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NG_007494.1(RHD):c.[4A>T;5G>C;6_7insG] with an RhD-negative phenotype

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1 | BACKGROUND

The *RHD* gene located on the short arm of chromosome 1 encodes RhD, a highly immunogenic blood group protein.[1] Several molecular mechanisms are known to cause a D-negative phenotype, with the *RHD* deletion being the most frequent one worldwide.[2] Another mechanism involves deletions or insertions of, for example, one, two, or four nucleotides, which alter the ribosome reading frame, resulting in premature termination of translation. The total number of these frameshift variants in the *RHD* gene currently stands at 51.[3] We report a 25-year-old Caucasian male donor with a D-negative phenotype harboring three single-nucleotide variants (SNVs), in *RHD* Exon 1, including a frameshift variant.

2 | BRIEF METHODS

Ethylenediaminetetraacetic acid–anticoagulated whole blood sample was collected with consent in Springe, Germany. We have previously used and described all methods (Appendix S1, available as supporting information in the online version of this paper).

3 | RESULTS

We analyzed a Ccdee blood donor harboring three novel SNVs in the *RHD* gene. His phenotype was reproducibly confirmed to be D-negative by adsorption/elution tests; two routine anti-D sera and two panels of 6 and 12 monoclonal anti-Ds, respectively, also tested

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AUTHOR CONTRIBUTIONS

Franz Friedrich Wagner instituted the *RHD* screen of blood donors in Springe and detected the blood donor. Andrea Doescher performed the original sequencing of the gDNA. Kshitij Srivastava performed the molecular testing of the mRNA. All authors analyzed molecular data. Kshitij Srivastava and Willy Albert Flegel wrote the article.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

negative (data not shown). Genomic DNA and cDNA sequencing revealed two transversion variants (c.4A>T and c.5G>C) and an insertion variant (c.6_7insG) in Exon 1 of the *RHD* gene (Figure 1A). The frameshift permits a full-length *RHD* transcript (Transcript 1, Figure 1B). Additional splicing isoforms that are normally seen in cDNA studies of *RHD* were also observed (Transcripts 2 to 5; Figure 1B). Zygosity testing revealed the donor to be hemizygous for the *NG_007494.1(RHD):c.[4A>T;5G>C;6_7insG]* allele with an *RHD* deletion in trans (*RHD*01N.01*). *RH*Ce* (*RHCE*02*) is the likely *RHCE* allele linked to the novel allele.

3.1 | Population frequency

We calculated the frequency of *NG_007494.1(RHD):c. [4A>T;5G>C;6_7insG]* among the 460 711 first-time blood donors (921 422 chromosomes; Figure S1). Its frequency is 2.58 in 1 000 000 chromosomes (mean, 1 in 386 843 chromosomes; 95% confidence interval, 1 in 72 674 to 1 in 7 585 157, Poisson distribution, two sided).

4 | SUMMARY

The novel *NG_007494.1(RHD):c.[4A>T;5G>C;6_7insG]* allele with an insertion of "G" between Nucleotides 6 and 7 in Exon 1 creates a premature termination codon at amino acid position 4. All five mRNA transcripts of different lengths and harboring a premature termination codon are predicted to translate a significantly truncated and dysfunctional RhD protein of only three amino acids. While the two SNVs c.4A>T and c.5G>C are missense variants when present alone, they still encode serine at amino acid position 2 of the RhD protein when present in tandem.

A possible mechanism for the origin of the *NG_007494.1(RHD):c.[4A>T;5G>C;6_7insG]* allele involves the error-prone human DNA polymerase zeta (pol ζ). Human pol ζ specializes in translesion DNA synthesis and lacks an intrinsic 3′-5′ exonuclease activity or proofreading function.[4] Pol ζ has been shown to be involved in the generation of multinucleotide mutations in a single event, including tandem mutations.[5] Around 13% of these tandem mutations have been associated with a de novo indel less than 10 bp away, as shown in yeast experiments.[6] Such an event would explain the occurrence of tandem mutation at nucleotide positions c.4 and c.5 with an insertion of nucleotide "G" nearby in our allele.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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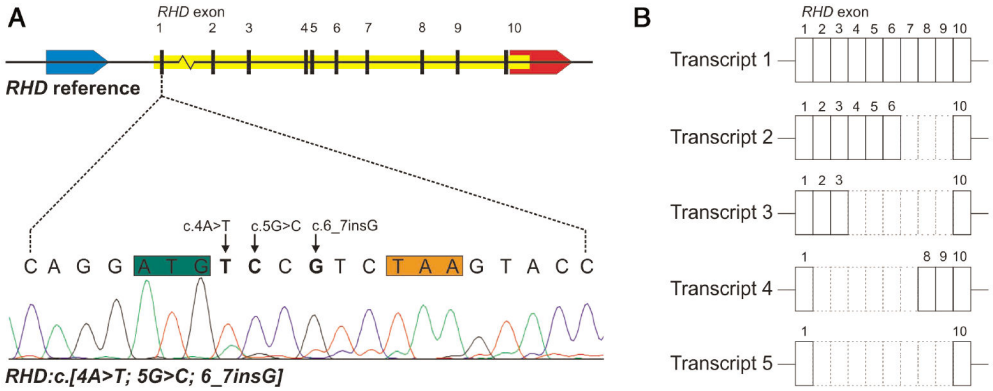

FIGURE 1.

Schematic representation of nucleotide substitutions and mRNA transcripts observed in the new *RHD* allele. The *RHD* gene comprises 10 exons (black bars) flanked by the upstream (blue) and downstream Rhesus box (red). The nucleotide sequences of the start codon (green box), premature termination codon (orange box) and the 3 variations (bold letters, with arrows) are shown along with their electropherograms, A. The resulting mRNA transcripts are symbolized as a concatenation of exons, B. Transcript 1 is full length and includes all 10 *RHD* exons (black outlined rectangles); Transcripts 2 to 5 lack different exons (gray outlined rectangles). GenBank entries: MT318152 (gDNA) and MT267527 (cDNA)