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Original Article

HLA haplotype in association with the low incidence C*07:66 allele found by case analysis of Taiwanese and mainland Chinese individuals

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ABSTRACT

Objectives: HLA-C*07:66 is a low-incidence HLA-C allele. The aim of the study is to report the Taiwanese and mainland Chinese ethnicities of individuals with C*07:66, together with its uniqueness and polymorphism.

Materials and Methods: A sequence-based typing method was employed to confirm this low-incidence allele. Polymerase chain reaction was performed to amplify exons 2, 3, and 4 of the HLA-A, HLA-B, and HLA-C loci and exon 2 of the HLA-DRB1 and HLA-DQB1 loci using group-specific primer sets. The amplicons were sequenced in both directions using BigDye Terminator Cycle Sequencing Ready Reaction kit. The blood donors in this study consisted of randomized Taiwanese and mainland Chinese individuals and family members with the C*07:66 allele.

Results: The DNA sequence of C*07:66 is identical to that of C*07:02:01:01 for exons 2, 3, and 4, except for residue 688 in exon 4. This nucleotide substitution causes a single amino acid alteration to the protein sequence of C*07:02:01:01. Confirmation of the DNA and protein sequences of C*07:66 and the Taiwanese and mainland Chinese ethnicities of individuals with this allele were established in this study. One probable HLA C*07:66-associated HLA haplotype may be deduced from these individuals.

Conclusion: The information on the ethnicity of the C*07:66 allele and the deduced probable HLA haplotype associated with the low-incidence C*07:66 allele reported in this study may aid in HLA testing laboratories for reference purposes. In addition, they can be used by stem cell transplant donor search coordinators to help create, for patients bearing this uncommon HLA allele, strategies for finding compatible donors using bone marrow donor registries comprising unrelated individuals.

1. Introduction

New 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, class II, and class III regions. The genes encoding the HLA alleles are located in the MHC class I and class II regions. 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 when performing 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 practice. To facilitate successful and comprehensive unrelated bone marrow hematopoietic stem cell donor search when patients are in need of hematopoietic stem cell transplantation, persistent efforts are needed to resolve unidentified, ambiguous, or low-incidence alleles to offer optimal HLA matching and donor selection.

Conflict of interest: none.

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The nucleotide sequence of HLA-C*07:66, which was initially detected in a Chinese individual with Han ethnicity, was first submitted to GenBank (accession number FJ629179) and the IMGT/HLA Database (submission number HWS10005968) in 2009, and the name HLA-C*07:66 was officially assigned by the World Health Organization HLA Nomenclature Committee [1]. A family study indicated that C*07:66 segregated as A*24:02- C*07:66-B*40:01-DRB1*12:02 haplotype [2]. Here we report the Taiwanese and mainland Chinese ethnicities of individuals bearing C*07:66 and the deduced probable HLA haplotypes found to be associated with C*07:66 based on HLA-A, HLA-B, HLA-C, and HLA-DRB1 alleles commonly shared across our randomized unrelated donors and family members with the C*07:66 allele. We further speculate that the deduced plausible HLA haplotypes associated with C*07:66 are restricted to individuals who are members of the Chinese or Taiwanese ethnic groups. In addition, through four family studies, we found that DRB1*12:02, present in the haplotype A*24:02-C*07:66-B*40:01-DRB1*12:02, is associated with DQB1*03:01.

2. Materials and Methods

Peripheral whole blood samples from a range of Taiwanese and Chinese ethnicity individuals were collected in acid citrate dextrose anticoagulant. A formal written consent was obtained from all the donors prior to blood collection. Whole blood samples with the anticoagulant were stored at −80°C until use. Peripheral blood genomic DNA was extracted using QIAamp DNA Blood Mini kits (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The DNA obtained was subjected to HLA genotyping for the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 loci using commercial polymerase chain reaction sequencing-based typing kits (Secore A/B/C/DRB1/DQB1 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 were used. These were firstly B-CG: M13-BIN1-CGG (sense): TGTAAAACGACGGCCAGTCGGGGGCGCAGGACCCGG and P3′ exon 5B (anti-sense): GCTCCGATGACCACAACTGCT and secondly B-TA: M13-BIN1-TGA (sense): TGTAAAACGACGGCCAGTGGCGGGGGCGCAG-GACCTGA and P3′exon 5B (anti-sense): GCTCCGATGACCACAACTGCT. The amplicons were subsequently sequenced in both directions using a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA) by following the manufacturer's instructions.

Determination of the deduced C*07:66-associated probable HLA haplotype in this study was performed by looking at the commonly shared HLA typing of the donors bearing C*07:66 across a sample of the randomized unrelated donors and family members. Where applicable, haplotype deduction based on HLA allelic homozygosity, as described previously, was employed [9,10].

3. Results

In this study, the oriental ethnicity of individuals with C*07:66 was identified. In addition, the study by Deng et al [2] is confirmed, which reported that the DNA sequence of C*07:66 is identical to that of C*07:02:01:01 in exons 2, 3, and 4 except for residue 688 (at codon 206; CTG->ATG) in exon 4 where cysteine (C) of C*07:02:01:01 is substituted by alanine (A) in C*07:66 (Fig. 1). This nucleotide replacement leads to an amino acid exchange wherein leucine (L) of C*07:02:01:01 is changed to methionine (M) in C*07:66 (Fig. 2).

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exon                    80         90         100        110        120        130        140        150        160        170
C*07:02:01:01        GCTCCCA CTCCATGAGG TATTTCGACA CCGCCGTGTC CCGGCCCGGC CGCGGAGAGC CCCGCTTCAT CTCAGTGGGC TACGTGGACG ACACGCAGTT
C*07:66              ------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

exon                   180        190        200        210        220        230        240        250        260        270
C*07:02:01:01     CGTGCGGTTC GACAGCGACG CCGCGAGTCC GAGAGGGGAG CCGCGGGCGC CGTGGGTGGA GCAGGAGGGG CCGGAGTATT GGGACCGGGA GACACAGAAG
C*07:66           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

exon                   280        290        300        310        320        330        340        350        360        370
C*07:02:01:01     TACAAGCGCC AGGCACAGGC TGACCGAGTG AGCCTGCGGA ACCTGCGCGG CTACTACAAC CAGAGCGAGG ACG|GGTCTCA CACCCTCCAG AGGATGTCTG
C*07:66           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---|------- ---------- ----------

exon                   380        390        400        410        420        430        440        450        460        470
C*07:02:01:01     GCTGCGACCT GGGGCCCGAC GGGCGCCTCC TCCGCGGGTA TGACCAGTCC GCCTACGACG GCAAGGATTA CATCGCCCTG AACGAGGACC TGCGCTCCTG
C*07:66           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

exon                   480        490        500        510        520        530        540        550        560        570
C*07:02:01:01     GACCGCCGCG GACACCGCGG CTCAGATCAC CCAGCGCAAG TTGGAGGCGG CCCGTGCGGC GGAGCAGCTG AGAGCCTACC TGGAGGGCAC GTGCGTGGAG
C*07:66           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

exon                   580        590        600        610         620        630        640        650        660        670
C*07:02:01:01     TGGCTCCGCA GATACCTGGA GAACGGGAAG GAGACGCTGC AGCGCGCAG|A ACCCCCAAAG ACACACGTGA CCCACCACCC CCTCTCTGAC CATGAGGCCA
C*07:66           ---------- ---------- ---------- ---------- ---------|- ---------- ---------- ---------- ---------- ----------

exon                   680        690        700        710        720        730        740        750        760        770
C*07:02:01:01     CCCTGAGGTG CTGGGCCCTG GGCTTCTACC CTGCGGAGAT CACACTGACC TGGCAGCGGG ATGGGGAGGA CCAGACCCAG GACACCGAGC TTGTGGAGAC
C*07:66           ---------- -------A-- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

exon                   780        790        800        810        820        830        840        850        860        870
C*07:02:01:01     CAGGCCAGCA GGAGATGGAA CCTTCCAGAA GTGGGCAGCT GTGGTGGTGC CTTCTGGACA AGAGCAGAGA TACACGTGCC ATATGCAGCA CGAGGGGCTG
C*07:66           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

exon                   880        890
C*07:02:01:01     CAAGAGCCCC TCACCCTGAG CTGGG
C*07:66           ---------- ---------- -----
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Fig. 1. The DNA sequence of C*07:66 is identical to C*07:02:01:01 in exons 2, 3 and 4 except for residue 688 (at codon 206; underlined) in exon 4 where C of C*07:02:01:01 is substituted by A (shaded) in C*07:66. Exons 2, 3 and 4 are separated by pipes (|) between nucleotides 343 and 344 and 619 and 620 respectively. Dashes indicate nucleotide identity with C*07:02:01:01.