REPORT OF NEW ALLELES OR ANTIGENS

Novel RHAG allele encoding the Rhnull phenotype in Brazil

Carine Prisco Arnoni,1 Janaina Guilhem Muniz,1 Diana Gazito,1 Rosangela de Medeiros Person,1 Tatiane Aparecida de Paula Vendrame,1 Lilian Castilho,2 and Flavia Roche Moreira Latini1

**Rhnull** is a rare phenotype characterized by the loss of Rh antigen expression. This phenotype can be related to several molecular backgrounds. In this study, we show a novel allele in a Brazilian pregnant woman encoding the Rhnull phenotype due to a change in RHAG exon 2 c.310G>C, which leads to a premature stop codon (Gln104Stop).

The Rh system is one of the most important and complex blood group systems and Rh antibodies can potentially cause hemolytic transfusion reactions and hemolytic disease of the fetus and newborn. The Rh system comprises two highly homologous genes, RHd and RHCE, which encode polypeptides that are expressed on the red blood cell (RBC) surface in a protein complex. Rhnull phenotype arises from two distinct genetic mechanisms, the regulator type and the amorph type. The amorph type is caused by homozygosity for silent genes at RHd and RHCE loci, resulting from inactivating mutations in RHCE and deletion of RHd, whereas the regulator type is caused by mutation in RHAG when in homozygous state or when in heterozygosity with another RHAG allele containing an inactivating mutation.2,23 The suppression of Rh antigen expression for regulator types is attributed to genetic variations, as missense point mutations, splice-site mutations, and small exonic deletions, which can affect the transcription and translation of RHAG protein that is essential for the assembly of the Rh proteins into the RBC membrane and for the integrity of RBC membranes.4

Rhnull syndrome is characterized by stomatocytosis and spherocytosis, increased osmotic fragility, altered cation transport, abnormal phospholipid organization, and chronic hemolytic anemia. We herein report a novel missense mutation of the RHAG gene, which resulted in Rhnull phenotype in a Brazilian pregnant woman.

We analyzed the genomic DNA of a 35-year-old pregnant woman without transfusion history, displaying a D-, C-, c-, E-, e- phenotype. Her serum presented an antibody reactive 4+ in gel indirect agglutination test with all RBCs except her own. She delivered a baby whose RBCs presented a positive direct agglutination test and an eluate reactive with all RBCs, with no signal of severe anemia.

**BRIEF METHODS**

Genomic DNA was isolated from peripheral blood with a commercially available purification kit (QiAamp blood mini kit, Qiagen, Inc., Valencia, CA). To determine the RH genotype, RHd, RHCE, and RHCEc alleles were amplified by allele-specific polymerase chain reaction (PCR). The presence of RHd gene was evaluated by multiplex PCR for RHd gene-specific regions in Introns 4 and Exon 7. Sequence of the entire RHCE, RHd, and RHAG gene coding region was performed using primers previously described.3-7 Sequencing analysis was performed on a genetic analyzer (3500xl, Applied Biosystems, Foster City, CA).

**RESULTS**

The proband was genotyped as RHd+ and RHCEc/RHCEc. Sequencing of RHCE and RHd showed no changes. The sequencing of the entire RHAG gene coding

From the 1Colsan-Associação Beneficente de Coleta de Sangue, São Paulo, SP, Brazil; and 2Hemocentro-Unicamp, Campinas, SP, Brazil.

*Address reprint requests to: Carine Arnoni, Colsan-Associação Beneficente de Coleta de Sangue, Avenida Janira 1220, Indianopolis-CEP 04080-066, Brazil; e-mail: carine. arnoni@colsan.org.br.*

Received for publication January 12, 2015; revision received May 19, 2015, and accepted May 25, 2015.

doi:10.1111/trf.13219
© 2015 AABB

TRANSFUSION 2015;0:000-00

Volume 00, Month 2015 TRANSFUSION 1