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### AUTHOR CONTRIBUTIONS

Zhi-Hui Feng carried out experiments. Shu-Xian Jiao collected the peripheral blood of this volunteer donor for China Marrow Donor Program. Peng Chen analyzed sequencing data with the soft GENTle and Expassy for translate. Zhi-Hui Feng wrote the manuscript and developed the figure. Manuscript final version was approved by Shutao Pang.

### CONFLICT OF INTEREST

The authors have declared no conflicting interests.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in IPD-IMGT/HLA at <https://www.ebi.ac.uk/ipd/imgt/hla/>, reference number HWS10061976.

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## Characterization of the novel *HLA-C\*17:01:18* allele by sequencing-based typing

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*HLA-C\*17:01:18* differs from *HLA-C\*17:01:01:05* by one nucleotide substitution in codon 18 in exon 2.

### KEYWORDS

HLA, *HLA-C\*17:01:18*, novel allele, sequencing-based typing

We report here a novel *HLA-C\*17* allele, now named *HLA-C\*17:01:18* that carries one nucleotide substitution in exon 2 when compared to the *HLA-C\*17:01:01:05* allele, identified in a volunteer bone marrow donor. The HLA typing was performed using next-generation sequencing (AllType NGS, One Lambda, Canoga Park, CA) on the Ion S5 system platform (ThermoFisher Scientific, Waltham, MA),<sup>1</sup> from exons 1 to 8. The reads were analyzed using the TypeStream Visual Software version 2.1 (One Lambda). This patient was found to have a new

*C\*17* allele and was consequently typed *A\*01:01, 11:01; C\*17:01:18, 17:03; B\*41:01, 41:02; DRB1\*07:01, 13:02; DRB3\*03:01; DRB4\*01:01; DQA1\*01:02, 02:01; DQB1\*03:03, 06:09; DPA1\*01:03, 01:03; and DPB1\*02:01, 104:01*. Using the IPD-IMGT/HLA Database,<sup>2</sup> nucleotide sequence alignment with HLA-C alleles shows that this new allele has one nucleotide change from *C\*17:01:01:05* in codon 18 in exon 2, where A→G (GGA→GGG, Figure 1), not resulting in a coding change. This nucleotide change was confirmed using other NGS reagents

AA Codon		5		10		15		20		25																
C*17:01:01:05	GC	TCC	CAC	TCC	ATG	AGG	TAT	TTC	TAC	ACC	GCC	GTG	TCC	CGG	CCC	GCC	CGC	GGA	GAG	CCC	CGC	TTC	ATC	GCA	GTG	
C*17:01:18	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
AA Codon		30		35		40		45		50																
C*17:01:01:05	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*17:01:18	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
AA Codon		55		60		65		70		75																
C*17:01:01:05	TGG	GTG	GAG	CAG	GAG	GGG	CCG	GAG	TAT	TGG	GAC	CGG	GAG	ACA	CAG	AAG	TAC	AAG	CGC	CAG	GCA	CAG	GCT	GAC	CGA	
C*17:01:18	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
AA Codon		80		85		90																				
C*17:01:01:05	GTG	AAC	CTG	CGG	AAA	CTG	CGC	GCC	TAC	TAC	AAC	CAG	AGC	GAG	GCC	G										
C*17:01:18	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

**FIGURE 1** Alignment of the sequence of exon 2 of *HLA-C\*17:01:18* with the sequence of *C\*17:01:01:05*. Dashes indicate nucleotide identity with the *HLA-C\*17:01:01:05* allele. Numbers above the sequence indicate codon position

provided by Immucor (Mia Fora NGS Flex, Norcross, GA) run on the Illumina MiSeq system (San Diego, CA) and analyzed with the Mia Fora Flex software (Immucor, version 5.1). We were confident in the phasing as the sample displayed a mean read length of 312 base pairs over all the loci, the mismatched G base was attributed 144 times to the new *HLA-C\*17* allele. HLA typing by Luminex reverse sequence-specific oligonucleotide (SSO) was performed (One Lambda Labtype XR, Canoga Park, CA).<sup>3</sup> With this assay (lot 002, catalog RSSOX1C\_002\_03 the HLA-typing of the *HLA-C\*17* allele was *HLA-C\*17:01*, *17:03* without any bead modification. Indeed, the IPD-IMGT/HLA Database 3.48 0.0 release describe very few HLA-C alleles displaying a GGG sequence in codon 18, explaining why the manufacturer did not include probes targeting this codon. The coding nucleotide sequence of the new allele has been submitted to the GenBank database (Accession No. ON185725) and to the IPD-IMGT/HLA Database (Submission No. HWS10061142). The name *C\*17:01:18* has been officially assigned by the WHO Nomenclature Committee for Factors of the HLA System in April 2022. This follows the agreed policy that, subject to the conditions stated in the most recent Nomenclature Report,<sup>4</sup> 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.

## AUTHOR CONTRIBUTIONS

Marine Cargou and Jonathan Visentin contributed to the design of the study. Marine Cargou and Jonathan Visentin participated in the writing of the paper. Marine Cargou, Vincent Elsermans, Isabelle Top, Elodie Wojciechowski and Jonathan Visentin participated in the performance of the research. Marine Cargou,

Vincent Elsermans, Isabelle Top, Elodie Wojciechowski and Jonathan Visentin participated in data analysis. Vincent Elsermans, Isabelle Top and Elodie Wojciechowski were involved in critical revision of the manuscript.

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## CONFLICT OF INTEREST


The authors confirm that there are no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. The sequence is freely available in the IPD-IMGT/HLA Database.

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## The novel *HLA-C\*17:64Q* allele characterized by two different sequencing-based typing techniques

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The novel allele *HLA-C\*17:64Q* differs from *HLA-C\*17:01:01:02* by insertion of a Lysine in exon 2.

### KEYWORDS

HLA, *HLA-C\*17*, nanotype sequencing, novel allele, sequencing-based typing

We report a novel allele *HLA-C\*17:64Q* identified in a recipient waiting for a renal transplant. It carries a three nucleotide insertion in exon 2 codon 67 when compared with the *C\*17:01:01:02* allele. The first HLA typing was performed by Next Generation Sequencing (NGSgo, GendX, Utrecht, NL) on the Illumina Miseq system platform (San Diego, CA). The fastQ files were analyzed using the NGSengine software version (2.23.1). The recipient was typed *A\*02:05*, *A\*30:01*, *B\*42:01*, *B\*53:01*, *C\*04:01*, novel *C\*17* allele, *DRB1\*03:02*, *DRB3\*01:62*, *DRB3\*03:01*, *DQA1\*04:01*, *DQB1\*04:02*, *DPA1\*01:03*, *DPA1\*02:02*, *DPB1\*01:01*, and *DPB1\*104:01*. Data metrics of the sample were excellent for all the loci, the mean read length was 138 base pairs, the lowest read depth was 392 and the maximum noise percentage was 5.8%. We observed a three nucleotide insertion AGA in the HLA-C sequence therefore the translation frame is maintained but shifted. This new allele was also confirmed by NanoTYPE™ sequencing based on the Nanopore technology<sup>1</sup> (Omicron Biocomputing Ltd, Budapest, HUN). Using the IPD-IMGT/HLA Database,<sup>2</sup> sequence alignment of the two *HLA-C\*17* alleles display one difference which is an additional amino acid encoded by AAG

(Lysine) resulting in a new codon 67 (Figure 1). This is an important change, since it could result in a protein sequence variation at the core, leading to questionable expression of the HLA protein. We were unable to perform serological typing using micro-lymphocyte cytotoxicity assay because of the lack of reagents for HLA-C typing. The localization of this insertion and its antibody accessibility was analyzed with pHLA3D database.<sup>3</sup> Residue 67 is not located at the protein surface, however, residue 66 is potentially surface accessible and close to the peptide binding groove. Thus changes at this position could have consequences in peptide presentation or in allogenic antibody triggering.

The nucleotide sequence of the exons 1 to 6 of this new allele was submitted to the GenBank database (Accession No. ON240061) and to the IPD-IMGT/HLA Database (Submission No. HWS10061164). The name *HLA-C\*17:64Q* has been officially assigned by the WHO Nomenclature Committee for Factors of the HLA System<sup>4</sup> in June 2022. This follows the agreed policy that, subject to the conditions stated in the most recent Nomenclature Report, names will be assigned to new sequences as they are identified. Lists of such new names