

## Dombrock genotyping in Brazilian blood donors reveals different regional frequencies of the HY allele

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**Background:** Dombrock blood group system genotyping has revealed various rearrangements of the Dombrock gene and identified new variant alleles in Brazil (i.e., DO\*A-SH, DO\*A-WL and DO\*B-WL). Because of the high heterogeneity of the Brazilian population, interregional differences are expected during the investigation of Dombrock genotypes.

**Objective:** The present study aims to determine the frequencies of Dombrock genotypes in blood donors from Minas Gerais and compare the frequencies of the HY and JO alleles to those of another population in Brazil.

**Methods:** The frequencies of the DO alleles in Minas Gerais, a southeastern state of Brazil, were determined from the genotyping of 270 blood donors. Genotyping involved polymerase chain reaction and restriction fragment length polymorphism analysis to identify the 323G>T, 350C>T, 793A>G, and 898C>G mutations, which are related to the HY, JO, DO\*A/DO\*B, and DO\*A-WL/DO\*B-WL alleles, respectively. Moreover, the frequencies of rare HY and JO alleles were statistically compared using the chi-square test with data from another Brazilian region.

**Results:** The HY allele frequency in Minas Gerais (2.4%) was almost twice that of the JO allele (1.5%). The frequency of the HY allele was significantly higher ( $p$ -value = 0.001) than that in another Brazilian population and includes a rare homozygous donor with the Hy- phenotype. In addition, the DO\*A-WL and DO\*B-WL alleles, which were first identified in Brazil, were found in the state of Minas Gerais.

**Conclusions:** The data confirm that the frequencies of DO alleles differ between regions in Brazil. The population of Minas Gerais could be targeted in a screening strategy to identify the Hy- phenotype in order to develop a rare blood bank.

**Keywords:** Blood donors; Polymerase chain reaction; Genotyping techniques; Blood group antigens; Phenotype; H-Y antigen/blood; Polymorphism, restriction fragment length; Alleles; Brazil

## Introduction

The Dombrock (DO) blood group system (ISBT 014) was first described in 1965; since then, several studies have revealed its complexity<sup>(1)</sup>. The analysis of gene polymorphisms was made possible after the DO2 gene was cloned and sequenced (GenBank accession number: AF 290204). Genotyping is an important tool for predicting Dombrock phenotypes. DNA analysis can explain some observed variations in reaction strength during the serologic characterization of different phenotypes and unusual antibody production<sup>(1)</sup>.

Single nucleotide polymorphisms (SNPs) in DNA are correlated with seven different antigens of this system: Doa, Dob, Hy, Joa, Gya, DOYA and DOMR<sup>(2-5)</sup>. Do antigens are carried on a 47- to 58-kDa glycoprotein attached to the red blood cell membrane via a glycosylphosphatidylinositol (GPI) bond<sup>(6)</sup>. Individuals with the null phenotype or Gy-(a) have no Dombrock antigens expressed in the red blood cell membrane; at least five molecular alterations have been described<sup>(7-10)</sup>.

Several authors have emphasized the importance of regional studies aiming to identify Dombrock genotypes because of the high variability of alleles (Table 1). In Brazil, three new alleles have already been identified: DO\*B-WL (898C>G), DO\*A-SH (624T>C), and DO\*A-WL (898C>G)<sup>(5,11)</sup>. In addition, molecular studies of the DO alleles revealed that JO is more common than HY in Brazil, whereas HY is more prevalent in New York<sup>(12)</sup>.

The 898G SNP in the DO\*B-WL and DO\*A-WL alleles in the heterogeneous Brazilian population demonstrates that this polymorphism is not restricted to one racial group. This polymorphism was previously identified only in association with the 323T SNP in the HY\*1 variant allele and was described as a mutation apparently restricted to black African individuals<sup>(13)</sup>.

Such diversity has also been observed in Africans tribes<sup>(14)</sup> after the identification of the variant alleles, DO\*B-SH-Q149K (445C>A) and DO\*B-I175N (524T>A), in which the protein contains altered nucleotides at different positions. Two other variant alleles, DO\*B-SH (624T>C) and DO\*A-HA (378C>T), were identified in a cohort comprising various ethnic groups including blood donors from New York<sup>(15)</sup>.

Table 1 - List of Dombrock alleles and their polymorphisms

Allele	Nucleotide								References
	323	350	378*	445	524	624*	793	898	
Position	323	350	378*	445	524	624*	793	898	
Amino acid position in resulting protein	(108)	(117)	(126)	(149)	(175)	(298)	(265)	(300)	
DOA	G (Gly)	C (Thr)	C	C	T (Ile)	T	A (Asn)	C	Gubin et al. <sup>(6)</sup>
DOB	G (Gly)	C (Thr)	T	C	T (Ile)	C	G (Asp)	C	Gubin et al. <sup>(6)</sup>
JO	G (Gly)	T (Ile)	T	C	T (Ile)	C	A (Asn)	C	Rios et al. <sup>(8)</sup>
HY1 <sup>†</sup>	T (Ile)	C (Thr)	C	C	T (Ile)	G	G	G (Val)	Rios et al. <sup>(8)</sup>
HY2	T (Ile)	C (Thr)	C	C	T (Ile)	C	G (Asp)	C	Rios et al. <sup>(8)</sup>
DOA-WL <sup>‡</sup>	G (Gly)	C (Thr)	NT	NT	NT	NT	A (Asn)	G (Val)	Baleotti et al. <sup>(5)</sup>
DOB-WL <sup>‡</sup>	G (Gly)	C (Thr)	T	C (Gln)	T (Ile)	C	G (Asp)	G (Val)	Baleotti et al. <sup>(11)</sup>
DOA-SH <sup>‡</sup>	G (Gly)	C (Thr)	C	C (Gln)	T (Ile)	C	A (Asn)	NT	Baleotti et al. <sup>(11)</sup>
DOB-SH <sup>‡</sup>	G (Gly)	C (Thr)	C	C (Gln)	T (Ile)	C	G (Asp)	C (Leu)	Hashmi et al. <sup>(15)</sup>
DOA-HA <sup>‡</sup>	G (Gly)	C (Thr)	T	C (Gln)	T (Ile)	T	A (Asn)	C (Leu)	Hashmi et al. <sup>(15)</sup>
DOB-SH-Q149K <sup>‡</sup>	G (Gly)	C (Thr)	C	A (Lys)	T (Ile)	C	G (Asp)	C (Leu)	Chapel-Fernandes et al. <sup>(14)</sup>
DOB-I175N <sup>‡</sup>	G (Gly)	C (Thr)	T	C (Gln)	A (Asn)	C	G (Asp)	C (Leu)	Chapel-Fernandes et al. <sup>(14)</sup>

\*Silent SNP, <sup>‡</sup>variant alleles. NT: Not tested

## Objective

Considering Brazilian miscegenation and the variability of the DO gene, the present study aims to determine the frequencies of Dombrock genotypes in blood donors from Minas Gerais with combinations of the following alleles: DO\*A (SNP 793A), DO\*B (SNP 793G), DO\*A-WL (SNP 793A, 898G), DO\*B-WL (SNP 793G, 898G), HY\*1 (SNP 323T, 898G), HY\*2 (SNP 323T, 898C), and JO (SNP 350T). In addition, it aims to compare the frequencies of the HY and JO alleles, which are related to the rare Hy- and Jo(a-) phenotypes, to those of another population in Brazil to identify possible regional differences in the frequencies of these alleles in order to guide the search for rare blood donors.

## Methods

Samples of peripheral venous blood were collected in EDTA from 270 randomly selected blood donors from the Blood Center of Belo Horizonte, Minas Gerais. Subjects provided informed consent and the study was approved by the institutional review board. The sample size was calculated assuming a significance level of 5% and 80% power in relation to the prevalence of the DO\*B-WL allele which has previously been identified in a Brazilian population<sup>(11)</sup>.

Genomic DNA was extracted from the leukocytes of blood samples using Ilustra TM blood genomicPrep Mini Spin Kit from GE Healthcare (Buckinghamshire, UK) according to the manufacturer's instructions.

The segments of the gene were amplified in a thermocycler (CT-412, Barloworld Scientific Techno, UK) using three pairs of primers<sup>(13)</sup> under the following conditions: 95°C for 5 minutes; 35 cycles at 94°C for 20 seconds, 62°C for nt 793, 58°C for nt 323 and 350, or 55°C for nt 898 for 20 seconds followed by 72°C for 20 seconds; and finally 72°C for 10 minutes. The polymerase chain reaction used 100 ng of DNA, 2.0 pmol of each primer, 200 M

of each dNTP, 3.5 mM of MgCl<sub>2</sub>, 0.8 U of Taq DNA polymerase, and buffer in a final volume of 20 µL. The amplification products were digested by restriction endonucleases to identify the following gene polymorphisms: Eam1105 I (MBI Fermentas®, NY, USA) for 793A>G (DO\*A/DO\*B), BseDI (MBI Fermentas, NY, USA) for 323G>T (HY), XcmI (New England Biolabs®, Ipswich, USA) for 350C>T (JO), and Alw26I (MBI Fermentas, NY, USA) for 898C>G (HY\*1, DO\*A-WL and DO\*B-WL). The concentrations of restriction enzymes and incubation temperatures were in accordance with the manufacturers' recommendations. For identification, the fragments of the enzymatic digestion were electrophoresed in 3% agarose gel, stained with ethidium bromide and revealed by ultraviolet light. The primer sequences, amplification products, and sizes of restriction fragments are listed in Table 2.

The statistical significance between the frequencies of the HY and JO alleles in Minas Gerais as well as a previous population analysis in Brazil was established using the Chi-square test for each allele.

## Results

The results of the Dombrock genotyping in blood donors from Minas Gerais are shown in Table 3.

The frequencies of the variant alleles, DO\*A-WL and DO\*B-WL, in a Brazilian population were previously reported to be 0.5% and 14%, respectively<sup>(5)</sup>. In the present population, the frequencies of DO\*A-WL and DO\*B-WL were approximately 0.7% and 7%, respectively. However, it was impossible to differentiate the genotypes DO\*A/DO\*B-WL and DO\*B/DO\*A-WL in 19 samples (7.04%) because the 898G SNP was associated with only one of these alleles.

The JO allele was heterozygous for the DO\*B and DO\*A alleles. In contrast, the HY allele was homozygous (HY\*1/HY\*1) in one sample.

The frequency of the HY allele in donors from Minas Gerais (2.4%) was significantly higher than previously reported

Table 2 - Primers used for polymerase chain reaction and product sizes after enzymatic restriction

Primer	Sequence: 5' to 3'	Uncut size (bp)	Enzyme	Restriction fragment size (bp)
DoX2F	5'-CACTTTAATGCCTACACAGGGACCACCAGTCGA-3'			
Do378R	5'-ATGTGCTCAGGTTCCCAGTTGACCTCAACGACAAC-3' (annealing temperature: 62°C)	257	<i>Eam</i> 1105	230, 27 196, 34 (DO*A) (DO*B)
DoEx3F	5'-TCAGTACCAAGGCTGTAGCA-3'			
DoEx3R	5'-AGTAAAGTCAGAATGAACATTGCTGCACAAT-3' (annealing temperature: 58°C)	220	<i>Bse</i> DI <i>Xcm</i> I	120, 92, 8 212, 8 (Wild type) (Variant) 167, 53 220 (Wild type) (Variant)
DoEx3F	5'-TCAATGGATAGATGAGGTAG-3'			
DoEx3R	5'-TGGTTTCAGCAGAAGTATGA-3' (annealing temperature: 55°C)	291	<i>Alw</i> 26I	291 170, 121 (Wild type) (Variant)

bp: base pairs

in another Brazilian population (0.7%) (p-value = 0.001)<sup>(12)</sup>. Moreover, a rare homozygous HY allele was identified.

There was no significant difference between the present and previous studies regarding the frequency of the JO allele (1.5% vs. 1.75; p-value = 0.735)<sup>(12)</sup>. In the previous study, the frequency of JO was approximately twice that of HY. However, in the present study in Minas Gerais, the frequency of the HY allele was higher (62%) than that of the JO allele (38%) only considering the total frequency of these two alleles.

### Discussion

This is the first report about regional differences in the frequencies of DO alleles in blood donors in Brazil. The results highlight the necessity of local studies in highly heterogeneous populations.

The Brazilian population has extremely mixed ancestry, which can be explained by miscegenation between Africans brought as slaves, European settlers, and native Indians. When molecular markers are evaluated, the contribution of each of these groups in Brazilian ancestry varies by region<sup>(16)</sup>. The huge size of Brazil and internal migration may also explain this heterogeneity.

Since monoclonal antibodies are unavailable for the routine hemagglutination technique, advances in molecular techniques have led to a better understanding of the DO blood group system and the inference of its phenotypes. Furthermore, unlike hemagglutination, molecular techniques enable the screening of large numbers of donors to find rare phenotypes.

This methodology revealed three novel DO alleles in the Brazilian population<sup>(5,11)</sup>. Although no new alleles were identified in the present study, the regional differences found in the frequencies of the rare HY allele highlight the expressive heterogeneity of the Brazilian population.

The Hy antigen is present in over 99% of black African individuals and 100% of individuals in most other populations; its absence, which is associated with the presence of the HY allele, is rare<sup>(13)</sup>. At least one case of active hemolysis by anti-Hy antibodies has been reported<sup>(17)</sup>. Moreover, the presence of a homozygous status reinforces the importance of including the 323G>T SNP in genotype screening protocols in Minas Gerais.

Table 3 - Dombrock genotyping results

Genotype	Nucleotide				Total (%)
	323	350	793	898	
DOA/DOB	G/G	C/C	A/G	C/C	87 (32.22)
DOB/DOB	G/G	C/C	G/G	C/C	80 (29.63)
DOA/DOA	G/G	C/C	A/A	C/C	25 (9.26)
DOB/DOB-WL	G/G	C/C	G/G	C/G	32 (11.85)
DOA/DOA-WL	A/A	C/C	G/G	C/G	4 (1.48)
DOB-WL/DOB-WL	G/G	C/C	G/G	G/G	3 (1.11)
DOA/DOB-WL (or DOA-WL/DOB)	G/G	C/C	A/G	C/G	19 (7.04)
HY1/DOA	T/G	C/C	G/A	G/C	6 (2.22)
JO/DOB	G/G	T/C	A/G	C/C	5 (1.85)
JO/DOA	G/G	T/C	A/A	C/C	3 (1.11)
HY1/DOB	T/G	C/C	G/G	G/C	3 (1.11)
HY2/DOB	T/G	C/C	G/G	C/C	2 (0.74)
HY1/HY1	T/T	C/C	G/G	G/G	1 (0.38)
Total					270 (100.00)

Although the Hy- phenotype has been described in people of African descent, the high frequency of the HY allele in Minas Gerais indicates the relatively higher probability of finding an individual homozygous for this allele, making this donor population a target for investigating the Hy- phenotype. However, self-identification does not accurately predict the degree of African ancestry of an individual and should not be applied as a variable in the screening of rare Hy- phenotypes in the mixed Brazilian population.

### Conclusion

The present study shows that donors from Minas Gerais are more similar to African-American donors from New York than the other previously investigated Brazilian population in respect to the frequency of the HY allele. Therefore, we can conclude that comparisons should be made on the basis of the Brazilian region investigated and not the entire country.

Furthermore, the presence of the DO\*A-WL and DO\*B-WL variant alleles in donors from Minas Gerais justify the inclusion of the 898C/G SNP in genotype screening protocols for this population.

In routine transfusions, compatibility between donors and recipients prevents both the appearance of antibodies and immune hemolytic conditions when these antibodies are already present. From the perspective of developing a rare blood bank, the present data highlight the relevance of using particular protocols for DO genotyping established from local population studies to identify rare phenotypes.

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