ORIGINAL REPORT

Molecular studies of *DO* alleles reveal that *JO* is more prevalent than *HY* in Brazil, whereas *HY* is more prevalent in New York

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Because of the scarcity of anti-Hy and anti-Jo^a, hemagglutination typing for the Dombrock blood group system antigens, Hy and Jo^a, is not feasible. The molecular bases associated with these antigens have been determined, making it possible to distinguish *HY* and *JO* from wild-type *DO*. This provides a tool to predict the probable phenotype of patients and to screen for antigen-negative donors. PCR-RFLP assays and a microchip assay were used to determine the frequency of *HY* and *JO* alleles in donors from Brazil and New York. DNA from random Brazilian donors, 288 by PCR-RFLP and 599 by the bead array method (BeadChip, BioArray Solutions, Warren, NJ), was tested to determine 323G/T (*HY*+/*HY*–) and 350C>T (*JO*+/*JO*–) single-nucleotide polymorphisms. In New York, 27,226 donors who self-identified as being African American were tested by hemagglutination with anti-Gy^a. Nonreactive and weakly reactive samples were tested by PCR-RFLP for the same alleles as listed above. In Brazil, 30 (3.4%) of the samples were *JO/DO* and 13 (1.4%) were *HY/DO*. In New York, of the samples that had *HY* or *JO* alleles, 14 were homozygous *HY/HY,* 132 were heterozygous *HY/DO,* 13 were heterozygous *HY/JO,* 14 were heterozygous *JO/DO,* and 3 were homozygous *JO/JO*. These results show that in donors from Brazil, *JO* (30 alleles) is more than twice as prevalent as *HY* (13 alleles), whereas in donors from New York, *HY* (173 alleles) was more than five times more common than *JO* (33 alleles). ***Immunohematology* 2008;24:135–137.**

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The Dombrock (Do) blood group system consists of five antigens: a pair of antithetical antigens (Do^a and Do^b) and three high-prevalence antigens (Gy^a, Hy, and Jo^a).[1] The Gy(a–) phenotype is the null of the Dombrock system.[2] The Hy-negative phenotype is associated with weak expression of Do^b and Gy^a, and absent or very weak expression of Jo^a, and the Jo(a–) phenotype is associated with weak expression of Do^a and Hy.[1,3]

In the transfusion setting, antibodies to antigens in the Dombrock blood group system have caused delayed, and rarely acute, transfusion reactions. In the prenatal setting, they have caused a positive direct antiglobulin test but not hemolytic disease of the newborn and fetus.[4] Antigens in the Dombrock blood group system are carried on the Dombrock glycoprotein, which is encoded by the *DO* gene, also known as *ART4*.[5] The molecular bases associated with the various Do phenotypes have been determined to be caused by single-nucleotide polymorphisms.[5–7] Our ability to type RBCs for Hy and Jo^a by hemagglutination has been severely limited because of the scarcity of suitable antibodies. As the molecular bases of these Dombrock blood group system antigens have been determined, the ability to distinguish *HY* and *JO* makes it feasible to predict the probable phenotype of patients and to screen for antigen-negative donors. Based on this knowledge, we used PCR-RFLP assays and a bead microchip assay to determine the relative frequency of *HY* and *JO* alleles in donors from Brazil and New York.

Materials and Methods

Genomic DNA was extracted from the buffy coat fraction from blood samples using a DNA extraction kit (QIAamp DNA Blood Mini Kit, QIAGEN, Inc., Valencia, CA). PCR was performed using the following conditions: 100 ng of each primer (synthesized by Life Technologies, Inc., Gaithersburg, MD), 200 µM of each dNTP, 2.5 mM (for nt 323 and nt 350

of *DO*) or 3.0 mM $MgCl_2$ (for nt 793 of *DO*), 1.0 U DNA polymerase (HotStar Taq, QIAGEN), and buffer in a total volume of 50 µL. Amplification was performed in a thermal cycler (GeneAmp PCR System 2400, Perkin Elmer, Norwalk, CT) with the following profile: 95°C for 15 minutes; followed by 35 cycles of 94°C for 20 seconds; 58°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.[7,8] PCR products were analyzed by electrophoresis in 1% agarose gel. PCR-RFLP assays were performed as previously described.[7,8] The sequence of primers, PCR product size, 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 in 8% polyacrylamide gel.

In Brazil, DNA samples from random Brazilian donors were tested by PCR-RFLP (n = 288) and by HEA bead microchip (n = 599; BeadChip, BioArray Solutions, Warren, NJ) to determine 793A>G (*DO*A/DO*B*), 323G>T (*HY*+/–), and 350C>T (*JO*+/–) single-nucleotide polymorphisms. The donors were predominantly of European and African descent, and a small number were of Asian descent. In New York, 27,226 (from September 1999 to April 2007) donors who self-identified as being African American were tested by hemagglutination with a polyclonal anti-Gy^a by the IAT. Nonreactive and weakly reactive samples were tested by PCR-RFLP for the same alleles as listed above.

Results

In Brazil, 21 (2.4%) typed as *JO/DO*B,* 9 (1.0%) as *JO/DO*A,* 5 (0.5%) as *HY/DO*A,* and 8 (0.9%) as *HY/DO*B.* Thus, of donors who had *HY* or *JO* alleles, 30 were heterozygous *JO* in *trans* to a *DO*B* or *DO*A*, and 13 were heterozygous *HY* in *trans* to *DO*A* or *DO*B* (Table 2). All donors with *HY* or *JO* alleles were Afro-Brazilians.

Table 2. Distribution of *HY* and *JO* alleles in donors of African descent in Brazil and New York

Allele Combinations	Brazilian Donors (n = 43)	New York Donors (n = 176)
HY/DO	13 (30%)	132 (75%)
HY/HY	0	14 (8%)
HY/JO	0	13 (7%)
JO/DO	30 (70%)	14 (8%)
JO/JO	0	3 (2%)

In New York, of the samples that had *HY* or *JO* alleles, 14 were homozygous *HY/HY,* 132 were heterozygous *HY* in *trans* to a *DO*A* or *DO*B*, 13 were heterozygous *HY/JO,* 3 were homozygous *JO/JO,* and 14 were heterozygous *JO* in *trans* to a *DO*A* or *DO*B* (Table 2).

Discussion

In this study, in the Afro-Brazilians, *JO* (30 alleles) is about twice as frequent as *HY* (13 alleles). In contrast, in African American donors from New York, *HY* (173 alleles) is more than five times as common as *JO* (33 alleles). It is likely these findings reflect that Africans brought to South America were from a different region of Africa than those brought to the East Coast of North America.

Antibodies to antigens in the Dombrock blood group system are difficult to identify, and the paucity of reliable monospecific antisera hampers studies involving the Dombrock blood group system. At least one anti-Hy has caused biphasic destruction of Hy+ RBCs.[9] Other examples of anti-Hy as well as anti-Gy^a and anti-Jo^a have caused moderate transfusion reactions (reviewed in Reid[1]). PCR-based testing for *DOA, DOB, HY,* and *JO* alleles provides a tool to predict the probable phenotype of patients and blood donors. This is an advantage for screening a large number of donors to find those who are Do(a–), Do(b–), Hy–, or Jo(a–), a feat not possible by hemagglutination. Thus, for Dombrock typing, DNA-based assays are not only feasible, they are more reliable

Table 1. Primers used for PCR-RFLP analyses

Primer Name	Primer Sequence (5´ to 3´)	Uncut Size (bp)	Restriction Enzyme (nt)	Restriction Fragment Size (allele)	
DoF	TACCTCACCTCAGCAATCCAGCTGCTGAGGAGAGAC	368	*Bse*RI	326, 42	268, 58, 42
DoR	TTTAGCAGCTGACAGTTATATGTGCTCAGGTTCC		(nt 793)	*(DOA)*	*(DOB)*
DoX2F	TCAGTACCAAGGCTGTAGCA	220	*Bsa*JI (nt 323)	120, 92, 8 (*DO*)	212, 8 (*HY*)
Do378R	AGTAAAGTCAGAATGAACATTGCTGCACAAT		*Xcm*I (nt 350)	167, 53 (*DO*)	220 (*JO*)

nt = nucleotide; bp = base pairs; *DO* = *DOA* or *DOB.*

than hemagglutination. Our findings emphasize the importance of testing populations with different ethnic backgrounds to define their *DO,* and other blood group, alleles.

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