TISSUE ANTIGENS
IMMUNE RESPONSE GENETICS

NEW ALLELE Alerts

A new human leukocyte antigen class II allele, *DRB1*09:12*

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Key words: *HLA-DRB1*09:12*; new allele; sequence-based typing

*HLA-DRB1*09:12* allele differs from *HLA-DRB1*09:01:02* by a single nucleotide substitution at codon 41 (AAG → AAC).

Human leukocyte antigen (HLA) genes, the most polymorphic genes of human genome, include HLA class I and HLA class II genes. To date, more than 1500 HLA class II-DRB1 alleles have been identified (1). In this report, we describe the identification of the new HLA class II allele, *HLA-DRB1*09:12*, that was discovered during routine sequence-specific oligonucleotide probe (SSOP) typing of a sample from a registered donor of China Marrow Donor Program (CMDP).

Genomic DNA of this sample was extracted from anticoagulated whole blood with DNA Extraction Reagent Kit (Tiangen, Beijing, China). HLA typing was performed by PCR-SSOP based on Luminex platform using LABType® SSO typing kits (One Lambda Inc., Los Angeles, CA) following the manufacturer's protocol for HLA-A, -B and -DRB1. The result showed *HLA-A*02, 11*; *B*51, 58*; *DRB1*09, 13,* but the HLA-DRB1 locus hybridization probe reaction patterns were unusual, which could not be completely assigned to a pair of DRB1 alleles, repeated typing also showed the same results, so suggesting the possible presence of a new *HLA-DRB1*09* allele.

With the aim to further confirm the alleles of HLA-DRB1, sequence-based typing (SBT) was then performed. HLA-DRB1 locus was amplified from exon 2 to exon 4 by SBTexcellerator® kit (Genome Diagnostics B.V., Arnhem, The Netherlands) and LongRange PCR kit (Qiagen, Hilden, Germany). The polymerase chain reaction (PCR) cycling parameters used for DRB1 locus consisted of an initial DNA denaturation of 3 min at 95°C, followed by a cycle of 15 s at 95°C, 30 s at 65°C, and 5 min at 68°C. The number of amplification cycles was 35. A final extension step was performed at 68°C for 10 min and held at 4°C until analysis.

Exon 2 was sequenced in forward and reverse directions using SBTexcellerator® sequence primer (Genome Diagnostics B.V., Arnhem), Big Dye® Terminator v3.1 Reaction Kit (Applied Biosystems, Torrance, CA) and running on ABI PRISM® 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). The SBTengine® software was used for analysis. The obtained heterozygous sequence showed the presence of a novel allele, as the nucleotide sequence did not match with any known allelic combination. To separate the two alleles, the sample was amplified with group-specific sequencing primer (GSSP), which was indicated by the analysis software. The

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(A)

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                  101                                123
DRB1*09:01:02      CA CGT TTC TTG AAG CAG GAT AAG TTT GAG TGT CAT TTC TTC
DRB1*09:12         -- --- --- --- --- --- --- --C --- --- --- --- --- ---
                  142
DRB1*09:01:02      AAC GGG ACG GAG CGG GTG CGG TAT CTG CAC AGA GGC ATC TAT
DRB1*09:12         --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                  184
DRB1*09:01:02      AAC CAA GAG GAG AAC GTG CGC TTC GAC AGC GAC GTG GGG GAG
DRB1*09:12         --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                  226
DRB1*09:01:02      TAC CGG GCG GTG ACG GAG CTG GGG CGG CCT GTC GCC GAG TCC
DRB1*09:12         --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                  268
DRB1*09:01:02      TGG AAC AGC CAG AAG GAC TTC CTG GAG CGG AGG CGG GCC GAG
DRB1*09:12         --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                  310
DRB1*09:01:02      GTG GAC ACC GTG TGC AGA CAC AAC TAC GGG GTT GGT GAG AGC
DRB1*09:12         --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                  352
DRB1*09:01:02      TTC ACA GTG CAG AGG CGA G
DRB1*09:12         --- --- --- --- --- --- -
```

(B)

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                  35      41
DRB1*09:01:02      RFLKQ DKFEC HFFNG TERVR YLHRG IYNQE
DRB1*09:12         ----- -N--- ----- ----- ----- -----
                  65
DRB1*09:01:02      ENVRF DSDVG EYRAV TELGR PVAES WNSQK
DRB1*09:12         ----- ----- ----- ----- ----- -----
                  95
DRB1*09:01:02      DFLER RRAEV DTVCR HNYGV GESFT VQRR
DRB1*09:12         ----- ----- ----- ----- ----- ----
```

Figure 1 The nucleotide sequence (A) and the amino acid sequence (B) of exon 2 of *HLA-DRB1*09:12* compared with the sequences of *DRB1*09:01:02*. Dashes (–) indicate identity with *HLA-DRB1*09:01:02*. The mutations at nucleotide 123 and codon 41 are indicated in bold.

sequence result showed that one allele was *DRB1*13:02:01*, and the other one was a new *HLA-DRB1*09* variant, *DRB1*09:12*. The high resolution HLA typing of HLA-A and -B was *A*02:01:01:01*, *11:01:01*; *B*51:01:01*, *58:01:01*.

The exon 2 sequence of *HLA-DRB1*09:12* has been submitted to GenBank with the accession number HQ896940. As shown in Figure 1, the *HLA-DRB1*09:12* allele differs from *DRB1*09:01:02* by a single nucleotide substitution at nucleotide 123 where G → C (codon 41 AAG → AAC) resulting in a coding change; 41 lysine (K) is changed to asparagine (N). The name *DRB1*09:12* has been officially assigned by the World Health Organization (WHO) Nomenclature Committee for Factors of the HLA system. This follows the agreed policy that, subject to the conditions stated in the most recent Nomenclature Report (2), names will be assigned to new sequences as they are identified. Lists of such new names will be published in the following WHO Nomenclature Report.

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doi: 10.1111/tan.12530

Acknowledgments

This study was supported in part by a grant from China Marrow Donor Program (CMDP) and Shenyang Science and Technology Foundation (F10-206-1-00).

Conflict of Interest

The authors have declared no conflicting interests.

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