Short deletion within the blood group Dombrock locus causing a Do\textsubscript{null} phenotype

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A new alteration of the blood group DO\textsuperscript{A} allele was identified in a female Do\textsubscript{null} donor from Reunion Island with allo-anti-DO3 in her serum; her parents are consanguineous. Because the amplification of the DO transcript failed, each exon and intron–exon junction from the DO gene were examined. After polymerase chain reaction (PCR) amplification and sequencing, the only deviation from the wild-type DO\textsuperscript{A} allele sequence was an 8-nucleotide deletion (nt 343-350) within exon 2. This short deletion generates a premature stop codon and encodes a truncated protein lacking the predicted functional motif of the adenosine diphosphate–ribosyltransferase enzyme and the glycosyl-phosphatidylinositol anchor motif essential for RBC membrane attachment. An allele-specific PCR to detect the DO(Δ8nt) deletion was developed. (Blood. 2002;100:1063-1064) © 2002 by The American Society of Hematology

Introduction

The antithetical Dombrock antigens (Do\textsuperscript{a}/Do\textsuperscript{b}) reside on a glycosyl-phosphatidylinositol (GPI)–anchored red blood cell (RBC) membrane glycoprotein that also carries 3 high-incidence antigens, Gregory (G\textsubscript{y}), Holley (H\textsubscript{y}), and Joseph (J\textsubscript{o}).\textsuperscript{1,2} The Dombrock gene locus (DO) consists of 3 exons spanning 14 kb on the short arm of chromosome 12, which predicts a 314-amino acid polypeptide containing an arginine-glycine-aspartic acid (RGD) motif (DO\textsuperscript{A+B} allele product only) commonly involved in cell-to-cell interactions involving integrin binding and the GPI anchor motif.\textsuperscript{3} A search of the GenBank database indicated that the full sequence is identical to adenosine diphosphate (ADP)–ribosyltransferase 4 (ART-4; GenBank accession numbers NM-021071 and AF290204),\textsuperscript{4} but enzymatic activity has not been demonstrated yet in any cell type including erythroid cells. The polymorphisms associated with DO\textsuperscript{A+B} alleles, the 2 C378T and T624C silent transitions, and the A793G transition resulting in an Asn265Asp amino-acid substitution located in the RGD motif have been reported.\textsuperscript{5,6} Recently, from unrelated donors with the rare Hy-negative phenotype [Do(a−b+w), G\textsubscript{y}(a+w), H\textsubscript{y}−, J\textsubscript{o}(a−)], it has been shown that both DO\textsuperscript{HY1}/DO\textsuperscript{HY2} alleles derive from a DO\textsuperscript{B} background with a G323T transition, resulting in a Gly108Val substitution and T378C silent substitution, but that they differ by a C898G ground with a G323T transition, resulting in a Gly108Val substitution.\textsuperscript{7} More recently, it was shown that the Joseph-negative phenotype [Do(a+b+w−), G\textsubscript{y}(a+), H\textsubscript{y}+(w), J\textsubscript{o}(−)] results from a JO allele with a DO\textsuperscript{A} background but through a C350T transition, resulting in a Thr117Ile substitution and a C378T silent mutation.\textsuperscript{8} The rare Do\textsubscript{null} phenotype, in which the RBCs lack all 5 antigens, may arise from a single nucleotide mutation in the acceptor splice site of intron 1, causing the skipping of exon 2 or from the absence of GPI-anchored proteins on RBCs from patients with paroxysmal nocturnal hemoglobinuria.\textsuperscript{10} In all cases, negative and null phenotypes can develop anti-Dombrock antibodies that may cause severe hemolytic transfusion reactions.\textsuperscript{11,12} Here, we report a new molecular mechanism from a patient with a Do\textsubscript{null} phenotype, identified as a short deletion in exon 2 altering the reading frame of a DO\textsuperscript{A} allele.

Study design

Reagents

Expand High Fidelity and primers were from Boehringer-Mannheim/Roche Diagnostics (Mannheim, Germany) and Genset (Paris, France), respectively. Nucleotide sequences were determined with BigDye Terminator Cycle Sequencing ready Reaction Kit and analyzed on an ABI-PRISM 310 Genetic Analyser (PE Applied Biosystem, Foster City, CA). Blood samples from the family of the Do\textsubscript{null} donor were collected after informed consent.

Genomic DNA analysis

Polymerase chain reaction (PCR) from 500 ng leukocyte genomic DNA extracted with the Wizard Genomic DNA Purification kit (Promega, Madison, WI) was performed to amplify each exon and intron–exon junction of the DO gene. For the first exon, PCR between primers SP-A (position −250 to −229, upstream of the initiation codon) and AS-B (position 67 to 50, downstream of exon 1) was performed under stringent conditions (94°C for 2 minutes [1 cycle]; 94°C for 30 seconds, 58°C for 30 seconds, 68°C for 1 minute [30 cycles]; 68°C for 7 minutes [1 cycle]) using Expand High Fidelity in a total volume of 50 μL. The same conditions were used to amplify the second and third exons with primers (1) SP-C (position −188 to −168, upstream of exon 2) and SP-D (position −177 to −144, upstream of exon 3), and (2) SP-E (position 41 to 20, upstream of exon 3) and SP-F (position 1054 to 1034). Then the final PCR products were subcloned into pCR2-1 vector from Invitrogen (Groningen, The Netherlands) and sequenced on both strands. All position primers refer to the first nucleotide of the genBank accession numbers AF290204 and AC007655, respectively.

PCR genotyping

To identify the Do(Δ8nt) variant, allele-specific PCR reactions were performed between the following sets of primers within exon 2: PCR-1 between the primers SP-1 (position 165-182) and AS-1 (position 363-343)
specific to the wild-type DO alleles and PCR-2 between the primers SP-1 and AS-2 (position 363-337, deleted of 350 to 343 nucleotides) specific of the DO*A(Δ8nt) allele, under the same conditions as above except that annealing was performed at 56°C. Expected PCR products were electrophoresed on a 3% (wt/vol) Metaphor gel (BMA, Rockland, ME) and were stained with ethidium bromide before analysis.

Results and discussion

The healthy female donor with a Donull phenotype was identified at the Reunion Island Blood Service (St Denis, France) and was typed as Do(a−b−), Gy(a−) with routine reagents (not shown) at the CNRGS (Paris, France) and is most likely also Hy(−) and Jo(−) because her serum contains an anti-DO antibody reacting with Hy(−) and Jo(−) cells that are not Do null. Two sisters of the propositus were phenotyped as Do(a−b+) because her serum contains an anti-DO antibody reacting with Hy(−) and Jo(−) cells that are not Do null. Two sisters of the propositus were phenotyped as Do(a−b+). PCR-genotyping indicated that the propositus and her sisters were homozygous for the DO*A and DO*B alleles, respectively. To determine the molecular defect occurring in the propositus, genomic DNA was analyzed because PCR amplification of the full-length DO cDNA failed repeatedly. PCR products for each of the 3 Dombrock exons, including intron–exon junctions, were amplified, subcloned, and sequenced as described in “Study design.” Sequence analysis revealed that the only deviation from the wild-type DO*A allele sequence was an 8-bp deletion (ATGAC-TAC) within exon 2 behind nucleotide 342 (Figure 1B). This short deletion causes a frameshift that generates a premature stop codon 17 amino acids further (Figure 1A). Thus, the resultant truncated protein without the predicted functional motifs of the ADP-ribosyltransferase enzyme and the GPI-anchor motif explains the loss of Dombrock blood group antigens on erythrocytes from this patient. To confirm this finding and to screen the DO(Δ8nt) deletion in random blood donors, an allele-specific PCR assay was developed (described in “Study design”). As expected, a 199-bp PCR product was detected only in the Do(a+b+) control as in the 2 sisters’ Do(a−b+) samples, whereas a 191-bp PCR product was obtained only in the propositus demonstrating the homozygosity of the short deletion in this Donull donor. Neither of the sisters carried the DO(Δ8nt) deletion because both inherited the normal DO*B allele from their heterozygous parents. In conclusion, a novel silent DO*A allele is described, but its frequency and geographic distribution are unknown. This second alteration of the DO locus suggests molecular heterogeneity of the Do null phenotype.

References

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