Brief communication

Characterization of a novel HLA-Cw*02 variant, Cw*0208, in a Caucasian individual

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Abstract: We describe an additional HLA-Cw*02 variant, HLA-Cw*0208, which has been identified in a renal transplant recipient of Caucasian origin (Italy). After performing preliminary serological typing, we analyzed exons 2 and 3 of the HLA-C locus polymorphism by cloning the amplified DNA and using a sequence-based typing method. The new allele differs from Cw*020202 by one nucleotide substitution at nucleotide 61 (G→A) of exon 2, which translates to a difference of one amino acid at residue 21 (His→Arg) of the HLA-C heavy chain. We propose that Cw*0208 was generated by a random point mutation in codon 21 from the Cw*020202 allele, or through gene conversion of Cw*020202 with another allele, probably the Cw*1205 and Cw*1602 alleles.

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Presumed to be one of the least polymorphic of the HLA loci, the HLA-C gene has been shown recently to display extensive sequence diversity through the routine application of molecular typing techniques. These techniques improve the discrimination of HLA-Cw alleles and allow several new subtypes to be identified. In particular, recent work has shown that sequence-based typing of the HLA-C locus is an adequate method for high-resolution typing (1).

One of the HLA-Cw specificities, the Cw*02 antigen group, has been subdivided according to the World Health Organization (WHO) Nomenclature for Factors of the HLA system (2) into 11 alleles: Cw*0201, 020201, 020202, 020203, 020204, 020205, 0203, 0204, 0205, 0206, and 0207. HLA-Cw2 is common in Blacks and Caucasians but is rare or absent in Japanese, Taiwanese, and Indian populations (3–5). In the Italian population, the allele frequency varies between 0.015 in Rome (6) and 0.051 in Bergamo (7).

We describe in this study an additional HLA-Cw*02 variant, Cw*0208. We identified this allele in a renal transplant recipient (DNA sample E805, E863) of Caucasian origin in Italy who showed the following genotypes: A*020101, 2501; B*1801, 4101;

Cw*0208, 120301; DRB1*04, 15; DQB1*06, 02; and DPB1*0301, 0401. After performing preliminary serological typing (A2;25 B18;41 Cw2 DR4;15 DQ2;6), we analyzed exons 2 and 3 of the HLA-C locus polymorphism using a sequence-based typing (SBT) method employing an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). This technique is based on the PCR amplification of a 906-kb fragment from genomic DNA, followed by direct sequencing of exons 2 and 3. We used the locus-specific primers 5CIn1-61 (5′-AGC GAG GKG CCC GCC CGG CGA-3′) and 3BCIn3-12 (5′-GGA GAT GGG GAA GGC TCC CCA CT-3′), previously described by Cereb et al. (8), for heterozygous amplification of exon 2, intron 2, and exon 3 of the C-locus. The DNA sequence analysis was performed in the forward and reverse orientations using Big Dye terminators. The primers used for exon 2 cycle sequencing were 5CIn1-61 in the sense direction and 3CIn2-1 in the reverse direction (5′-CGT CCG TGGGGG ATG RGG-3′). Sequencing of exon 3 in the sense direction was performed using the primer 5CIn2-237 (5′-TCG GGG GAC CGG GCT GAC C-3′) and the primer 3BCIn3-12 mentioned above was used for reverse sequencing. We used the protocol described elsewhere (9). To remove unincorporated dye terminators, sequencing products were purified using a spin column (CentriSep; Applied Biosystems). Allele assignment was performed using the HLA MatchTools and MT navigator Software from the MatchTools Allele Identification Packet (Applied Biosystems), which detects the heterozygous positions within each electropherogram and assesses the typing based on the alignment of the processed sequence with the updated HLA sequence library. To verify this new HLA-Cw variant, the amplified DNA was cloned to separate the alleles using the pGEM-T Easy Vector System II cloning kit (Promega Italia, Milan, Italy). DNA inserts from the cloned allele were directly amplified from a lysate containing the bacterial clone and sequenced. Both strands of DNA were sequenced for each allele.

The novel HLA-Cw*02 gene was submitted to GenBank and is available under accession numbers AY230856 and AY230857. The designation Cw*0208 was officially named by the WHO Nomenclature Committee in April 2003 (10). This follows the agreed policy that, subject to the conditions stated in the most recent Nomenclature Report (2), names will be assigned to the new sequences, as they are identified.

As summarized in Table 1, the sequence analysis revealed that the new allele differs from Cw*020202 by one nucleotide substitution at nucleotide 61 (G→A), which is located in exon 2 (Fig. 1). We identified two nucleotide differences that translated to one or two amino acid differences with other Cw2 variants (Cw*020202, 020201, 020203, 020204, and 0204) (Table 2). This exchange translates to a difference of one amino acid at residue 21 of the HLA-C heavy chain: histidine in Cw*020202 to arginine in Cw*0208 (Table 1), both of which are negatively charged amino acids. This residue is polymorphic in HLA-C locus but it is constant in other HLA class I loci except for one substitution in the HLA-B locus (HLA-B*5205). It is located on the β2-pleated sheet of the HLA-C α1 domain and sits adjacent to residue 22, which is involved in the binding with the side chain P6 into pocket C (11, 12) and to the loop between strands 1 and 2 (Fig. 2).

It is likely that Cw*0208 may have been generated by a random point mutation in codon 21 from the Cw*020202 allele, or through recombination of Cw*020202 with another C-locus allele. This gene conversion could occur via several alleles because G is located at nucleotide 61 in many other HLA-Cw allelic groups (e.g., Cw*01, *04, *05, *06, *07, *08, *12, *14, *16, *17, and *18). The best candidates

Alignment of the novel allele Cw*0208 with the most similar Cw*02 variants (Cw*020202, 020201, 020203, 020204, and 0204) at the nucleotide positions 18–20, 60–62, and 240–242 in exon 2, and at positions 294–296 and 411–413 in exon 3

	Nucleotide positions				
Cw*	18–20 (exon 2)	60–62 (exon 2)	240–242 (exon 2)	294–296 (exon 3)	411–413 (exon 3)
0102 (reference)	TAT	CGC	CTG	TGT	ACC
0208	TAT	CGC	CTG	TAC	ACA
020202	TAT	C**A**C	CTG	TAC	ACA
020201	TAT	C**A**C	CT**A**	TAC	ACA
020203	TAT	C**A**C	CTG	TA**T**	ACA
020204	TAT	C**A**C	CTG	TAC	AC**G**
0204	T**G**T	C**A**C	CTG	TAC	ACA

The numbers start from the beginning of the coding region.

Table 1

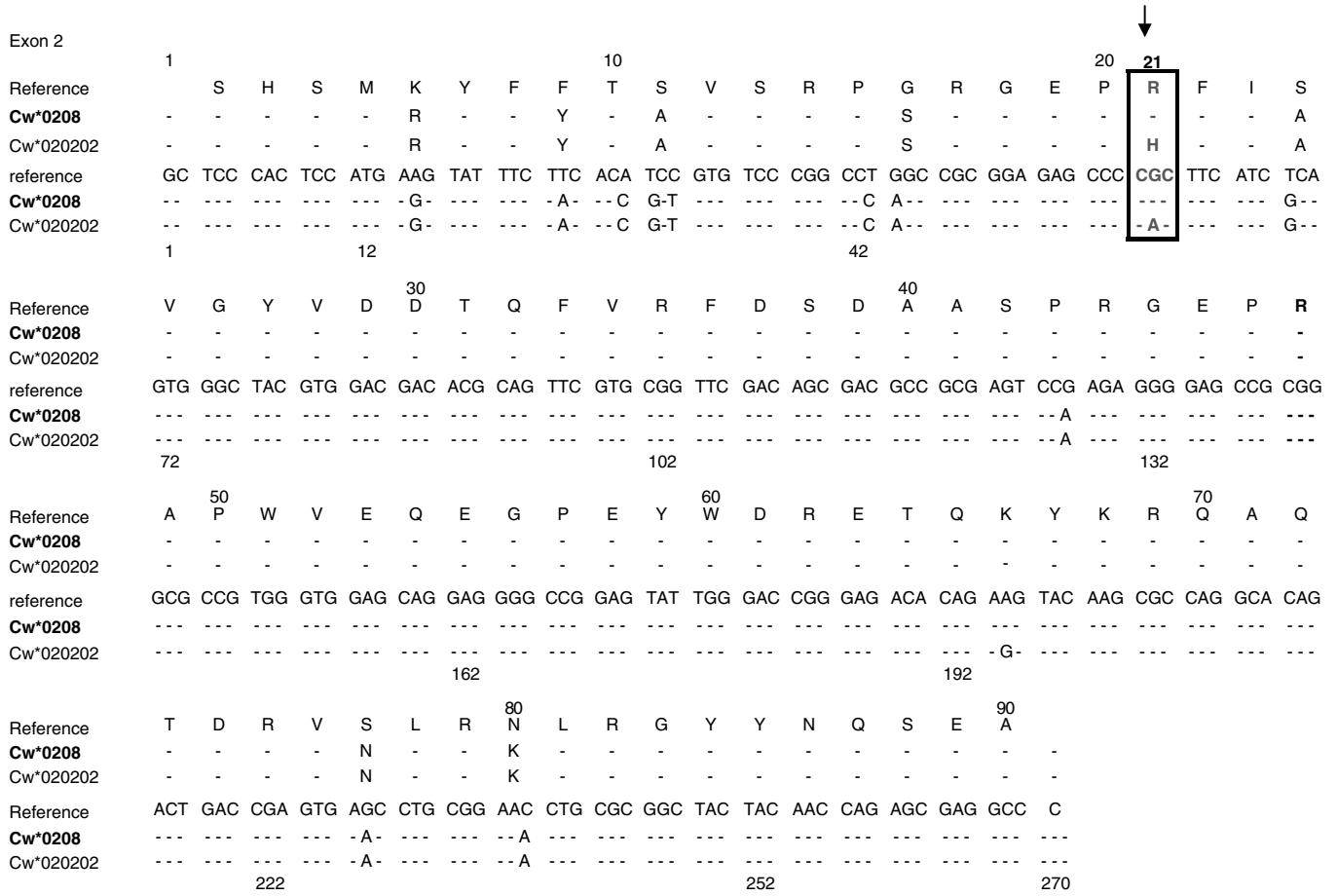

Fig. 1. **Nucleotide and predicted amino acid sequences of exon 2 of the new Cw*0208 allele (aa 1–90) compared with the Cw*020202 allele.** The Cw*0102 allele was used as a reference sequence. The numbers above indicate the corresponding amino acid and the numbers below refer to the nucleotide position, with exon 2 starting at nucleotide 1.

might be the Cw*1205 and Cw*1602 alleles, which share an extended fragment with the new allele (codons 16–91 of exon 2). The relatively high evolutionary divergence between the new allele Cw*0208 and these two other HLA-Cw alleles (13) would support the first hypothesis.

In conclusion, application of SBT is proved a useful tool to investigate the high degree of polymorphism in HLA-C locus, a locus whose antigens play an important role in the stimulation of allograft rejection and, as receptors for NK cells, in stimulating cellular immune responses. The discovery of this new allele adds

Deduced amino acid sequences from the sequences given in Table 1

Cw*	Residue 7	Residue 21	Residue 81	Residue 99	Residue 138
0102 (reference)	Tyr	Arg	Leu	Cys	Thr
0208	Tyr	Arg	Leu	Tyr	Thr
020202	Tyr	**His**	Leu	Tyr	Thr
020201	Tyr	**His**	Leu	Tyr	Thr
020203	Tyr	**His**	Leu	Tyr	Thr
020204	Tyr	**His**	Leu	Tyr	Thr
0204	**Cys**	**His**	Leu	Tyr	Thr

Table 2

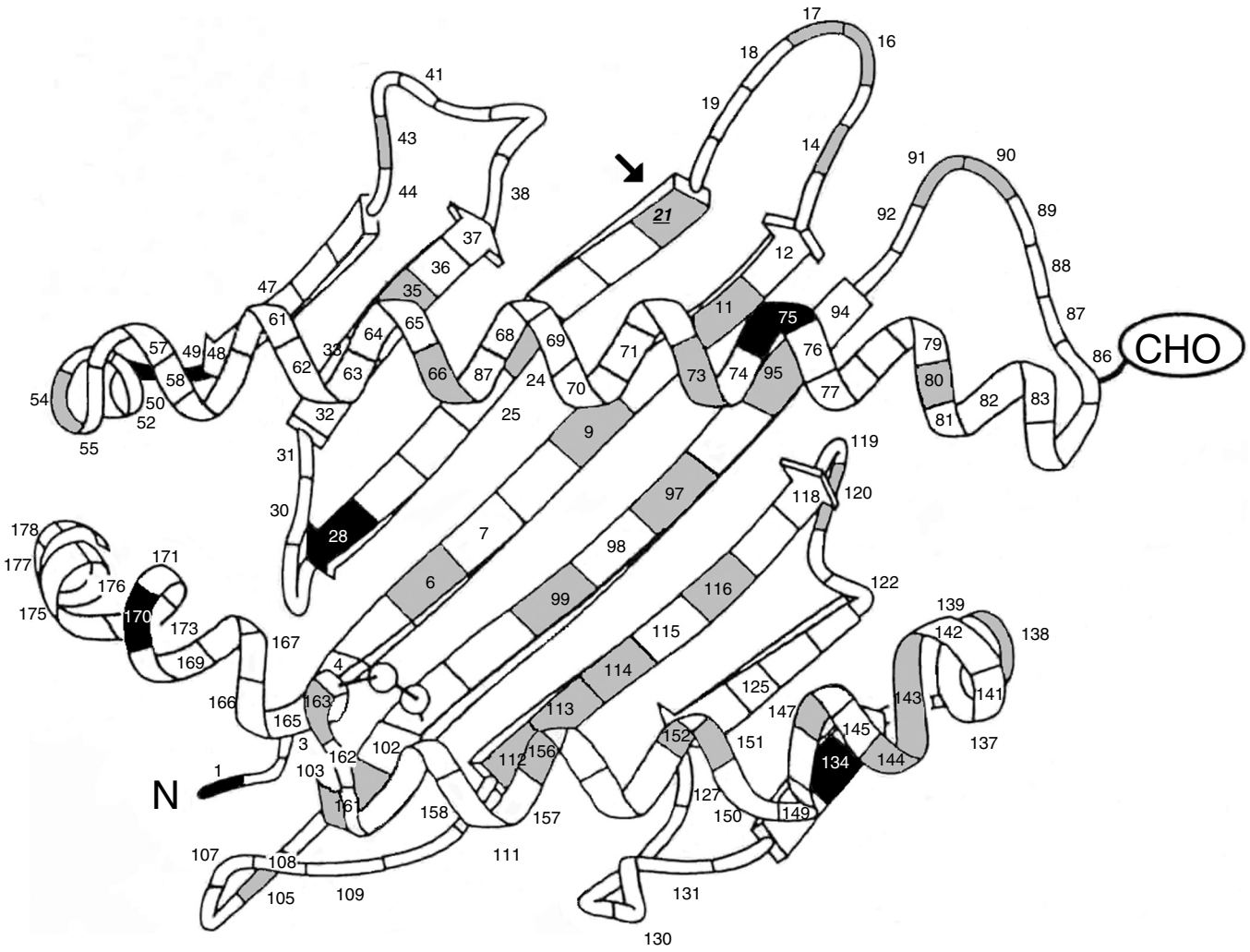

Fig. 2. **A ribbon diagram of the HLA class I heavy chain illustrating polymorphic numbered positions located within the HLA class I molecules.** The residues unique to the HLA-C molecules are colored black, those which are additionally polymorphic in other class I genes are white, and those common in all three loci are gray (IMGT/HLA Sequence Alignments, 12 January 2005). An arrow shows residue 21, which is described in the article.

complexity to the HLA-C locus, in particular to the Cw*02 allelic group, and may have implications in transplantation field and HLA-associated diseases, particularly in ankylosing spondylitis (14–16).

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