Novel *RHAG* allele encoding the Rh_{null} phenotype in Brazil

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Rh_{null} 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 Rh_{null} phenotype due to a change in *RHAG* exon2 c.310C>T, 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.[1] 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.[1,2] Rh_{null} 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.[2,3] 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]

Rh_{null} 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 Rh_{null} 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*, *RHCle*, and *RHE/e* 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 Intron 4 and Exon 7. Sequence of the entire *RHCE*, *RHD*, and *RHAG* gene coding region was performed using primers previously described.[5-7] Sequencing analysis was performed on a genetic analyzer (3500xL, Applied Biosystems, Foster City, CA).

RESULTS

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

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