Original article

Genotyping of Dombrock and Lutheran blood group systems in blood donors from the southwestern region of the state of Paraná, Southern Brazil

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\textbf{ABSTRACT}

Background: Lutheran and Dombrock are two blood group systems with low immunogenic antigens; they can cause mild-to-moderate transfusion reactions. For both, immunophenotyping is not performed in the pretransfusion routine in Brazil. In addition, the distribution of their antigenic frequencies is an important marker of ethnicity. Thus, the goal of this study was to carry out the genotyping of the LU\textsuperscript{01}, LU\textsuperscript{02}, DO\textsuperscript{01} and DO\textsuperscript{02} alleles of the Lutheran and Dombrock blood group systems in blood donors from the southwestern region of the state of Paraná, Southern Brazil.

Method: Genotyping was performed for 251 blood donors by specific allele-polymerase chain reaction. The genotype and allele frequencies were obtained through direct counting and compared with other Brazilian populations using the chi-square test with Yates correction.

Results: The distribution of genotype frequencies for LU were 0.4% for LU\textsuperscript{01}/LU\textsuperscript{01}, 6.8% for LU\textsuperscript{01}/LU\textsuperscript{02} and 92.8% for LU\textsuperscript{02}/LU\textsuperscript{02} and for DO, they were 19.9% for DO\textsuperscript{01}/DO\textsuperscript{01}, 44.6% for DO\textsuperscript{01}/DO\textsuperscript{02} and 35.5% for DO\textsuperscript{02}/DO\textsuperscript{02}. The allele and genotype frequencies of LU and DO were similar to those expected for Caucasians, but the DO\textsuperscript{01}/DO\textsuperscript{01} genotype frequency was different to other Brazilian populations. The rare LU\textsuperscript{01}/LU\textsuperscript{01} genotype was found in a loyal blood donor.

Conclusion: The genotyping techniques allowed the evaluation of the LU\textsuperscript{01}, LU\textsuperscript{02}, DO\textsuperscript{01} and DO\textsuperscript{02} alleles in blood donors registered in the Hemotherapy Center of the southwestern region of Paraná, Southern Brazil, and contributed to a genotyped blood donor database.

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Introduction

Erythrocyte blood group antigens are carried by surface proteins and glycoproteins on the red blood cell (RBC) membrane lipid bilayer. These antigens are polymorphic and play important biological functions, including ion transport, red cell structure, cytokine chemotaxis, ligand of complement proteins and others. Knowing RBC antigens is essential in blood transfusion practices, because their immunogenicity is related to the formation of alloantibodies and adverse clinical reactions. Currently, 346 RBC antigens have been described with most of them being distributed in 36 blood group systems. New antigens of blood group systems are always being identified (http://www.isbtweb.org).

Immunophenotyping is routinely used to identify RBC antigens and has high specificity and sensitivity. However, some limitations prevent its use, such as in the presence of RBCs from a donor in a recipient’s circulation, when RBCs are coated with alloantibodies or autoantibodies, as well as the lack of commercial antisera. In these situations, genotyping is an important tool: the characterization of the genes that codify RBC antigens and their variants allows phenotype inference with a high degree of precision. Two blood group systems that express RBC antigens with low immunogenicity and are involved in mild-to-moderate transfusion reactions and mild hemolytic disease of the fetus and newborn are the Lutheran and Dombrock blood groups. The frequencies of RBC antigens vary in different populations and ethnic groups with the distribution of the Dombrock genotype being useful as a genetic marker. Because the Brazilian population has great miscegenation, and its colonization varies according to region, the knowledge of the distribution of these RBC antigens could contribute to the understanding of the genetic constitution of Brazilians and to build a representative blood bank.

The Lutheran blood group system (LU - ISBT 005) comprises 22 antigens of a glycoprotein known as basal cell adhesion molecule (BCAM). BCAM is a 597-amino acid protein, belonging to the Ig superfamily transmembrane receptor for laminin α5. This protein is coded by the LU gene located on chromosome 19q13.2, which contains 12.5 kilobases and 15 exons. The molecular basis for most LU’01 and LU’02 alleles is the result of a single nucleotide polymorphism (SNP) 230 G>A, which results in the substitution of the amino acid at position 77 (exchange of arginine by histidine) of the glycoprotein. This glycoprotein is widely expressed in tissues.

The Dombrock blood group system (DO - ISBT 014) consists of seven antigens of a 314-amino acid glycoprotein linked to the RBC membrane by glycosylphosphatidylinositol (GPI). The ART4 gene located on chromosome 12p13.2-p12 encodes a Dombrock glycoprotein (ADP-ribosyltransferase: DO, ART4, CD297) which expresses eight different antigens. The DO’01 and DO’02 alleles differ in three nucleotides in exon 2 at the positions 376C>T, 624T>C and 793A>G. The first two polymorphisms are silent, while the 793 A>G SNP encodes the substitution of a asparagine amino acid for aspartic acid at position 265 of the protein characterizing the respective antiesthetic antigens Doa and Doβ. These antigens are mainly expressed in the membranes of RBCs and lymphocytes.

Considering that there are no genotyping studies for the LU and DO blood group systems in the southwestern region of the state of Paraná, and their importance, the objective of this study was to genotype the LU’01, LU’02, DO’01 and DO’02 alleles in blood donors registered in the Hemotherapy Center of the southwestern region of Paraná, Southern Brazil.

Methods

This study was conducted in accordance with the standards recommended by the Ethics Committee of the Universidade Estadual de Maringá (UEM), (P 581/2011 – COPEP) according to the Resolution of the Brazilian Health Council (CNS 466/12) and all subjects gave their informed consent.

Patients

Two hundred and fifty-one unrelated red blood donors of the Hemonclúeo Regional de Francisco Beltrão in the southwestern region of Paraná participated in this study. They were enrolled from September 2012 to July 2013. Fifty-one per cent were males and the mean age was 34.35 ± 10.41 years. The state of Paraná is located in the southern region of Brazil, between 22°29’30”–26°42’59”S and 48°02’24”–54°37’38”W. Paraná’s ethnic constitution was previously described with the majority being White of European origin (80.6%), with a small contribution of African (12.5%) and Amerindian (7.0%) genes. However, a larger percentage of individuals declared themselves as White in the southwestern region of the state (88%) vs. 80.6%). The high prevalence of Caucasians in this area is explained as this region was colonized by descendants from Italians, Germans and Poles who first inhabited other Southern Brazilian regions, such as Rio Grande do Sul and Santa Catarina.

Serologic tests

RBC phenotyping for the Lutheran blood group system was performed using the ID-Perfil II gel card (Luα-Luβ-ctl) with monoclonal antibody, according to the manufacturer’s recommendations (Bio-rad®, California, USA). The RBCs were prepared at a ratio of 1:50 using Diluent 1 (bromelin solution).

Genotyping of the LU’01, LU’02, DO’01 and DO’02 alleles

Genomic DNA was extracted from peripheral blood collected in ethylenediaminetetraacetic acid (EDTA) using the salting-out technique. The concentration and quality of the DNA were analyzed by NanoDrop 2000® technology (Thermo Scientific, Wilmington, USA).

A polymerase chain reaction (PCR) technique was performed as previously described to genotype the LU’01, LU’02, DO’01 and DO’02 alleles. Human growth hormone (HGH) was used as an internal control for the reaction. The final volume of the reaction was 25 μL, constituted by 50 ng of DNA, 50 pmol of each primer, 1 nmol of each dNTP and 2.5 U of Taq DNA polymerase (Invitrogen®, Carlsbad, CA, USA). The amplification cycles were performed in a PCR 9700 System and Veriti thermal cyclers (Applied Biosystems, Foster City, CA, USA).
Table 1 – Sequences of primers used for the genotyping of the LU*01, LU*02, DO*01, and DO*02 alleles.

| Allele | Primer | Sequence                        |
|--------|--------|---------------------------------|
| LU*01  | LU     | antisense                       |
|        | LU*    | sense                           |
| LU*02  | LU     | antisense                       |
|        | LU*    | sense                           |
| DO*01  | DO-F   | sense                           |
|        | DO*    | antisense                       |
| DO*02  | DO-F   | sense                           |
|        | DO*    | antisense                       |

For the LU*01 and LU*02 alleles, the amplification cycles consisted of denaturation at 94 °C for 9 min and 32 cycles of 30 s at 94 °C, 30 s at 62 °C and 30 s at 72 °C, followed by an extension of 5 min at 72 °C. For the DO*01 and DO*02 alleles, the amplification cycles consisted of denaturation at 94 °C for 9 min and 32 cycles of 30 s at 94 °C, 30 s at 67 °C and 30 s at 72 °C, followed by an extension of 5 min at 72 °C. The analysis of PCR products was performed in 2% agarose gel, with SYBR Safe DNA Gel Stain (Invitrogen Life Technologies, Grand Island, NY, USA) in a SSP gel System Micro Vat (One Lambda®, Canoga Park, CA, USA). The primers used are shown in Table 1.

Statistical analysis

The genotype and allele frequencies were obtained by direct counting and recorded on Excel spreadsheets (Microsoft Office Excel 2010). The comparison between different populations was determined using the chi-square test with Yates correction or Fisher’s exact test using a 2 x 2 contingency table in the SISA software (http://www.quantitativeskills.com/sisa/statistics/twoby2-.htm). p-Values ≤0.05 were considered significant. The Hardy-Weinberg equilibrium15 was tested by calculating the expected genotype frequencies and comparing them to the observed values.

Results

The distributions of genotype frequency ratios for all analyzed genes were in Hardy-Weinberg equilibrium. For the Lutheran blood group system, 100% of concordance was found between immunohemotyping and genotyping and for Dombrock, genotyping was previously validated.14

The genotype and allele frequency distributions of the Lutheran and Dombrock in blood donors from the southwestern region of the state of Paraná are shown in Table 2. The distribution of the LU genotype frequencies were 0.4% for LU*01/LU*01, 6.8% for LU*01/LU*02 and 92.8% for LU*02/LU*02 and the allele frequencies were 4.0% for LU*01 and 96% for LU*02. For DO the genotype frequencies were 19.9% for DO*01/DO*01, 44.6% for DO*01/DO*02 and 35.5% for DO*02/DO*02 and the allele frequencies were 42% for DO*01 and 58% for DO*02. These frequencies were compared with other Brazilian populations16–18 (Table 2). Differences were observed for the DO*01/DO*01 genotype frequency which was higher in the populations from the southwestern region of the state compared to all other Brazilian populations. The rare LU*01/LU*01 genotype was found in a loyal blood donor.

Discussion

Identifying and understanding RBC antigen distribution is needed when one considers the great variability of allele and genotype frequencies in the different ethnic groups and regions. The Brazilian population is extremely heterogeneous resulting from crosses between people from different continents: Amerindians, Europeans and Africans.13 This study is the first to report the distribution of the LU and DO blood systems in the population of the southwestern region of Paraná, Southern Brazil.

The distribution of genotype frequencies for the LU system in this population was similar to the one expected for Caucasians and those found in other Brazilian populations. It was between 92% and 95% for the LU*02/LU*02 genotype or the Lu(a−b+) phenotype.16,17,20,21 It is important to highlight the fact that among the blood donors of the Hemonúcleo Regional de Francisco Beltrão who are considered loyal donors and give blood at least three times a year one had the LU*01/LU*01 genotype which is rare. The blood units of this rare genotype could be suitable for patients presenting the anti-LuB antibody. In Brazil, the Lu phenotype is not routinely investigated and the risk of LuA and LuB alloimmunization should be considered, because Lu incompatibility could cause late hemolytic reactions. Anti-LuB antibodies are rarely correlated to hemolytic disease of the fetus and newborn because the presence of the Lutheran glycoprotein of fetal origin in placenta tissue adsorbs the maternal anti-Lu antibodies.20,21 The null phenotype Lu(a−b−) was not found in this population. This rare phenotype [http://www.redcrossblood.org] was found in 2.6–3.15% of Iraq22 and Indian donors.23,24

The distribution of genotype frequencies for the DO system in this study (19.9% for DO*01/DO*01, 44.6% for DO*01/DO*02 and 35.5% for DO*02/DO*02) was similar to what is expected for Caucasians: 18% for DO*01/DO*01, 49% for DO*01/DO*02 and 33% for DO*02/DO*02.11 However, it was different to other Brazilian populations (p-value <0.05) where genotype frequencies are similar to those of Blacks (11% for DO*01/DO*01, 44% for DO*01/DO*02 and 45% for DO*02/DO*02).11 These findings can be attributed to a higher predominance of European descendants in the state of Paraná and more specifically in the southwestern region of Paraná when compared with the other Brazilian regions where African descendants played an impor-
Table 2 - Distribution of LU and DO genotype and allele frequencies in blood donors from the southwestern region of the state of Paraná and in other Brazilian populations.

| Genotype and allele | Inferred phenotype | Southwestern region of Paraná\(^a\) \(n = 251\) | Northeastern region of Brazil\(^b\) \(n = 196\) | São Paulo Brazil\(^c\) \(n = 948\) | Minas Gerais Brazil\(^d\) \(n = 192\) |
|---------------------|--------------------|---------------------------------|---------------------------------|----------------------|----------------------|
|                     |                    | \(f(n)\)                         | freq.                            | p-value              | freq.                | p-value              | freq.                | p-value              |
| Lutheran system     |                    |                                 |                                 |                      |                      |                      |                      |                      |
| \(LU^01/LU^01\)     | \(Lu(a+b-)\)       | 0.004 (1)                       | 0.090                           | 0.004                | 0.090                | 0.004                | 0.910                | 0.050                |
| \(LU^01/LU^02\)     | \(Lu(a+b+)\)       | 0.068 (17)                      | 0.910                           | 0.068                | 0.910                | 0.068                | 0.950                | 0.050                |
| \(LU^02/LU^02\)     | \(Lu(a-b+)\)       | 0.928 (233)                     | 0.928                           | 0.928                | 0.928                | 0.928                | 0.928                | 0.928                |
| \(LU^01\)           | \(Lu^a\)           | 0.04 (19)                       | 0.045                           | 0.004                | 0.045                | 0.004                | 0.020                |                      |
| \(LU^02\)           | \(Lu^b\)           | 0.96 (483)                      | 0.955                           | 0.020                | 0.955                | 0.020                | 0.980                |                      |
| Dombrock system     |                    |                                 |                                 |                      |                      |                      |                      |                      |
| \(DO^01/DO^01\)     | \(Do(a+b-)\)       | 0.199 (50)                      | 0.100                           | 0.001                | 0.140                | 0.03                 | 0.130                | 0.04                 |
| \(DO^01/DO^02\)     | \(Do(a+b+)\)       | 0.446 (112)                     | 0.430                           | 0.450                | 0.450                | 0.450                |                      |                      |
| \(DO^02/DO^02\)     | \(Do(a-b+)\)       | 0.355 (89)                      | 0.470                           | 0.410                | 0.410                | 0.410                | 0.420                |                      |
| \(DO^01\)           | \(Do^a\)           | 0.42 (212)                      | 0.315                           | 0.04                 | 0.370                | 0.360                |                      |                      |
| \(DO^02\)           | \(Do^b\)           | 0.58 (290)                      | 0.685                           | 0.630                | 0.630                | 0.640                |                      |                      |

freq.: allele or genotype frequency.

\(^a\) Southwestern region of Parana, Southern Brazil (this study).

\(^b\) The northeastern region of Brazil.\(^c\)

\(^c\) São Paulo, Southeast Brazil.\(^d\)

\(^d\) Minas Gerais, Southeast Brazil.
tantal role in the formation of the population. Differences in the distribution of various SNPs of the DO blood group system have been reported in blood donor samples from the state of São Paulo and Minas Gerais, demonstrating these patterns of regional differences in Brazil. These and the findings of this study suggest that the DO blood group system can be considered an ethnic marker. The anti-Do antigens and anti-Do antibodies have been reported in early and late hemolytic reactions, but although some neonates had a positive human antoglobulin test, there are no reports correlating anti-Do to hemolytic disease of the newborn. Low titers of Do antibodies added to other alloantibodies in plasma of multitransfused patients make it difficult to detect. Therefore, it is possible that transfusion reactions are not reported due to anti-Do.

LU and DO genotyping revealed allele and genotype frequencies similar to those for Caucasians. However, they showed frequency differences when compared with other Brazilian studies highlighting the ethnic mixture in the constitution of Brazilians. The inclusion of the investigation of LU and DO alleles could contribute to ensure transfusion safety, especially for multitransfused patients.

The presence of the low frequency genotype LU’01/LU’01 in individuals from the southwestern region of Paraná and the significant differences for the DO’01/DO’01 genotypes when compared with other Brazilian regions, alert to the need of including LU and DO genotyping. Rare or low frequency alleles and genotypes were previously found for the Rh, Kell, Duffy, Kidd and Diego blood group systems in this same population. Thus, our results contribute to the database of genotyped donors and could contribute to matching RBCs for patients with less common phenotypes.

**Conclusion**

The genotyping techniques evaluated the LU’01, LU’02, DO’01 and DO’02 alleles in blood donors registered in the Hemotherapy Center of the southwestern region of Paraná and contributed to a genotyped blood donor database.

**Conflicts of interest**

The authors declare no conflicts of interest.

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