haplotype in association with C\*03:187 in Taiwanese is as A\*24:02-B\*35:01-C\*03:187-DRB1\*11:01. **Conclusion:** The data on the deduced plausible HLA haplotype in association with the low-frequency C\*03:187 allele that we described in this report are valuable for immunogenetics laboratories for reference purposes. In addition, they can be utilized by search coordinators in hematopoietic stem cell transplant programs to determine a strategy for locating compatible donors in unrelated bone marrow donor registries for a patient with this unusual HLA allele.

**KEYWORDS:** Haplotype, HLA-C\*03:187, Human leukocyte antigen, Sequence-based typing, Taiwanese

## INTRODUCTION

Major histocompatibility complex (MHC) was discovered originally as a genetic locus controlling rapid rejection of tissue grafts expressing surface antigens. In humans, these transplantation antigens are referred to as human leukocyte antigens (HLAs). The discovery of MHC restriction and molecular identification of MHC genes and their products has subsequently led to a unified theory of the principal physiological functions of MHC molecules to act as guidance molecules for immune responses. The genes found within the HLA system are involved in recognition of foreign antigen in association with HLA gene products that are referred to as restriction recognition. Moreover, it is a process that has evolved in a manner that enables the immune system in mammals to respond effectively to foreign antigens while at the same time to recognize but not respond to self-antigens [1,2].

The overall size of the MHC is about 4000 kb. It contains the most polymorphic genes and the highest diversity of functional gene cluster of the human genome harboring over 270 genes. Today, at least 22,000 different HLA alleles have been reported [1,2]. Low-incidence HLA alleles are still being revealed every day. Recognition of the new- and low-frequency alleles has enriched our appreciation on the complexity and the importance of the HLA system clinically and scientifically [1].

### \*Address for correspondence:

Prof. Kuo-Liang Yang,  
Buddhist Tzu Chi Stem Cells Centre, Hualien Tzu Chi Hospital, Buddhist  
Tzu Chi Medical Foundation, 707, Section 3, Chung-Yang Road, Hualien,  
Taiwan.  
E-mail: edward@tzuchi.com.tw

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To date, many ethnic diversities in allogenic HLA loci are not fully recognized due to the nature of extensive HLA polymorphism. To elucidate the essence of the individual HLA allele and its variants in terms of clinical implications, it is imperative to unambiguously define variants of HLA allele. Information from this will assist in evaluating the degree of compatibility between donor and recipient in transplantation, as well as providing a measurable criterion to make a prognosis for the outcome of transplantation [3].

The nucleotide sequence of HLA-C\*03:187 (cell ID HG00005395) was first reported to the IPD-IMGT/HLA Database, and the term of HLA-C\*03:187 was assigned by the World Health Organization HLA Nomenclature Committee officially in June 2013 [4]. Nevertheless, the information on the ethnicity of C\*03:187 is lacking, and its HLA-associated haplotype is undermined. Yang and Lin [5] reported the detection of C\*03:187 in a Taiwanese individual in 2018. Nevertheless, due to the lack of specimens from the family members of the blood donor bearing the C\*03:187, a deduced probable HLA haplotype associated with C\*03:187 was impossible. We report here the Taiwanese ethnicity of C\*03:187 and its deduced plausible HLA haplotypes associated with C\*03:187 based on the HLA-A, -B, -C, and -DRB1 alleles shared in common by the C\*03:187-bearing donor (ID HG00005395) reported to the IPD-IMGT/HLA Database [4] and the donors with C\*03:187 in our registry. In addition, we further speculate that the deduced probable HLA haplotype associated with C\*03:187 is only found in the Taiwanese population.

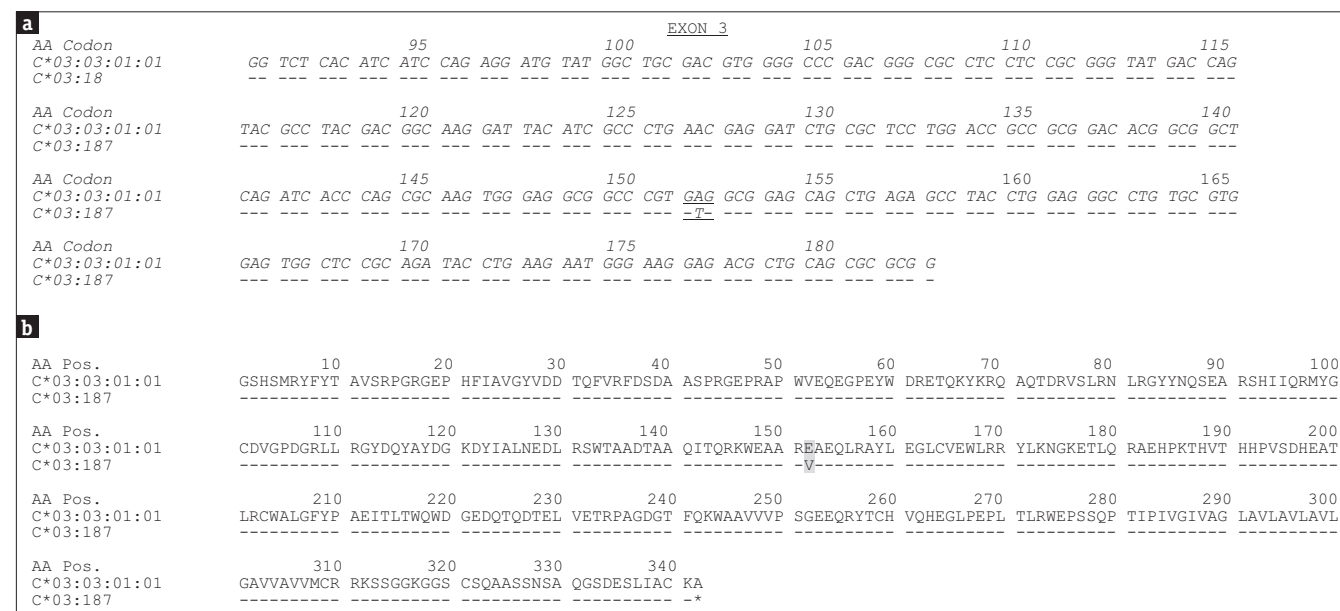
## MATERIALS AND METHODS

We use sterile tubes with acid citrate dextrose (ACD) or ethylenediaminetetraacetic acid (EDTA) anticoagulant to collect the peripheral blood samples from unrelated bone

marrow hematopoietic stem cell donors with Taiwanese ethnicity. Formal written consents were signed by the donors before blood collection. The ACD- or EDTA-anticoagulated whole blood samples were stored at -80°C until use. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kits according to the instructions provided by the manufacturer (Qiagen, Hilden, Germany). The DNA material 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 (TBG, Medigen Biotechnology, Taipei, Taiwan). Since the deduction of HLA haplotype in this study is a common routine practice, institution review is exempt in our institute.

## RESULTS

We confirmed that the DNA sequence of C\*03:187 is identical to C\*03:03:01:01 from exon 1 to exon 7, except in codon 152 of exon 3 where GAG of C\*03:03:01:01 is replaced by GTG in C\*03:187 [Figure 1a]. The nucleotide replacement causes one amino acid substitution to the protein sequence of C\*03:03:01:01 at position 152 of exon 3 in which glutamic acid (E) of C\*03:03:01:01 is exchanged by a valine (V) in C\*03:187 [Figure 1b]. The extended HLA-A, -B, -C, and -DRB1 typing of our donors with C\*03:187 are shown in Table 1. Together with the HLA typing (A\*24:BMD, A\*24:KNHR, B\*35:MVJD, B\*51:FSSY, C\*03:187, C\*12:04:02, and DRB1\*07:01:01, DRB1\*11:CTPB) of the donor HG00005395 reported to the IPD-IMGT/HLA Database [4], we deduced the probable HLA-A, -B, -C, and -DRB1 haplotype in association with C\*03:187 in our Taiwanese donors as A\*24:02-B\*35:01-C\*03:187-DRB1\*11:01 [Table 1]. We further speculate that individuals bearing the A\*24:02-B\*35:01-C\*03:187-DRB1\*11:01 haplotype are Taiwanese.



**Figure 1:** (a) The DNA sequence of C\*03:187 is identical to C\*03:03:01:01 from exon 1 to 7, except for codon 152 of exon 3 (shown here), where GAG (underlined) of C\*03:03:01:01 is substituted by GTG (underlined) in C\*03:187. (b) The nucleotide substitution introduces one amino acid replacement at the residue 152 where glutamic acid (E) (shaded) of C\*03:03:01:01 is replaced by a valine (V) (shaded) in C\*03:187. Dashes indicate nucleotide or amino acid identity with C\*03:03:01:01

**Table 1: Human leukocyte antigen-A, -B, -C, and -DRB1 alleles of donors with C\*03:187 and the deduced probable human leukocyte antigen-A-B-C-DRB1 haplotypes in association with C\*03:187**

Donor	HLA-A*		HLA-B*		HLA-C*		HLA-DRB1*		C*03:187-associated haplotypes
Donor 1	24:02		35:01	40:01	03:187	07:02	09:01	11:01	A*24:02-B*35:01-C*03:187-DRB1*11:01
Donor 2	24:02	26:01	35:01	40:02	03:187	03:03	11:01	15:01	A*24:02-B*35:01-C*03:187-DRB1*11:01
IMGT <sup>x</sup>	24:BMD	24:KNHR	35:MVJD	51:FSSY	03:187	12:04:02	11:CTPB	07:01:01	A*24-B*35-C*03:187-DRB1*11

<sup>x</sup>IPD-IMGT/HLA Database (HG00005395), IMGT: Immunogenetic

## DISCUSSION

In this study, we confirmed the DNA sequence in exons 2 and 3 of C\*03:187 reported to the IPD-IMGT/HLA Database previously [4,5]. In addition, we confirmed the DNA sequences of C\*03:03:01:01 and C\*03:187, as reported by Yang and Lin [5], that are identical in exon 1 and from exon 4 to exon 7, except for codon 152 of exon 3 where GAG of C\*03:03:01:01 is replaced by GTG in C\*03:187 [Figure 1a]. We also confirmed that the matured amino acid sequence encoded by C\*03:187 and C\*03:03:01:01 is identical except for position 152, where glutamic acid (E) of C\*03:03:01:01 is substituted by a valine (V) in C\*03:187 [Figure 1b] that was described in the IPD-IMGT/HLA Database [4,5]. The DNA sequence of C\*03:187 was initially reported to the IPD-IMGT/HLA Database with neither any information of its ethnic group [4] nor the HLA haplotype in association with the allele [4,5]. In this current report, we deduced the plausible C\*03:187-associated HLA haplotypes as A\*24:02-B\*35:01-C\*03:187-DRB1\*11:01, based on the commonly shared HLA typing of the two donors carrying C\*03:187 listed in our registry and the donor reported to the IPD-IMGT/HLA Database (HG00005395) [Table 1]. We further speculate that individuals carrying A\*24:02-B\*35:01-C\*03:187-DRB1\*11:01 haplotype are probably Taiwanese since both donors with this haplotype in our registry are Taiwanese.

Direct approach to determine HLA haplotype via family study when test materials from a number of key family members are available is the most classical practice. Otherwise, population study may be employed when a significant number of unrelated donors is available alternatively [6,7]. However, the haplotypes deduced via population study are considered as likely or most probable. In this present investigation, due to the lack of availability of necessary test materials from the family of the donors with C\*03:187, we opted to determine the haplotype by looking at the HLA alleles carried in common by unrelated donors bearing the same alleles of interest. Similarly, if the determination of plausible HLA haplotypes is for low frequency or rare HLA alleles, the alleles shared in common by unrelated individuals may be employed to deduce associated plausible haplotypes [8-12].

In Taiwanese population, the frequency of C\*03:187 allele is about 1 in 40,000 according to our HLA typing procedure. It was neither detected in a total of 812,211 Chinese individual donors in Mainland China tested [13], nor was it found in a total of 5,104,477 German individuals studied [7]. In addition, to date, the Allele Frequency Net Database ([http://www.allele-frequencies.net/hla6006a.asp?hla\\_locus\\_type=Classical#](http://www.allele-frequencies.net/hla6006a.asp?hla_locus_type=Classical#)) has yet to show the presence of C\*03:187 in the world population.

Therefore, we think the probable C\*03:187-associated HLA haplotype in Taiwanese that we deduced in this study is highly accountable.

The essence to determine the ethnicity of individuals with C\*03:187 and its HLA-associated plausible haplotypes is that the data may be employed for anthropological investigation of races in addition to allowing search coordinators in unrelated bone marrow donor registries to find suitable unrelated bone marrow hematopoietic stem cell donors for their recipients carrying this low-incidence HLA allele.

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## Conflicts of interest

There are no conflicts of interest.

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