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NEW ALLELE ALERT

Identification of a Novel HLA-G Allele, HLA-G*01:62, Using PolyseqOne and Oxford Nanopore Sequencing

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ABSTRACT

HLA-G*01:62 differs from HLA-G*01:04:01 by one nonsynonymous nucleotide substitution in codon 269 in exon 4.

HLA-G is a non-classical class I molecule of the human MHC class Ib family, featuring low protein but relatively high DNA-level polymorphism. As of IPD-IMGT/HLA Database Release 3.60 (April 2025), 177 alleles encoding 52 proteins have been identified [1, 2]. We report a novel allele, HLA-G*01:62, which differs from HLA-G*01:04:01 by a single nucleotide change in exon 4, identified in a Chinese individual.

Genomic DNA was extracted from peripheral blood using the iPure DNA HS kit (IGE Biotechnology, Guangzhou, China). HLA typing at 12 loci (HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQA1, -DQB1, -DPA1, -DPB1, -G) was performed via long-range multiplex PCR using ApexHF HS DNA Polymerase (Accurate Biology, AG12204) and primers from DAFEI Biotech. Routine typing was conducted on the PolyseqOne nanopore platform (www.polyseq.com), and novel alleles were confirmed using Oxford Nanopore Technologies (ONT). PolyseqOne libraries were prepared with PY-DTB101/102 and PY-BLP101 kits, sequenced on PY-NFC001 flow cells for 12 h, and basecalled with Kant v1.0.1 (high-accuracy mode, 420 bp/s). ONT libraries were prepared with ONT Adaptor Mix, sequenced on R10.4 flow cells (FLO-PRO114M) using the SQK-NBD114-96 protocol. FastQ files from both platforms were error-corrected with NanoFix-AI, and HLA genotyping was conducted using NanoHLA-Resolve Assign v1.0.5 (DAFEI Inc. Guangzhou, China) referencing the IPD-IMGT/HLA Database. The error-corrected consensus sequence of HLA-G*01:62 from both platforms was identical across the full 7.1 kb, with no nucleotide differences.

HLA-G*01:62 is a novel allele differing from HLA-G*01:04:01 by a single nucleotide change at position 805 in exon 4 (G>A), resulting in a codon change from GCA to ACA and an amino acid substitution from Alanine to Threonine (Figure 1). The 7.1 kb amplicon includes key regulatory regions, and sequence analysis shows that both the promoter and 3′-UTR are identical to those of HLA-G*01:04:01:01, which may impact gene expression [3]. The complete HLA genotyping of the individual with this novel allele was: HLA-A*24:02:01, 30:01:01; -B*13:02:01, 40:01:02; -C*06:02:01, 07:02:01; -DRB1*07:01:01, 15:01:01; -DRB4*01:03:01, -DRB5*01:01:01; -DQB1*02:02:01, 06:10; -DQA1*01:02:01, 02:01:01; -DPB1*13:01:01, 17:01:01; -DPA1*02:01:01, 02:02:02; -G*01:05:01N, 01:62.

The novel allele HLA-G*01:62 was submitted to GenBank (accession no. PV607041) and the IPD-IMGT/HLA Database (HWS10100924). It was officially named HLA-G*01:62 by the WHO Nomenclature Committee in May 2025, following current naming guidelines [4].

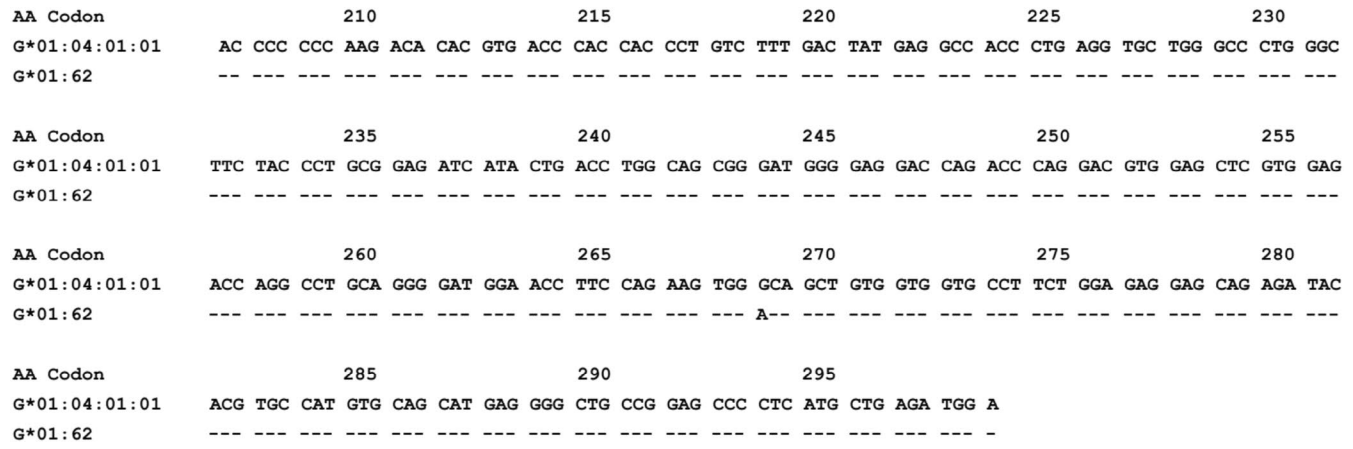

FIGURE 1 | Nucleotide sequence alignment of exon 4 from HLA-G*01:62, compared with the closely related HLA-G*01:04:01 allele. Dashes (–) indicate nucleotides identical to HLA-G*01:04:01. Codon positions are shown above the sequence.

Author Contributions

Jianrong Chen wrote the manuscript; Guorong Liao performed the experiments, Jun Qian collected the sample; Yuan Yao and Runjun He analysed the sequencing data; Yongguang Liu revised the manuscript; all the authors approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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