

Exon 2 (270 bp)

AA Codon		5		10		15		20		25															
C*03:04:01:01	GC	TCC	CAC	TCC	ATG	AGG	TAT	TTC	TAC	ACC	GCT	GTG	TCC	CGG	CCC	GGC	CGC	GGG	GAG	CCC	CAC	TTC	ATC	GCA	GTG
C*03:539	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
AA Codon		30		35		40		45		50															
C*03:04:01:01	GGC	TAC	GTG	GAC	GAC	ACG	CAG	TTC	GTG	CGG	TTC	GAC	AGC	GAC	GCC	GCG	AGT	CCG	AGA	GGG	GAG	CCG	CGG	GCG	CCG
C*03:539	A	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
AA Codon		55		60		65		70		75															
C*03:04:01:01	TGG	GTG	GAG	CAG	GAG	GGG	CCG	GAG	TAT	TGG	GAC	CGG	GAG	ACA	CAG	AAG	TAC	AAG	CGC	CAG	GCA	CAG	ACT	GAC	CGA
C*03:539	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
AA Codon		80		85		90																			
C*03:04:01:01	GTG	AGC	CTG	CGG	AAC	CTG	CGC	GGC	TAC	TAC	AAC	CAG	AGC	GAG	GCC	G									
C*03:539	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

FIGURE 1 Alignment of the exon 2 nucleotide sequences of C*03:04:01:01 and C*03:539. Identity to the C*03:04:01:01 allele is shown by dashes (-). The single nucleotide change at codon 26 is highlighted

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the IPD-IMGT/HLA database at <https://www.ebi.ac.uk/ipd/imgt/hla/>, IMGT/HLA Acc No:HLA29031.

ORCID

John J. Yang  <https://orcid.org/0000-0002-7739-1009>

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How to cite this article: Yang JJ, Kwon O-J, Oh H-B. The HLA-C*03:539 allele identified in a kidney transplantation recipient. *HLA.* 2021;97: 158–159. <https://doi.org/10.1111/tan.14121>

Hemizygous amplification and complete Sanger sequencing of HLA-C*07:37:01:02 from a South European Caucasoid

Judit Asenjo | Elisa Cisneros | Carlos Vilches 

Immunogenetics and Histocompatibility Laboratory, Instituto de Investigación Sanitaria Puerta de Hierro—Segovia de Arana, Majadahonda, Spain

Correspondence

Carlos Vilches, Servicio de Inmunología, Hospital Universitario Puerta de Hierro, Manuel de Falla 1, 28222 Majadahonda, Spain.
Email: carlos.vilches@yahoo.com

Funding information

Consejería de Sanidad de la Comunidad de Madrid, Grant/Award Number: 2019;

The novel C*07:37:01:02 was completely sequenced after haplo-specific amplification from a European Caucasoid carrying B*07:02.

KEYWORDS

genomic DNA, HLA-C, human variation, new allele, sequence-based typing

Fundación Científica Asociación Española
Contra el Cáncer, Grant/Award Number:
GCB15152947MELE; Ministerio de
Ciencia e Innovación/Agencia Estatal de
Investigación, Grant/Award Numbers:
PID2019-110609RB-C22/
AEI/10.13039/501100011033,
SAF2016-80363-C2-2-R

Multiple strategies have been designed for high resolution HLA typing and characterization of newly discovered HLA alleles, owing to the intrinsic difficulty of isolating and accurately defining extremely polymorphic and highly homologous HLA genes. These features and the combinatorial nature of HLA polymorphism challenge the capacity for cis/trans phasing of allelic polymorphisms of even modern technologies, including simple next-generation sequencing approaches.¹ We and others have previously reported that the full genomic sequence of a given HLA class I allele can, in most instances, be routinely amplified in isolation from the accompanying allele.^{2,3} This is achieved with primers targeting allelic polymorphisms in the 5'/3'-untranslated regions (UTR), method designated allele Group-Specific Amplification sequence-based typing (GSA-SBT) or allele-specific hemizygous Sanger sequencing. Here, we use this strategy to characterize the complete genomic sequence of allele *C*07:37:01:02*, of which partial sequences had been obtained previously from Japanese individuals (Dr. Masahiro Satake lab, Tokyo Red Cross Blood Center, GenBank accession nos. AB248242 and LC361334, unpublished).

We tentatively identified *C*07:37* using a probe-hybridization method in the course of an external proficiency test (EPT). The sample, derived from a Caucasoid individual from Palencia (Spain), was provided, without previous HLA-phenotype selection, by the EPT scheme organizer (GECLID, Garantía Externa de Calidad para Laboratorios de Inmunología Diagnóstica). To obtain allelic resolution, and, eventually, characterize the allele complete genomic sequence, GSA-SBT was performed as previously published.² In parallel, a novel forward primer (FHg-196, 5'-ggaggcgccgctgg-3'), recognizing a distinctive polymorphism in the 5'-UTR of *C*07* and *C*18*, was substituted for FHC-20. Sanger sequencing of the two amplicons with intronic primers in the core facility of our research institute yielded co-incident results, and permitted the characterization of the *HLA-C* genomic sequence (3266 bp), including exons 1-8, the intervening introns, and part of the flanking UTR. The new sequence was submitted to the GenBank database (accession number: MG739377) and to the WHO Nomenclature Committee for Factors of the HLA System, which, following the rules established in the last Nomenclature report,⁴ designated

it officially as *HLA-C*07:37* (*C*07:37:01:01* in IPD-IMGT/HLA v3.42). Unfortunately, the sequence originally deposited in GenBank and IPD-IMGT/HLA v3.32 through v3.42 databases was found in October 2020 to contain two typographical errors (two adjacent C>T changes in intron 2); after correction, it was shown to be identical and was reassigned to a recently reported allele, *C*07:37:01:02*, as it will appear in the next database release.

The *C*07:37:01:02* genomic sequence differs from that of *C*07:02:01:03* at a single nucleotide (T>G at position 915, exon 3), which determines substitution of arginine for leucine 156. This residue is located in an alpha-helix of the alpha-2 domain, where it can interact with the T-cell receptor, antigen peptides, and alloantibodies (Figure 1). Classical HLA class I molecules are highly polymorphic at position 156, where five different amino acids are commonly observed (aspartate, leucine, arginine, glutamine, and tryptophan). Within *HLA-C*, arginine 156 is seen in the majority of *C*01*, **04*, **05*, **14*, **18* alleles, while leucine is mainly found in *C*03*, **07*, **08*, **15*, and **17*. Arginine 156 of *C*07:37* is therefore an exception among *C*07* alleles.

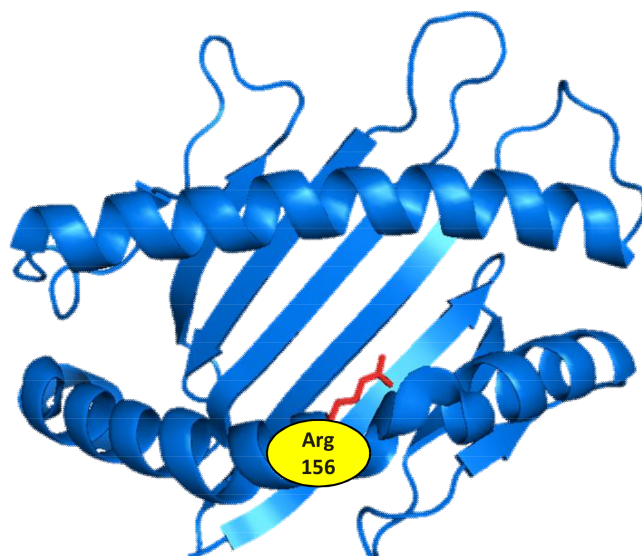


FIGURE 1 An HLA-*C*07:37* model highlighting arginine 156. The latter residue was represented with PyMOL (DeLano Scientific, San Francisco, CA) within a crystal structure of *C*07:02* (Protein Data Bank ID: 5VGE)

Unfortunately, family segregation of the donor was unfeasible. However, his complete HLA type (*A*02:01P*; *B*07:02:01,*44:05:01*; *C*02:02:02G,*07:37:01:02*; *DRB1*15:01:01G,*16:01:01*; *DRB5*01:01:01G,*02:02:01*; *DQB1*05,*06*; *DPB1*02:01:02G,*04:01P*) suggests that *C*07:37:01:02* emerged from *C*07:02* within the well-conserved haplotype *C*07:02-B*07:02-DRB1*15:01*. This is in contrast with the Japanese reference donors, who seemed to share a *C*07:37-B*67:01* haplotype.

Polymorphism at position 156 of HLA class I molecules affects both peptide and heavy chain conformation, hence modifying peptide repertoire and T-cell recognition.⁵ Therefore, position 156 is considered an important determinant of alloreactivity. In particular, HLA-C leucine 156 vs arginine 156 mismatch was pointed out as a risk factor for severe acute graft vs host disease and malignancy relapse after hemopoietic stem cell transplant.⁶

ACKNOWLEDGMENTS

We thank Rosario Solís and Esperanza Rodríguez, technicians of the Immunogenetics & Histocompatibility laboratory, Dr. Elvira Ramil, from the DNA sequencing core facility of *Instituto de Investigación Sanitaria Puerta de Hierro—Segovia de Arana*, and Dr. Carmen Martín, from GECLID, for continued support. This study was partially funded by grants SAF2016-80363-C2-2-R from the Spanish *Ministerio de Ciencia e Innovación* (MCINN, *Agencia Estatal de Investigación-AEI/FEDER*, EU), and GCB15152947MELE from the *Asociación Española contra el Cáncer* Foundation. Judit Asenjo is hired by grant PID2019-110609RB-C22/AEI/10.13039/501100011033 (AEI/FEDER, EU) to Carlos Vilches. Publication costs are defrayed by a nominative grant to support research from *Consejería de Sanidad de la Comunidad de Madrid* in 2019 to *Fundación de Investigación Biomédica Puerta de Hierro*.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Carlos Vilches  <https://orcid.org/0000-0002-0300-9225>

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How to cite this article: Asenjo J, Cisneros E, Vilches C. Hemizygous amplification and complete Sanger sequencing of *HLA-C*07:37:01:02* from a South European Caucasoid. *HLA*. 2021;97:159-161. <https://doi.org/10.1111/tan.14095>

A novel variant of *HLA-C*12*, *HLA-C*12:130*, detected in a Taiwanese individual

Kuo-Liang Yang^{1,2}  | Chi-Cheng Li¹ | Py-Yu Lin¹

¹Laboratory of Immunogenetics, Tzu Chi Cord Blood Bank, and Buddhist Tzu Chi Bone Marrow Donor Registry, Buddhist Tzu Chi Stem Cells Centre, Hualien Tzu

One nucleotide substitution in codon 40 of *HLA-C*12:02:01* results in a novel allele, *HLA-C*12:130*.