Case report: DNA testing resolves unusual serologic results in the Dombrock system

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Typing for antigens in the Dombrock blood group system and identifying the corresponding antibodies are notoriously difficult tasks. The reagents are scarce and the antibodies are weakly reactive. When RBCs from family members of a patient with an antibody to a high-prevalence Dombrock antigen were tested for compatibility, an unusual pattern of inheritance was observed: RBCs from the patient's children and one niece, in addition to those from some of the patient's siblings, were compatible. This prompted the performance of DNA-based assays for *DO* alleles and the results obtained were consistent with and explained the compatibility test results. It was possible to study this large kindred because of the cooperation of family members, hospital personnel, and reference laboratory staff. ***Immunohematology* 2006;22:69–71.**

Key Words: blood groups, Dombrock, transfusion medicine, DNA-based assay, molecular typing

Antibodies to antigens in the Dombrock blood group system are likely to be more clinically significant than is currently documented. This is because of the lack of typed RBCs on antibody identification panels, a dearth of monospecific antibodies with a reasonable strength of reactivity, and the absence of in vitro characteristics that are usually associated with delayed hemolytic transfusion reactions.[1] The cloning of the gene for Dombrock and the identification of the molecular basis associated with Dombrock antigens provide other means by which to study them.[2–5]

The *DOA* and *DOB* alleles can be distinguished by a mutation at nucleotide (nt) 793(A>G) of *DO,* which is predicted to encode a change of Asn265Asp. The Hy– phenotype is associated with a change of G>T at nt 323 (Gly108Val). The *HY* allele also carries 793G, which explains why the Hy– phenotype is invariably Do(a–b+). The Jo(a–) phenotype, encoded by *JO,* is associated with a single-nucleotide change of 350 C>T (Thr117Ile). 350T is on an allele carrying 793A and most Jo(a–) phenotype RBCs are Do(a+).[1]

We describe here the use of PCR amplification followed by restriction fragment length polymorphism (RFLP) analyses to resolve unusual serologic results in a patient with an antibody to a high-prevalence Dombrock antigen.

Case Report

A 55-year-old, group B, D+ African American woman was hospitalized with congestive heart failure (CHF) and anemia. Her antibody screen was negative and she received two RBC transfusions and was discharged with a Hct of 29.6%. Tests performed to identify the cause of the anemia were within the normal range for each test and there were no signs of bleeding or hemolysis. Eight days later, the patient was readmitted with CHF and a Hct of 27%. Two units were ordered but the antibody screen was positive (2+) by the IAT. The antibody reacted with all RBCs tested except a Hy– sample. After 6 days in the hospital, she was discharged with a Hct of 24.7%. Twelve days later, she was readmitted with CHF again, chronic renal failure, and unstable angina. While the antibody was being investigated, the primary care physician was informed that compatible blood was not available and was asked to determine if the patient had siblings. The patient's son arranged for five siblings and 13 other relatives to have blood samples collected for testing with the patient's serum. Two ABO-compatible siblings were crossmatch compatible as were three of her five children and one niece. Compatible blood was transfused without incident. Difficulties with antibody identification, lack of sufficient volumes of anti-Hy and anti-Jo^a, and the unusual inheritance pattern prompted us to perform DNA analysis.

Materials and Methods

Blood samples were collected from consenting family members. The IATs were performed by hemagglutination in tubes.

Table 1. Primers used for PCR-RFLP analyses

Primer	Sequence 5´ to 3´	Uncut size	Restriction enzyme (nt)	Restriction fragment size (allele)	
DoF	TACCTCACCTCAGCAATCCAGCTGCTGAGGAGAGAC	368 bp	BseR I (nt 793)	326, 42	268, 58, 42
DoR	TTTAGCAGCTGACAGTTATATGTGCTCAGGTTCC (annealing temperature 62°C)			(*DOA*)	(*DOB*)
DoX2F	TCAGTACCAAGGCTGTAGCA				
Do378R	AGTAAAGTCAGAATGAACATTGCTGCACAAT (annealing temperature 58°C)	220 bp	*Bsa*J I (nt 323)	120, 92, 8 (wild type)	212, 8 (*HY*)
			Xcm I (nt 350)	167, 53 (wild type)	220 (*JO*)

Genomic DNA was extracted using the QIAamp DNA Mini Blood Kit (QIAGEN, Valencia, CA). PCR was performed using the following conditions: 100 ng of each primer, 200 µM of each dNTP, 2.5mM (for nt 323 and nt 350 of *DO*) or 3.0mM MgCl2 (for nt 793 of *DO*), 1.0 U HotStar Taq DNA polymerase (QIAGEN), and buffer in a total volume of 50 µl. Amplification was performed in the 9700 thermal cycler (Perkin Elmer, Norwalk, CT) with the following profile: 95°C for 15 minutes; followed by 35 cycles of 94°C for 20 seconds; 55°C (for nt 323 and nt 350 of *DO*) or 62°C (for nt 793 of *DO*) for 20 seconds and 72°C for 20 seconds; then 72°C for 7 minutes.[5] PCR products were analyzed by electrophoresis in 1% agarose gel.

PCR-RFLP assays for these three polymorphisms were performed using *Bsa*JI for the polymorphism at nt 323, *Xcm*I for the polymorphism at nt 350, and *Bse*RI for the *DOA/DOB* polymorphism at nt 793.[3,6] The sequence of primers, PCR annealing temperature, restriction enzyme used to digest each PCR-amplified product, and expected restriction-fragment sizes are given in Table 1. Digested products were analyzed by electrophoresis on an 8% polyacrylamide gel.

Results

The results of PCR-RFLP analyses of the three *DO* single nucleotide polymorphisms (SNPs) are shown in Table 2. The patient (II-10), who had one *HY* and one *JO* allele, is predicted to have the phenotype Do(a+^{w}b+^{w}) Hy+^{w} Jo(a–) and to have produced anti-Jo^{a}. Her two compatible siblings (II-4 and II-7) and one compatible child (III-9) were *JO/JO.* The other two compatible children (III-8 and III-11) were *HY/JO* and the compatible niece (III-2) was *HY/HY.* Thus, RBCs from all six compatible family members are predicted to be Jo(a–). Of the ABO-compatible but crossmatch-incompatible family members, each had one *DOA* or *DOB* allele together with either an *HY* or a *JO* allele. A summary of ABO types, compatibility testing with the patient's serum by the IAT, and *DO* alleles is given in the pedigree (Fig. 1).

Discussion

The cooperation among family members, hospital personnel, and reference laboratory staff made it possible not only to provide blood for the patient but also to study this large kindred by PCR-based methods. The presence of combinations of *DOA, DOB, HY,* and *JO* alleles was consistent with the compatibility testing

Table 2. Results of PCR-RFLP analyses

Identification	323 (G>T) Hy+>Hy–	350 (C>T) Jo(a+)>Jo(a–)	793 (A>G) Do^{a}/Do^{b}	Alleles	Predicted phenotype
II-2	G	T	A	*JO/JO*	Do(a+b–) Hy+ Jo(a–)
II-4	G	T	A	*JO/JO*	Do(a+b–) Hy+ Jo(a–)
II-6	G/T	C	A/G	*DOA/HY*	Do(a+b+W) Hy+W Jo(a+W)
II-7	G	T	A	*JO/JO*	Do(a+b–) Hy+ Jo(a–)
II-9	G/T	C	A/G	*DOA/HY*	Do(a+b+W) Hy+W Jo(a+W)
II-10	G/T	C/T	G/A	*HY/JO*	Do(a+Wb+W) Hy+W Jo(a–)
II-11	G	C/T	G/A	*DOB/JO*	Do(a+ W b+) Hy+ Jo(a+W)
III-1	G	C/T	G/A	*DOB/JO*	Do(a+ W b+) Hy+ Jo(a+W)
III-2	T	C	G	*HY/HY*	Do(a–b+W) Hy– Jo(a–)
III-4	G/T	C/T	G/A	*HY/JO*	Do(a+Wb+W) Hy+W Jo(a–)
III-5	G	C/T	G/A	*DOB/JO*	Do(a+Wb+) Hy+ Jo(a+W)
III-6	G/T	C	G	*DOB/HY*	Do(a–b+) Hy+ Jo(a+W)
III-8	G/T	C/T	G/A	*HY/JO*	Do(a+Wb+W) Hy+W Jo(a–)
III-9	G	T	A	*JO/JO*	Do(a+b–) Hy+ Jo(a–)
III-11	G/T	C/T	G/A	*HY/JO*	Do(a+Wb+W) Hy+W Jo(a–)
III-12	G	C/T	G/A	*DOB/JO*	Do(a+Wb+) Hy+ Jo(a+W)
IV-1	G/T	C	G	*DOB/HY*	Do(a–b+) Hy+W Jo(a+W)
IV-2	G/T	C	G	*DOB/HY*	Do(a–b+) Hy+W Jo(a+W)
IV-3	G	C/T	G/A	*DOB/JO*	Do(a+Wb+) Hy+ Jo(a+W)

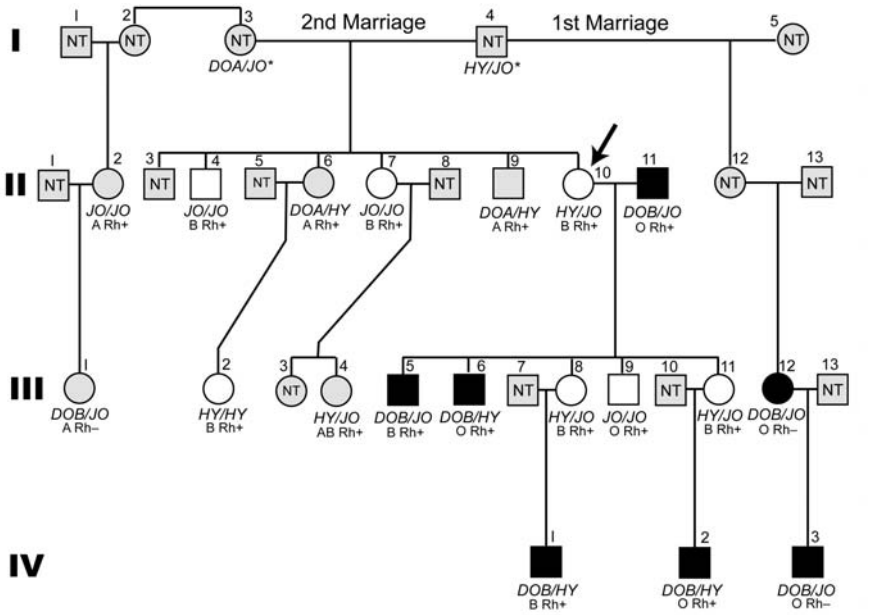

Fig. 1. Pedigree of the family studied. The ABO and Rh types and *DO* alleles are indicated under the family members who were tested. * = presumed; NT = not tested. Circles represent women and squares represent men. Open symbols indicate serologically compatible family members, black symbols indicate ABO-compatible but serologically incompatible family members, and grey symbols indicate either ABO-incompatible family members or those from whom blood samples were not obtained.

results and provided an explanation for the initial, apparently unusual inheritance pattern of compatible donors. This study also revealed a surprisingly high number of negative alleles (*HY* or *JO* or both) in one kindred.

The Do status of the RBC samples could not be determined because of the lack of appropriate antibodies. This study highlights the value of using PCR-based analyses in conjunction with classic hemagglutination. This is particularly relevant when studying blood group antigens that are expressed weakly and when reagents are scarce. As we have previously advocated,[1,7] PCR-based analyses can be invaluable for typing reagent RBCs and for screening for antigen-negative donors.

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