RHD del28Phe (DMW) encoded by a novel in-frame deletion resulting in reduced D antigen expression

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Red blood cells (RBCs) of variant Rh antigens, including weak D or partial D phenotypes, may cause anti-D immunization following transfusion or pregnancy.[1] Partial D individuals are at risk for producing allo-anti-D against the missing epitopes when they are exposed to normal D-positive cells, with the potential for eliciting hemolytic transfusion reactions and HDFN.[1,2] We have identified a novel *RHD* allele variant in a pregnant Caucasian woman with a considerably reduced D expression on her red blood cells (RBCs).

BRIEF METHODS

Rh phenotyping was performed by standard gel card matrix techniques (Micro Typing ID System, Bio-Rad Medical Diagnostic GmbH), including monoclonal (Diaclon ABO/Rh, ABO/D and Rh-subgroups + K), and human antibodies (ABO/Rh and Rh-subgroups + Cw + K). The presence of maternal irregular antibodies was investigated by indirect antiglobulin test prenatally and 6 weeks after delivery. A commercial partial RHD typing set (Bio-Rad Medical Diagnostic GmbH) was used for further characterization. Extended D epitope mapping was done employing 50 different human monoclonal anti-D as previously described.[3] The D antigen density was determined by flow cytometry using five primary anti-Ds: Brad-3, P3x290, P3x241, P3x249, and ESD1.

RHD was genotyped using RHD detection tests (RBC-Ready Gene CDE and RBC-Ready Gene Zygofast, Inno-train Diagnostik GmbH). DNA sequencing of *RHD* Exons 1 to 10 and flanking intronic regions was performed.[4] *RHD* mRNA isoforms were analyzed. The identified sequence was deposited under EMBL Accession number LR214928. The designation DMW refers to the identification of the allele by the authors Matzhold and Wagner.

A direct antiglobulin test of the newborn was performed.

RESULTS

Standard serology determined a C + c + D + E–e + phenotype, with weak positive reactions for the D antigen (3+) in both the monoclonal and human gel card. Accordingly, the RBCs of the proposita were found to have a reduced D antigen density of 1.001 D sites per cell.

The commercial monoclonal anti-D panel resulted in at least 2+ positive reactions with all the anti-D, except anti-D LHM59/19 (IgG) recognizing EpD8.1, where only a marginal positive reaction (+/−) was observed. The extended D epitope mapping demonstrated a near-normal, albeit weakened, epitope profile. Consistently, LHM59/19 reacted very weakly (1+). Several maternal antibody screening tests were negative and no allo-anti-D was detected in the proposita's serum.

Sequencing analysis revealed the presence of a novel *RHD* allele (DMW), characterized by a deletion of TCT at nucleotide position 83 (c.83delTCT, p.28delPhe) in Exon 1 (Fig. 1). The major *RHD* cDNA isoform confirmed the del28Phe alteration, resulting in a shortened protein of 416 instead of 417 amino acids.

Neonatal cord blood typing revealed blood group O, D-positive, and a negative direct antiglobulin test.

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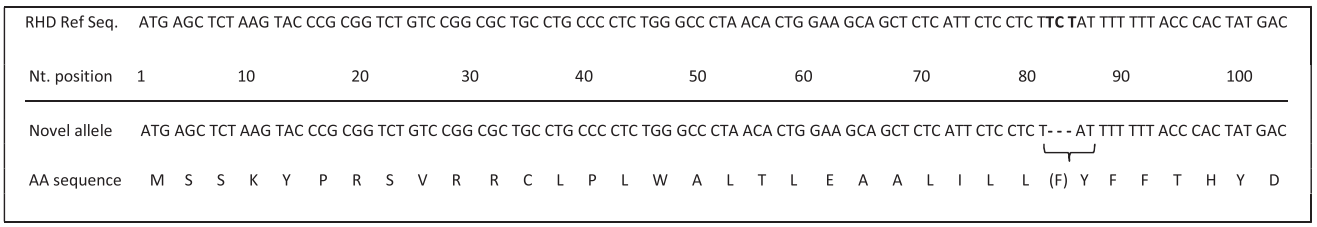

Fig. 1. Partial nucleotide and amino acid sequences of *RHD* Exon 1. The identified novel in frame mutation c.83delTCT is indicated by hyphens. The encoded D protein is characterized by a deletion of phenylalanine (F) as shown in brackets. The *RHD* reference sequence NM_016124 (mRNA) is used. AA = amino acid; Nt. = nucleotide.

SUMMARY

The novel in-frame deletion is associated with a considerably reduced expression of D antigen and a variant RhD phenotype.

The mutation occurs in close proximity to the first exofacial loop of the protein which is mainly encoded by sequences of Exon 1. Structural changes caused by the deletion may reach the surface of the protein in this region. Consistent with the substantially weakened agglutination reactions observed with anti-D LHM59/19, the presence of an altered Loop 1–dependent epitope 8.1[5] appears to be possible. Although all the anti-Ds we used to examine the epitope pattern reacted positive with the proposita's RBCs, the presence of a qualitatively altered D antigen may not be excluded.

The primigravida had not received prenatal or postnatal RhIG prophylaxis. Though pregnant with a D-positive fetus, no immunization was observed; however, whether this novel D variant permits anti-D immunization remains unclear. Hence, D-negative transfusion in carriers and prophylactic use of RhIG in pregnancy is recommended.

DMW expands the exceptionally rare *RHD* in-frame deletions reported and should be regarded as a putative partial D allele.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

WEB-BASED RESOURCES

The following Web-based resource was used:

http://www.ebi.ac.uk/ena/data/view/LR214928. Accessed January 3, 2019.

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