Tzu Chi Medical Journal 28 (2016) 59–62

Contents lists available at ScienceDirect

Tzu Chi Medical Journal

journal homepage: www.tzuchimedjnl.com

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

Kuo-Liang Yang [a, b, *]

[a] Laboratory of Immunogenetics, Tzu Chi Cord Blood Bank and Buddhist Tzu Chi Marrow Donor Registry, Buddhist Tzu Chi Stem Cells Centre, Hualien Tzu Chi Hospital, Hualien, Taiwan
[b] Department of Laboratory Medicine, Buddhist Tzu Chi University, Hualien, Taiwan

ARTICLE INFO

Article history:
Received 24 February 2016
Received in revised form
14 March 2016
Accepted 18 March 2016
Available online 27 May 2016

Keywords:
Haplotype
Hematopoietic stem cell
Human leukocyte antigen
Sequence-based typing
Transplantation

ABSTRACT

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.

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

Conflict of interest: none.

* Corresponding author. Buddhist Tzu Chi Stem Cells Centre, Hualien Tzu Chi Hospital, 707, Section 3, Chung-Yang Road, Hualien, Taiwan. Tel.: +886 38561825x3373; fax: +886 38567851.
E-mail address: edward@tzuchi.com.tw.

http://dx.doi.org/10.1016/j.tcmj.2016.04.004

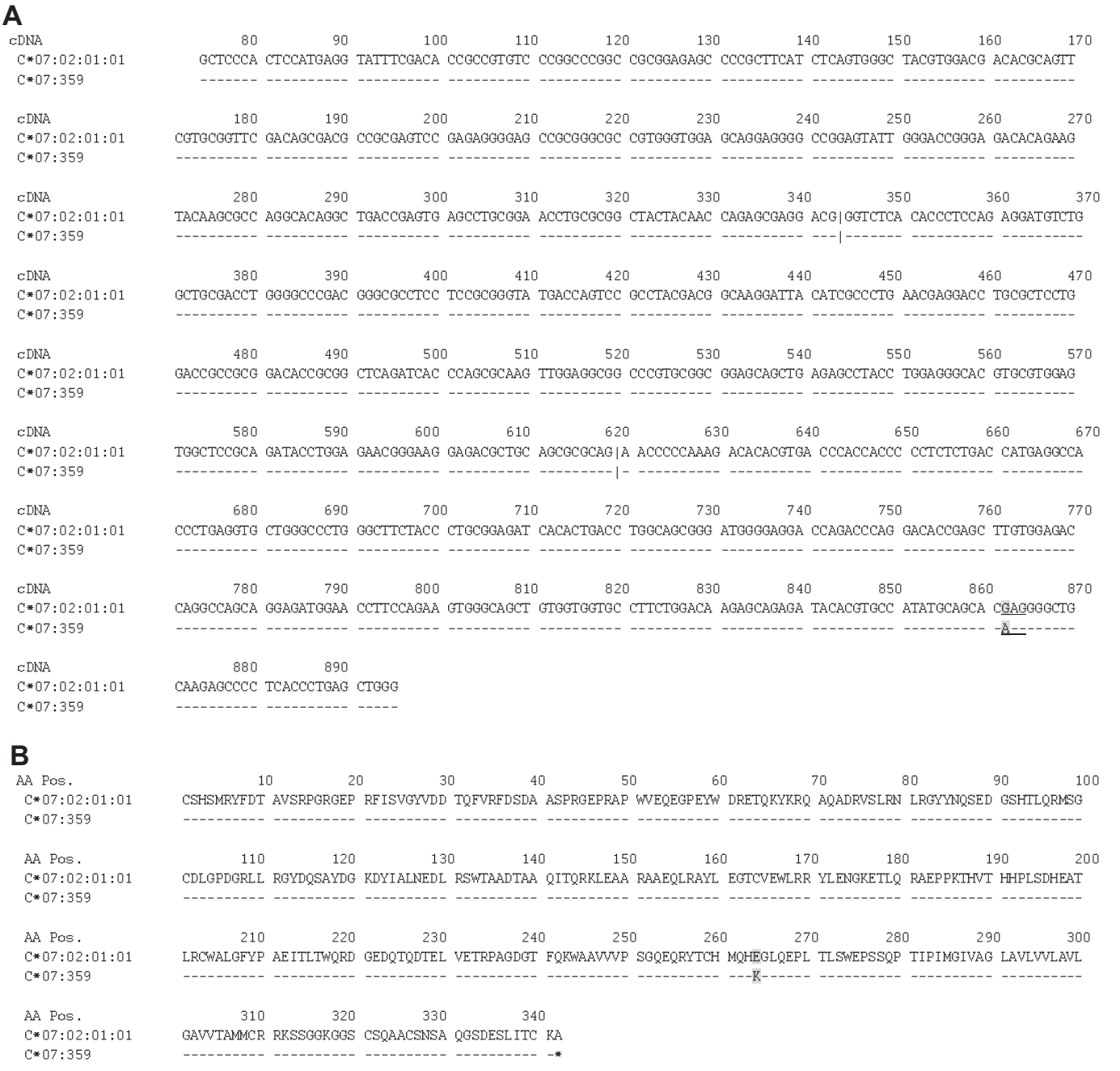

Fig. 1. (A) The DNA sequence of C*07:359 is identical to that of C*07:02:01:01 in exons 2, 3, and 4 except at residue 862, where the G of C*07:02:01:01 is substituted by the A in C*07:359 (shaded; codon 264; underlined). Exons 2 and 3 are separated by pipes (|) between nucleotides 343 and 344, and exons 3 and 4 are separated by pipes (|) between nucleotides 619 and 620. (B) 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 (shaded). The dashes indicate nucleotide or amino acid identity with C*07:02:01:01.

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°C until use. Genomic DNA was extracted using the QIAamp DNA Blood Mini kits according to the manufacturer's instructions

Table 1
Deduced probable HLA-A, HLA-B, and HLA-C haplotypes associated with C*07:359 in Taiwanese.

Donor ID	HLA-A*		HLA-B*		HLA-C*		HLA-DRB1*		Ethnicity	Deduced probable C*07:359-associated HLA haplotype
313413	11	24	39	40	03:04	07:359	04	15	Taiwanese	B*39-C*07:359
331307	11	02	39	46	01:08	07:359	12	16	Taiwanese	B*39-C*07:359
357339	02:01	24:02	39:01	40:01	07:02	07:359	04:04	04:05	Taiwanese	B*39-C*07:359
368267	11:02	24:02	39:01	40:01	07:02	07:359	04:04	04:05	Taiwanese	B*39-C*07:359
371042	24:02	—	39:01	40:01	03:04	07:359	04:04	04:05	Taiwanese	B*39-C*07:359
R39387	24:02	34:01	39:01	40:01	04:82	07:359	04:04	04:05	Taiwanese	B*39-C*07:359
AB3586	24:02	33:03	15:25	39:01	04:03	07:359	04:04	12:02	Taiwanese	B*39-C*07:359
295076	24	—	39	56	01:02	07:359	04	15	Taiwanese	B*39-C*07:359
ABAL772993 (1)	02	03:01	07:02	08:01	07:02	07:359	03:01	04:05	Unknown	?

HLA = human leukocyte antigen.

(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): TGTAAAACGACGGCCAGTCGGGGGCGCAGGACCCGG; P3′ exon 5B (antisense): GCTCCGATGACCACAACTGCT and (2) B-TA: M13-BIN1-TGA (sense): TGTAAAACGACGGCCAGTGGCGGGGGCG-CAGGACCTGA; P3′ exon 5B (antisense): GCTCCGATGACCA-CAACTGCT. The amplicons were sequenced in both directions using a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA).

Determination 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 homozygosity was carried out as described previously [8,9]. For example, if a donor is typed for HLA-A, HLA-B, and HLA-DRB1 as having *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 the 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 of C*07:02:01:01 is substituted by the A 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 bear 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 linked 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 individuals 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.

Acknowledgments

I am indebted to all the volunteer donors who willingly joined the Taiwan Buddhist Tzu Chi Bone Marrow Donor Registry and gave their consent for this research project. Their unselfishness together with their effort to help needy patients is most respected. I would like to offer sincere thanks to Dharma Master Cheng Yen, the founder of the Buddhist Compassion Relief Tzu Chi Foundation, for his continuing support and kind encouragement, both intellectually and spiritually. Furthermore, the generosity and camaraderie of my colleagues are also greatly and deeply appreciated.

References

[1] Robinson J, Mistry K, McWilliam H, Lopez R, Parham P, Marsh SG. The IMGT/HLA database. Nucleic Acids Res 2011;39:D1171–6.
[2] Hung W, Liu X, Li E, Zhao C, Liu Q, Liang Z, et al. Identification of a novel DRB1 allele through intergenic recombination between HLA-DRB1 and HLA-DRB3*02 in a Chinese family. Hum Immunol 2013;74:1603–9.
[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] Yang KL, Lee SK, Lin CC, Jiang S, Chiu HM, Lin S, et al. Oriental HLA-A*11:90 detected in a Taiwanese cord blood sample and the haplotype in association with A*11:90 allele. Int J Immunogenet 2011;38:543–6.
[5] Yang KL, Lee SK, Kao RH, Lin CL, Lin PY. Discovery of the novel HLA-DRB1*03:77 allele in a Taiwanese unrelated hematopoietic 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 haplotype associated with DRB1*16:16. Int J Immunogenet 2012;39:445–7.
[7] Yang KL, Lee SK, Kao RH, Lin CL, Lin PY. Discovery of the novel HLA-DRB1*10:04 allele in a Taiwanese volunteer bone marrow donor and identification of the probable HLA-A, -B, -C and -DRB1 haplotype in association with DRB1*10:04. Int J Immunogenet 2012;39:448–50.
[8] 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.
[9] Yang KL, Lin PY. Determination of HLA-A, -B and -DRB1 haplotypes based on allelic homozygosity data in selected bone marrow donors of the Taiwanese marrow donor registry. Int J Immunogenet 2007;34:385–92.
[10] Yang KL, Lee SK, Lin PY. Discovery of a novel HLA-B*51 variant, B*51:112, in a Taiwanese bone marrow donor and identification of the plausible HLA haplotype in association with B*51:112. Int J Immunogenet 2012;39:451–3.
[11] Yang KL, Lee SK, Lin PY. Identification of the novel HLA allele, HLA-B*40:159, in a Taiwanese hematopoietic stem cell donor and the probable haplotype in an association with B*40:159. Int J Immunogenet 2012;39:520–3.
[12] Yang KL, Lee SK, Kao RH, Lin CL, Lin PY. Recognition of HLA-A*24:137 allele in a Taiwanese unrelated bone marrow stem cell donor and the plausible HLA haplotype associated with A*24:137. Int J Immunogenet 2012;39:530–1.
[13] Yang KL, Lin PY. 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 Taiwan population. Tzu Chi Med J 2013;25:218–20.