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


Because of the scarcity of anti-Hy and anti-Jo, hemagglutination typing for the Dombrock blood group system antigens, Hy and Jo, 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-Gya. 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.

Key Words: blood groups, DNA testing, Dombrock, molecular basis

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. Antigens in the Dombrock blood group system are carried on the Dombrock glycoprotein, which is encoded by the DO gene, also known as ART4. The molecular bases associated with the various Do phenotypes have been determined to be caused by single-nucleotide polymorphisms. Our ability to type RBCs for Hy and Jo 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 MgCl₂ (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. PCR products were analyzed by electrophoresis in 1% agarose gel. PCR-RFLP assays were performed as previously described. 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 PCR 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, 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. Other examples of anti-Hy as well as anti-Gy⁺ and anti-Jo⁺ have caused moderate transfusion reactions (reviewed in Reid). 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

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Primer Sequence (5’ to 3’)</th>
<th>Uncut Size (bp)</th>
<th>Restriction Enzyme</th>
<th>Restriction Fragment Size (allele)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DoF</td>
<td>TACCCTACCTCAGAATCTCAGCTGAGGAGAGAC</td>
<td>368</td>
<td>BseRI</td>
<td>268, 32, 152 (DO)</td>
</tr>
<tr>
<td>DoR</td>
<td>TTTAGCAGCTGACAGTTAGTTGTGTCAGGTGTC</td>
<td>(nt 793)</td>
<td>(DOA)</td>
<td></td>
</tr>
<tr>
<td>DoX2F</td>
<td>TCGTACCTGAGCTCTGAGCTGAGC</td>
<td>220</td>
<td>BseI (nt 323)</td>
<td>120, 92, 8 (DO)</td>
</tr>
<tr>
<td>Do378R</td>
<td>AGTAAATGCATTGAGCTGAGGAGAGAC</td>
<td>167, 53 (DO)</td>
<td>XcmI (nt 350)</td>
<td>220 (JO)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Allele Combinations</th>
<th>Brazilian Donors (n = 43)</th>
<th>New York Donors (n = 176)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HY/DO</td>
<td>13 (30%)</td>
<td>132 (75%)</td>
</tr>
<tr>
<td>HY/HY</td>
<td>0</td>
<td>14 (8%)</td>
</tr>
<tr>
<td>HY/JO</td>
<td>0</td>
<td>13 (7%)</td>
</tr>
<tr>
<td>JO/DO</td>
<td>30 (70%)</td>
<td>14 (8%)</td>
</tr>
<tr>
<td>JO/JO</td>
<td>0</td>
<td>3 (2%)</td>
</tr>
</tbody>
</table>

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

**Acknowledgments**

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**References**

2. Banks JA, Hemming N, Poole J. Evidence that the Gy<sup>a</sup>, Hy and Jo<sup>a</sup> antigens belong to the Dombrock blood group system. Vox Sang 1995; 68:177–82.


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