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Characterization of the HLA-C*07:01:01G allele group in European and African-American cohorts

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ABSTRACT

The HLA-C*07:01:01G allele group consists of three nonsynonymous alleles, C*07:01:01, C*07:06 and C*07:18, plus C*07:01:02, which is synonymous to C*07:01:01. All of these alleles have identical exons 2, 3 and 4, but differ in exons 5 or 6. Therefore routine sequence-based typing (SBT) of exons 2 and 3 is unable to resolve these subtypes, resulting in ambiguous typing results in population and disease cohort studies. In the present study, we fully characterized C*07:01:01G subtypes in European and African Americans and examined their relative frequency distributions. In European Americans C*07:01:01G is predominantly represented by C*07:01:01 (94.4%), whereas C*07:01:02 (1.1%) and C*07:18 (4.5%) were detected relatively infrequently. In African Americans C*07:18 (42.4%) showed a high frequency similar to that of C*07:01:01 (44.7%) whereas C*07:06 was detected at a low frequency (4.7%). C*07:06 was found exclusively on B*44:03 carrying haplotypes in both ethnic groups, but C*07:18 showed multiple linkage relationships with HLA-B. These results demonstrate that C*07:01:01G as defined by routine SBT is a heterogeneous group of alleles, especially among individuals of African origin. If C*07:01:01G subtypes prove to bear divergent functional significance, it would be necessary to include these subtypes in routine HLA-C typing for clinical transplantation and disease association studies.
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1. Introduction

HLA-C*07 is one of the most common, divergent, and polymorphic HLA-C lineages with 281 synonymous, non-synonymous and null alleles recognized so far (http://www.ebi.ac.uk/imgt/hla/, Release 3.7.0, 12-January-2012). C*07:01:01G is the name for the allele group comprising eight subtypes sharing identical exons 2 and 3. Four of them, C*07:01:01, C*07:01:02, C*07:06 and C*07:18 share an identical nucleotide sequence from exon 1 through exon 4 but differ in exons 5 or 6. The other four, C*07:01:09, C*07:52, C*07:153 and C*07:166, differ from C*07:01:01 only in exon 4. According to the National Marrow Donor Program (NMDP, http://bioinformatics.nmdp.org/) database, C*07:01:01G was commonly detected in all major populations in the US: 17% in European Americans, 12% in African Americans, 10% in Hispanics, and 4% in Asian and Pacific Islanders. Three of the C*07:01:01G subtypes, C*07:01:01, C*07:06 and C*07:18 are on the ASHI CWD allele list.

The genes encoding C*07:01:01, C*07:01:02, C*07:06 and C*07:18 share an identical sequence from exon 1 through exon 4 but differ in exons 5 and 6 (Fig. 1), which encode the transmembrane segment and cytoplasmic tail of the HLA-C molecule, respectively [1]. Though little is known about the functional significance of the polymorphisms outside of the peptide binding regions (PBR), the potential effect of amino acid variations in the transmembrane segment and cytoplastic tail on HLA expression and intracellular signaling, as well as their potential influence on transplantation and disease association, have drawn interest [2–4].

C*07:06 was first identified in 1996 by cDNA cloning, where only the coding sequence was published [5]. The full-length sequence of C*07:06 has been characterized more recently in greater detail, and the allele was found to have two non-synonymous substitutions relative to C*07:01:01 at nt992 T > A in exon 5 and nt1043 C > T

Abbreviations: HLA, human leukocyte antigen; ASHI, American Society for histocompatibility and immunogenetics; CWD, common and well-documented; NMDP, national marrow donor program; SBT, sequence based typing; PBR, peptide binding region.

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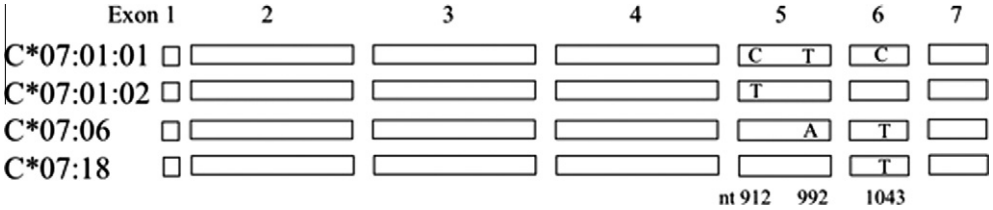

Fig. 1. Nucleotide variations of C*07:01:01G subtypes detected in the present study. The four subtypes in the C*07:01:01G allele group share identical exons 1 through 4 and 7 but differ in three positions in exons 5 and 6.

in exon 6, plus four additional substitutions in intron 3, intron 4, and the 3′-UTR [6]. The coding sequence of C*07:18, which differs from C*07:01:01 by a single nucleotide at nt1043 C > T in exon 6, was first detected in 2003 [4]. In a recent study C*07:18 was indicated as one of the risk factors for severe cutaneous adverse reactions (SCAR), a condition of severe hypersensitivity to a variety of medicines [7]. C*07:01:02 differs from C*07:01:01 by a single synonymous substitution at nt912 C > T in exon 5.

Routine HLA class I tying has focused on the 4-digit PBR polymorphism encoded by exons 2 and 3, whereas other coding and non-coding polymorphisms of these genes have largely been neglected. Therefore, little information has been generated about the frequency distribution of C*07:01:01G subtypes from population and disease-association studies [8–10], though C*07:01:01, C*07:06 and C*07:18 are all on the ASHI CWD allele list [11]. Previous studies did show strong linkage disequilibrium relationships of C*07:01:01G subtypes with HLA-B alleles. In particular, C*07:06 and C*07:18 were found to be associated with B*44 and B*58:01, respectively [6,12,13], the latter of which associates with protection against HIV [14].

We have recently characterized C*07:01:01G subtypes in Northern and Southern Chinese populations and found that C*07:01:01 and C*07:06 were both represented with C*07:06 having a slightly higher frequency. C*07:18 was completely absent from the 1795 individuals tested [6]. Interestingly, C*07:18 was detected as the second most common C*07:01:01G allele after C*07:01:01 in Ugandans from East Africa, whereas C*07:06 was only found in a single individual of the 175 subjects examined [12]. These limited studies suggest that the distribution of C*07:01:01G subtypes is highly divergent across ethnic groups. Greater knowledge of the subtype distribution may carry significance in transplantation, population, and disease association studies.

In the present study we fully characterize C*07:01:01G subtypes and investigate their relative frequencies in African American and European American subjects, providing an estimate of how much of the diversity in this grouping was actually missed in previous HLA typing across population and disease studies.

2. Materials and methods

2.1. Study subjects

A total of 226 C*07:01:01G-positive individuals from cohorts we genotyped previously for exons 2 and 3 were selected for full characterization of C*07:01:01G subtypes. The details of the cohorts have been previously described [15]. The study subjects were grouped into two panels for separate analyses. The first panel consisted of randomly selected C*07:01:01G-positive individuals of 80 European Americans and 81 African Americans for estimating the relative frequencies of C*07:01:01G subtypes in the two cohorts. The second panel were individuals with both C*07:01:01G and B*44, and was used to evaluate the previously reported linkage relationship of C*07:06 with B*44. The second panel included 11 B*44 positive individuals from the first panel of randomly selected C*07:01:01G-positive European or African Americans plus an additional 65 individuals of mixed ethnicities that were selected specifically for having both C*07:01:01G and B*44. A total of 76 individuals (59 European Americans, 8 African Americans, 5 Hispanics, 2 Asians and 2 of unknown ethnicity) in the second panel were available for examining the linkage relationship between C*07:06 and B*44.

The study subjects have been previously typed for HLA-C by routine SBT of exons 2 and 3. These subjects were retyped for HLA-C by sequencing exons 2 through 6 in the present study.

2.2. Sequencing of exons 2, 3 and 4

Genomic DNA was used to examine HLA-C variation by SBT in this study. PCR amplification of exons 2, 3 and 4 in a single amplicon was achieved using HLA-C specific primers that match the sequences in the 5′UTR and intron 4, generating a PCR fragment of about 2000 bp in size. The PCR primers and conditions have been described previously [6]. PCR amplicons were purified using the Omega Mag-Bind® EZPure commercial kit. Sequencing reactions of exons 2, 3 and 4 were performed separately in both orientations using published primers (exon 2: Forward 5′-GGGTCTCAGCCMCTCCTC-3′, Reverse 5′-GCC GTC CGT GGG GGA TG-3′; exon 3: Forward 5′-GCCCCAGTCRCCTTTAC-3′, Reverse 5′-TTCCTCCCCTCCTCGTG-3′; exon 4: Forward 5′-TTC TCA GGA TRG TCA CAT G-3′, Reverse 5′-CCYCATYCCCCTCCTTAC-3′) [16]. Sequences were analyzed on an ABI 3730XL DNA Sequencer (Applied Biosystem, Foster City, CA).

2.3. Sequencing of exons 5 and 6

Sequencing of exons 5 and 6 followed our in-house SBT protocol [6] with a modification to the forward PCR primer. An amplicon of 1050 bp was obtained using primers spanning exons 5 through 8 (forward 5′- GTA AGG AGG GGR ATG RGG GGT-3′; reverse 5′- AAT CCT GCA TCT CAG TCC CAC-3′). PCR was carried out in a 25 μl volume containing 12.5 μl of 2 × GC Buffer, 0.2 μl of each dNTP (25 mM), 1 μl of each PCR primer (10 μM), 100 ng of genomic DNA, and 2.5 U of Genomic LA Taq polymerase (Clontech, 1290 Terra Bella Avenue Mountain View, CA 94043,USA). PCR conditions were: 95 °C for 2 min followed by 35 cycles of 30 s at 95 °C, 30 s at 62 °C, and 1.5 min at 72 °C, and a final extension at 72 °C for 15 min. Sequencing was performed using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit and our in-house sequencing primers (exon 5: forward 5′-GTCAGGGCTGAGGCTTG-3′, reverse 5′-GATGGTGCTTCCAGTAAC-3′;exon 6: forward 5′-TCCAAGACTAG-GAGGTTC-3′, Reverse 5′-AAAAAGACCTGGTCAGAG-3′).

2.4. Assignment of HLA-C alleles and HLA-B/C haplotypes

HLA-C alleles were assigned with the help of the ASSIGN 3.5 HLA typing software (Conexio Genomics, Fremantle, Western Australia). Since family members were not available for segregation analysis, all C*07:01:01G-related HLA-B/C haplotypes described in this study were elucidated by maximum likelihood estimation

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