A PCR-Based Strategy for Dombrock Screening in Brazilian Blood Donors Reveals a Novel Allele: The DO* A-WL

Wilson Baleotti Jr.,^{1*} Rodrigo Buzinaro Suzuki,¹ Milena Polotto,² Marcelo Ortega Ruiz,¹ Antonio Fabron Jr.,¹ and Lilian Castilho³

¹Hemocentro, Faculdade de Medicina, Marília, São Paulo, Brazil

²FAMERP, São José do Rio Preto, São Paulo, Brazil

³Hemocentro Unicamp, Campinas, São Paulo, Brazil

Background: Determination of the molecular basis underlying the antigens in the Dombrock blood group system has shown various rearrangements between the alleles associated with DO* A and DO* B. Based on this, we employed a PCR-based strategy to screen DO alleles (DO* A, DO* B, HY* 1, HY* 2 and JO) in Brazilians. **Methods:** We tested DNA of 278 Brazilian blood donors by PCR-RFLP on plates of 96 wells to determine the 793A/G (DO* A/DO* B), 323G/T (HY), 350C/T (JO) and 898C/G (HY* 1/HY* 2) single nucleotide polymorphisms. In order to confirm the results sequence analysis was also performed. **Results:** When samples of these donors were analyzed, a novel allele combination, the DO* A allele (793A and 323G)

associated with 898G was identified and designated as DO* A-WL allele. This new allele encoding 300Val is the same as HY* 1 at nucleotide 898 on the molecular background of DO* A. Among the 556 alleles analyzed by PCR-RFLP, 3 were DO* A-WL and 78 were DO* B-WL. This represents an overall frequency of 0.5% for DO* A-WL and 14% for DO* B-WL across the population studied. **Conclusion:** Molecular screening of Brazilians revealed one novel allele, the DO* A-WL. Our data highlight the importance of testing a cohort of different populations to determine DO haplotypes and to establish reliable genotyping tests for predicting Do^a/Do^b status. J. Clin. Lab. Anal. 25:79–82, 2011. © 2011 Wiley-Liss, Inc.

Key words: molecular assays; Dombrock alleles; Brazilians

INTRODUCTION

The Dombrock blood group system is composed of the major antigens Do^a and Do^b and the high-incidence antigens Hy and Jo^a that are closely related at the phenotypic level and are carried on a glycosylphosphatidylinositol (GPI)-linked glycoprotein. The Gy(a–) phenotype, associated with Hy-negative and Jo(a–), represents the null phenotype. The Hy-negative phenotype is associated with weak expression of Do^b and Jo^a antigens, whereas Jo(a–) is associated with weak expression of the Do^a and Hy antigens (1–5). The Dombrock antibodies are weakly reactive and are generally found in serum samples with multiple antibodies; moreover, the antigens can be weakly expressed on the red blood cell membrane (6–8). Therefore, Dombrock typing has severe limitations because the antibodies (especially anti-Do^b) are rarely available as a single specificity with strength and volume to make accurate typing possible (7). As the molecular basis of the Dombrock antigens

has been determined, the ability to distinguish DO* A and DO* B makes it possible to type donors and patients (9,10). Antigens in the Dombrock blood group system are carried on the Dombrock glycoprotein, which is encoded by the DO gene, also known as ART4 (4). The molecular bases associated with the various Do phenotypes have been determined to be due to single nucleotide polymorphisms (2,4,9). The molecular background of the null phenotype is varied, with four molecular causes responsible for this variation (11–13). There are two forms of the allele encoding the Hy

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*Correspondence to: Wilson Baleotti Jr., Hemocentro, FAMEMA, Rua Lourival Freire 240, CEP: 17519-050, Fragata C, Marília, São Paulo, Brazil. E-mail: baleotti@famema.br

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negative phenotype, one with 898 G (named *HY1*) encoding 300Val and the other with 898 C (designed *HY2*) encoding 300Leu (2). It has been suggested that the 898 C/G single nucleotide polymorphism (SNP) was a possible reason for the observed weakened expression of Gy^a and Do^b on red blood cells from *HY1* homozygous individuals (2), but specific studies addressing this issue have not been conducted. In a previous study, we found a high frequency (13.4%) of 898G not invariably associated with the presence of *HY*1* allele on the molecular background of *DO*B* allele, and this new allele was designated *DO*B-WL* allele (14).

Silent polymorphisms have also been previously associated with either *DO*A* or *DO*B* alleles, which are now recognized as simply variation within the *DO* gene (2,4,10). Three new *DO* alleles—*DO*B-SH*, *DO*A-HA* and *DO*A-SH*—were identified using a bead array format for typing alleles associated with blood group antigens (14,15). Molecular *DO* screening in a native Congolese cohort revealed a high frequency of *HY* and *JO* alleles and two novel alleles, one on the *DO*B* (or *DO*B-WL*) and one on the *DO*B-SH* (16). Some SNPs associated with *DO* alleles are given in Table 1.

Determination of the molecular basis underlying the antigens in the Dombrock blood group system has shown that the allelic diversity is greater than expected. Based on this knowledge, and in our previous finding that 898G is not invariably associated with the presence of *HY*1* allele (14) we performed a systematic nucleotide analysis of *DO* alleles on DNA samples from Brazilian blood donors. During this analysis a novel allele combination, the *DO*A* allele (793A and 323G) associated with 898G was identified and designated as *DO*A-WL* allele.

MATERIALS AND METHODS

Blood Samples

Peripheral blood samples from 278 Brazilian blood donors, representing a high inter-racial mixture, including Europeans, Japanese, South Americans and African-Americans, were collected in EDTA after obtaining signed informed consent, as approved by the Institutional Review Board.

PCR-RFLP

PCR assays using plates of 96 wells were performed on 278 DNA samples from Brazilian blood donors using 100–200 ng of DNA, 10 pmole of each primer, 0.8 nmole of each dNTP, 0.2 U of *Taq* DNA polymerase, and buffer, in a total volume of 20 µl. For each assay, PCR amplification was performed with the same reaction and cycling conditions as described previously, except for the annealing temperature (2,14). Amplified DNA fragments were digested with four different restriction enzymes (*Eam*I 105I for the polymorphism at nucleotide 793, *Bsa*II for the polymorphism at nucleotide 323, *Xcm*I for the polymorphism at nucleotide 350, and *Alw*26I for the polymorphism at nucleotide 898), according to the conditions recommended by the manufacturers, and were analyzed on 3% agarose gels after being stained with ethidium bromide.

Sequence Analysis

In order to confirm the new allele result obtained by PCR-RFLP, three samples carrying the *DO*A-WL* allele were sequenced. We also sequenced six samples

TABLE 1. Some SNPs Associated With *DO* Alleles

Allele	Nucleotides								Ref.
	NT (aa) 323 (108)	NT (aa) 350 (117)	NT 378	NT (aa) ⁺ 445 (149)	NT (aa) ⁺ 524 (175)	NT 624	NT (aa) 793 (265)	NT (aa) 898 (300)	
<i>DO*A</i>	G(Gly)	C(Thr)	C	C(Gln)	T(Ile)	T	A(Asn)	C(Leu)	(2)
<i>DO*B</i>	G(Gly)	C(Thr)	T	C(Gln)	T(Ile)	C	G(Asp)	C(Leu)	(2)
<i>HY*1</i>	T(Val)	C(Thr)	C	C(Gln)	T(Ile)	C	G(Asp)	G(Val)	(2)
<i>HY*2</i>	T(Val)	C(Thr)	C	C(Gln)	T(Ile)	C	G(Asp)	C(Leu)	(2)
<i>JO</i>	G(Gly)	T(Ile)	T	C(Gln)	T(Ile)	T	A(Asn)	C(Leu)	(2)
<i>DO*A-SH</i>	G(Gly)	C(Thr)	C	C(Gln)	T(Ile)	C	A(Asn)	NT	(14)
<i>DO*B-WL</i>	G(Gly)	C(Thr)	T	C(Gln)	T(Ile)	C	G(Asp)	G(Val)	(14)
<i>DO*A-HA</i>	G(Gly)	C(Thr)	T	C(Gln)	T(Ile)	T	A(Asn)	C(Leu) ⁺	(15)
<i>DO*B-SH</i>	G(Gly)	C(Thr)	C	C(Gln)	T(Ile)	C	G(Asp)	C(Leu) ⁺	(15)
<i>DO*B-SH-Q149 K</i>	G(Gly)	C(Thr)	C	A(Lys)	T(Ile)	C	G(Asp)	C(Leu)	(16)
<i>DO*B-I175 N</i>	G(Gly)	C(Thr)	T	C(Gln)	A(Asn)	C	G(Asp)	C(Leu)	(16)
<i>DO*A-WL</i>	G(Gly)	C(Thr)	C	C(Gln)	T(Ile)	T	A(Asn)	G(Val)	Novel allele

NT—not tested, Highlighted row—*DO*A-WL* molecular basis, Nucleotides 378 and 624—silence SNPs recognized as simply variation within the *DO* gene.

⁺Nucleotide analysis published in reference (16).

carrying the *DO***A*, *DO***B*, *DO***A-WL* and *DO***B-WL* alleles in order to identify the polymorphisms 445C>A and 524 T>A recently reported (16). *DO***A-WL* samples were amplified with primers DoEx3F/DoEx3R located in the intronic region flanking the exon 3 (2,14) and the six samples with different *DO* genotypes were amplified with primers DoX2f/DoABR located in exon 2.

PCR products were purified by elution from 1% agarose gels by using a gel extraction kit (Qiaex II, Qiagen, Valencia, CA) and were sequenced directly on a sequencer (3130, with Big Dye reagent BD Half-term, Applied Biosystems, Foster City, CA).

RESULTS

PCR-RFLP Results

When samples of these 278 Brazilian blood donors were analyzed, a novel allele combination, the *DO***A* allele (793A, 323G and 350C) associated with 898G (previously described on molecular background of the *HY**1 and *DO***B-WL* alleles) was identified and designated as the *DO***A-WL* allele. This new allele encoding 300Val is the same as *HY**1 or *DO***B-WL* at nucleotide 898 on the molecular background of *DO***A*. Among the 556 alleles analyzed by PCR-RFLP, 3 were *DO***A-WL* and 78 were *DO***B-WL*. This represents an overall frequency of 0.5% for *DO***A-WL* and 14% for *DO***B-WL* across the population studied.

Sequencing Results

Sequencing studies on three DNA samples that displayed the *DO***A-WL* allele revealed the presence of 898G associated with 793A (*DO***A* allele) confirming previous results obtained by PCR-RFLP (Fig. 1). Sequencing on six samples with different *DO* alleles used to identify the polymorphisms 445C>A and 524 T>A were all 445C and 524T (Fig. 1).

DISCUSSION

The Dombrock blood group system has been recognized for several genetic polymorphisms, and this characteristic is very interesting. Since identification of the Dombrock protein and its molecular bases, many polymorphisms have been identified. We confirmed results on frequencies of *DO* alleles in Brazilian blood donors found in previous report and observed the occurrence of the previously described allele (*DO***B-WL*) (14,17). During this study, we found another novel *DO* allele, namely *DO***A-WL*, characterized by the association of the SNP 793A, 378C and 624T (*DO***A* allele) with SNP 898G (like *HY**1 and *DO***B-WL*). It was postulated that 898G (which encodes 300Val within the sequence motif for GPI linkage) may cause reduced efficiency of protein anchoring to the red blood cell membrane, thereby weakening the expression of Gy^a and Do^b antigens (2) The frequency of *DO***A-WL* was

Fig. 1. (A) Identification of the polymorphisms 378T, 445C, 524T and 624C on a *DO***B-WL/DO***B-WL* sample. (B) Partial sequence diagram of exons 2 and 3 to show the polymorphisms 378C, 445C, 524T and 624T the presence of 898G on a *DO***A* sample (*DO***A-WL*).

demonstrated to be 0.5% and were found in three heterozygous samples, DO^*A/DO^*A-WL .

Determination of the molecular basis underlying the antigens in the Dombrock blood group system has shown various rearrangements between the alleles associated with DO^*A and DO^*B . The various SNPs combinations could be explained as a normal variation within the DO gene and therefore they were expected in a genetically diverse group of individuals, like the Brazilian population.

In our sequence analysis of six samples with different DO genotypes (1 DO^*A/DO^*A , 1 DO^*B/DO^*B , 1 DO^*B/DO^*B-WL , 1 DO^*B-WL/DO^*B-WL AND 2 DO^*A/DO^*A-WL) we did not find the polymorphisms 445A and 524A as previously identified in a Congolese cohort (16). This finding suggests that these polymorphisms are not necessarily associated to DO^*A-WL or DO^*B-WL alleles.

In summary, we found another novel DO allele, namely DO^*A-WL , probably the antithetical allele of the DO^*B-WL , previously described (14). These findings demonstrate high heterogeneity of DO alleles in the Brazilian population and highlight the importance of testing DNA samples from distinct ethnic groups to achieve a better understanding of the genetic basis associated with blood group antigens. We demonstrated the possibility to employ PCR-RFLP based assays using plates not only to screen DO alleles (DO^*A , DO^*B , $HY1$, $HY2$, JO and now, DO^*B-WL and DO^*A-WL) but also to screen DO blood group SNPs that can identify new alleles in donor cohorts.

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