Identification of Four Novel HLA-A Alleles from an East African Population by High-Resolution Sequence-Based Typing

Kulvinder K. Gill, Aleksandra Leligdowicz, Ma Luo, Thomas Bielawny, Robert Brunham, and Francis A. Plummer

ABSTRACT: We report here four novel human leukocyte antigen (HLA)–A alleles identified among an East African population during sequence-based HLA-A typing. The novel alleles were confirmed by sequencing two separate polymerase chain reaction products and by molecular cloning and sequencing multiple clones. The new allele A*9202 is identical to A*0202 at exon 2 and exon 3 except for a single nucleotide difference at codon 43 (CGG→CAG), resulting in a coding change from Arginine to Glutamine. The second new allele has a synonymous change at codon 139 (GCA→GCG), that differentiates it from A*680101. The new allele has been named by the World Health Organization nomenclature committee as A*680105. The novel allele A*2630 is identical to A*2603 at exon 2 and exon 3 except for a nonsynonymous change at codon 90 (GAC→GCC), changed from Aspartic acid to Alanine. The fourth new allele is identical to A*290201 except for a single nucleotide difference at codon 138 (ATG→GTG), resulting in a coding change from Methionine to Valine. The new allele has been named by the World Health Organization nomenclature committee as A*2915. Identification of these novel HLA-A alleles reflects the genetic diversity of this East African population. *Human Immunology 67, 833–838 (2006).* © American Society for Histocompatibility and Immunogenetics, 2006. Published by Elsevier Inc.

KEYWORDS: A*2630; A*2915; A*680105; A*9202; SBT; TBSA; HLA-A

Human leukocyte antigen (HLA) is the most polymorphic genetic system known in humans. Among HLA loci, HLA-A is the second most polymorphic locus with 429 HLA-A alleles reported in the latest ImMunoGeneTics (IMGT)/HLA database [1]. As the "origin of humanity," Africa is the most genetically diverse region of the world. A large number of HLA alleles have been identified, and many novel alleles have been reported in the African population [2, 3]. During sequence-based HLA-A typing of an East African population, we identified four novel HLA-A alleles. In this study, we describe the identification, cloning, and sequencing of these four novel alleles.

Genomic DNA was prepared from peripheral blood mononuclear cells (PBMCs) using a QIAamp DNA Mini Kit (QIAGEN Inc., Mississauga, Ontario, Canada). Exon 2, intron 2, and exon 3 were amplified for HLA-A genes using primers from Cereb *et al.* [4, 5] by polymerase chain reaction (PCR).The resulting PCR product was purified using Amicon Microcon-PCR Centrifugal filter device (Millipore, Bedford, MA). The purified PCR products were sequenced with gene-specific primers [3] using ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kits (Applied Biosystems, Foster City, CA). The sequence was analyzed with ABI3100 Genetic Analyzer (Applied Biosystems). HLA-A alleles were typed with computer software, CodonExpress, developed based on a taxonomy-based sequence analysis [6, 7].

The A*9202 allele was identified in a woman from Bukoba, Tanzania. The patient was typed as A*0202 and A*new for a nucleotide difference at codon 43(CGG→CAG), resulting in a coding change from Arginine to Glutamine (Figure 1). HLA-A was ream-

---

From the Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada (K.K.G., A.L., M.L, T.B., F.A.P.); the National Microbiology Laboratory, Winnipeg, Manitoba, Canada (M.L., F.A.P.); and BCCDC, Vancouver, British Columbia, Canada (R.B.).

Address reprint requests to: Dr. Ma Luo, Department of Medical Microbiology, University of Manitoba, 730 William Avenue, Winnipeg, Manitoba Canada R3E 0W3, ; Fax: (204) 789-3926 E-mail: mluo@cc.umanitoba.ca

The names listed for the sequences have been officially assigned by the WHO Nomenclature Committee. This follows the agreed policy that, subject to the conditions stated in the most recent Nomenclature Report [1], 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.

Received May 12, 2006; revised June 26, 2006; accepted July 11, 2006.

Human Immunology **67**, 833–838 (2006)

Published by Elsevier Inc.

0198-8859/06/$–see front matter
doi:10.1016/j.humimm.2006.07.007

```
                            Intron 1
A*9202  CGGGCCCGCCTGGCGGGGGCGCAGGACCCGGGAAGCCGCGCCGGGAGGAGGGTCGGGCGGGTCTCAGC
A*9202  CACTCCTCGTCCCCAG
                        5                   10                  15
A*0202  GC TCT CAC TCC ATG AGG TAT TTC TTC ACA TCC GTG TCC CGG CCC GGC CGC
A*9202  -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                20                  25                  30
A*0202 GGG GAG CCC CGC TTC ATC GCA GTG GGC TAC GTG GAC GAC ACG CAG TTC GTG
A*9202 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
        35                  40                  45                  50
A*0202 CGG TTC GAC AGC GAC GCC GCG AGC CGG AGG ATG GAG CCG CGG GCG CCG TGG
A*9202 --- --- --- --- --- --- --- --- -A- --- --- --- --- --- --- --- ---
                    55                  60                  65
A*0202 ATA GAG CAG GAG GGT CCG GAG TAT TGG GAC GGG GAG ACA CGG AAA GTG AAG
A*9202 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
            70                  75                  80                  85
A*0202 GCC CAC TCA CAG ACT CAC CGA GTG GAC CTG GGG ACC CTG CGC GGC TAC TAC
A*9202 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                        90
A*0202 AAC CAG AGC GAG GCC G               Intron 2
A*9202 --- --- --- --- --- - GTGAGTGACCCCGGCCCGGGGCGCAGGTCACGACCTCTCATCCCCCACG
A*9202 GACGGGCCAGGTCGCCCACAGTCTCCGGGTCCGAGATCCGCCCCGAAGCCGCGGGACCCCGAGACCCTTGC
A*9202 CCCGGGAGAGGCCCAGGCGCCTTTACCCGGTTTCATTTTCAGTTTAGGCCAAAAATCCCCCCAGGTTGGTC
A*9202 GGGGCGGGGCGGGGCTCGGGGGACCGGGCTGACCGCGGGGTCCGGGCCAG

                       95                 100                 105
A*0202  GT TCT CAC ACC CTC CAG AGG ATG TAT GGC TGC GAC GTG GGG TCG GAC TGG
A*9202  -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
               110                 115                 120
A*0202 CGC TTC CTC CGC GGG TAC CAC CAG TAC GCC TAC GAC GGC AAG GAT TAC ATC
A*9202 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
       125                 130                 135                 140
A*0202 GCC CTG AAA GAG GAC CTG CGC TCT TGG ACC GCG GCG GAC ATG GCA GCT CAG
A*9202 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

A*0202 ACC ACC AAG CAC AAG TGG GAG GCG GCC CAT GTG GCG GAG CAG TTG AGA GCC
A*9202 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

A*0202 TAC CTG GAG GGC ACG TGC GTG GAG TGG CTC CGC AGA TAC CTG GAG AAC GGG
A*9202 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

A*0202 AAG GAG ACG CTG CAG CGC ACG G        Intron 3
A*9202 --- --- --- --- --- --- --- - GTACCAGGGGCCACGGGGCGCCTCCCTGATCGCCTGTA
A*9202 GATCTCCCGGGCTGGCCTCCCACAAG
```

FIGURE 1 Alignment of nucleotide sequences of the new HLA-A allele, A*9202, with A*0202. The Genbank accession number for the new allele is **DQ494174**. A dash indicates identity between sequences. Codons are numbered according to Marsh *et al.* [1].

plified, and the PCR products were cloned with TOPO TA cloning Kit (Invitrogen Co. Carlsbad, CA). Among 38 clones sequenced, 21 clones were typed as A*0202 and 17 clones were typed as A*new with a new sequence of CAG at codon 43 (Figure 1). The new HLA-A allele was designated as A*9202 by the World Health Organization (WHO) Nomenclature Committee (see footnote). The HLA-B alleles of this individual are B*1516 and B*5802, and the HLA-C alleles are Cw*0602 and Cw*140201. The class II alleles of this woman are: DRB1*080401, DRB1*130201, DRB3*030101, DPA1*0301, DPA1*010301, DPB1*020102, DPB1*0402, DQA1*020101, DQA1*040101, DQB1*0401, and DQB1*060401.