Weak D antigen expression caused by a novel *RHD* allele in Argentineans

Carolina Trucco Boggione,[1,3] Melina Eliana Luján Brajovich,[1,3] Ane Caroline Gaspardi,[2] Emilia Sippert,[2] Stella Maris Mattaloni,[1,3] Mónica Leri,[4] Claudia Biondi,[3] Lilian Castilho,[2] and Carlos Miguel Cotorruelo[1,3]

Variant D phenotypes are evidenced as serologic discrepancies with different anti-D reagents or reduced intensity of the hemagglutination reaction or if anti-D alloantibodies are detected in D+ individuals. The serologic assignment of the D status may be hindered in such situations. Molecular analysis allows the characterization of the allele involved in the aberrant expression of the D antigen and facilitates decision making by transfusion specialists and obstetricians.[1] This report describes a novel *RHD* allele responsible for a variant D phenotype associated with normal c, E, and e antigens expression.

BRIEF METHODS

A total of 289 samples obtained from both unrelated patients and donors from different hospitals and donor centers around Argentina were submitted to our reference laboratory for molecular typing because they typed weakly positive for the D antigen. The D status was evaluated with four commercially available monoclonal anti-D reagents: one blended IgM and IgG (Clones TH-28/MS-26) from Wiener Lab and three different IgM (Clones MS-201, RUM-1, and LDM1/ESD1M) from Rediar. D antigen expression was further investigated using the Advanced partial D typing kit from ALBAclone (Alba Bioscience). Samples were also tested with anti-C (Clone MS24), anti-c (Clone MS33), anti-E (Clones MS80/MS258), and anti-e (Clones MS16 + MS 21 + MS63) from Rediar. Screening of unexpected red blood cells alloantibodies was performed in all samples. Genomic DNA was isolated from peripheral blood with a commercially available purification kit (ADN PuriPrep-S, INBIO Highway). Most common weak D and partial D alleles were investigated by allele-specific polymerase chain reaction (PCR) techniques.[2] DNA array analysis was performed with the *RHD* BeadChip platform from BioArray Solutions (Immucor), which uses probes directed to polymorphic sites for many *RHD* variants.[3] Direct automated sequencing of the 10 *RHD* exons and flanking intron regions was performed by the Sanger dideoxy method.[4] Ten of the samples carrying a new *RHD* polymorphism found were also analyzed by flow cytometry with IgM anti-D clones MS-201, RUM-1, and LDM1/ESD1M as described.[5]

RESULTS

Among the 289 variant D samples, 122 Ccee, 104 ccEe, 48 ccee, eight CCee, six CcEe, and one CeEE phenotypes were encountered. Allele-specific PCR, DNA array, and sequencing studies identified already known alleles responsible for the aberrant D antigen expression observed in most of the samples. A novel missense mutation c.359C>A in *RHD* Exon 3 (GenBank Accession Number KU847398) was found in 28 samples carrying a ccEe phenotype. The nucleotide change is shown in Fig. S1 (available as supporting information in the online version of this paper). This new allele, named in this paper *RHD*359A*: (ISBT allele designation: Weak D type 93), leads to the amino acid substitution p.Ala120Asp, predicted to be in the fourth transmembrane region of the RhD protein. The 28 *RHD*359A* samples showed weak hemagglutination

From the [1]IDICER-CONICET, Rosario, Argentina; [2]Hemocentro–Unicamp, Campinas, Brazil; [3]Laboratorio de Inmunohematología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina; and the [4]Banco Central de Sangre-SIPROSA, Tucumán, Argentina.

Address correspondence to: Carolina Trucco Boggione, Laboratorio de Inmunohematología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, 2000 Rosario, Argentina; e-mail: ctrucco@fbioyf.unr.edu.ar.

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reactions (2+) with anti-D clones TH-28/MS-26, MS-201, RUM-1, and LDM1/ESD1M after immediate spin and slightly weaker reactions (1+/2+) with all clones from the advanced partial D typing kit (ALBAclone) in the indirect antiglobulin test. No unexpected alloantibodies were found in any of these 28 samples. A relative D epitope density between 4.1 and 14.2% was quantified by flow cytometry analysis. Weak D Type 1 and weak D Type 3 variants analyzed for comparison presented a similar relative D epitope density range.

*RHD*359A* allele was found in 25.2% (28/111) of the samples with weak D antigen expression carrying E antigen. To note, 22 of the samples carrying the *RHD*359A* variant were obtained from patients and donors from the north region of the country where the Amerindian influence in the genetic background of the population is higher than in other areas. Serologic and molecular findings strongly suggest a genetic association (in cis) between this new *RHD* variant and the *RHcE* allele since it was only found in samples with ccEe phenotype. The weak positive reactivity obtained with the panel of monoclonals and the absence of anti-D alloantibodies suggest that this variant does not represent a partial D allele.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig. S1. *RHD* exon 3 sequencing diagrams of a D positive sample (top) and a *RHD*359A* sample (bottom). The nucleotide change position is indicated by an arrow.