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Duffy blood group system: New genotyping method and distribution in a Brazilian extra-Amazonian population

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ABSTRACT

Duffy blood group system is of interest in several fields of science including transfusion medicine, immunology and malariology. Although some methods have been developed for Duffy polymorphism genotyping, not all of them have been sufficiently described and validated, and all present limitations. At the same time, the frequency of Duffy alleles and antigens in some densely populated regions of the world are still missing.

In this study we present new tests for genotyping the major alleles of the Duffy blood system and describe Duffy alleles and antigens in blood donors and transfusion-dependent patients in Minas Gerais, Brazil. A simple and reproducible strategy was devised for Duffy genotyping based on real-time PCR that included SNPs rs12075 and rs2814778. No significant differences between the allele frequencies were observed comparing blood donors and patients. Among the blood donors, the phenotype Fy(a−b+) was the most common and the Fy(a−b−) phenotype, associated with populations of African descent, was remarkably less common among subjects who self-identified as black in comparison to other ethnoracial categories. However, the African ancestry estimated by molecular markers was significantly higher in individuals with the allele associated to the Duffy null phenotype. The genotyping method presented may be useful to study Duffy genotypes accurately in different contexts and populations. The results suggest a reduced risk of alloimmunization for Duffy antigens and increased susceptibility for malaria in Minas Gerais, considering the high frequency of Duffy-positive individuals.

1. Introduction

The Duffy blood group system was first described in 1950 [9] and since then it has been of interest to diverse fields of science such as transfusion medicine, genetics, evolutionary science, immunology, malariology and anthropology.

The Duffy blood group antigen is carried by a transmembrane glycoprotein encoded by the gene *DARC* (Duffy antigen receptor for chemokines), located on the long arm of chromosome 1 (1.q22–1.q23) [23]. This glycoprotein is expressed on erythroid and non-erythroid cells, such as neurones; endothelial cells from the brain; and kidney, spleen and lung epithelial cells [12].

The Duffy blood group has two major codominant alleles, *FY*A* and *FY*B*, differentiated by a base substitution (G125A, rs12075), resulting in the main blood group variants Fy^a and Fy^b, which differ by a single amino acid (Gly42Asp) [16,43]. Antibodies to Duffy antigens are usually clinically significant and have been reported to cause immediate and delayed haemolytic transfusion reactions [10] and haemolytic diseases of the foetus and newborns [15].

An additional polymorphism in the GATA promoter region of *DARC* gene (−46T > C, rs2814778), prevents the Duffy antigen's expression only on erythroid cells [17,43], resulting in the null phenotype Fy(a−b−). This polymorphism (FY^*B^{ES} allele) is haplotypically associated with *FY*B*, and its frequency is at or near-fixation in most sub-Saharan African populations, being common

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in populations with African ancestry, but is very rare outside Africa.

Since DARC is the main receptor for *Plasmodium vivax*, individuals with a null phenotype present resistance to malaria, suggesting that this allele has been the target of natural selection [13,14,24]. Moreover, DARC has been shown to be a receptor for chemokines [26], playing a crucial role in the regulation of circulating chemokine levels [18,33]. The absence of DARC on erythrocytes is also associated with lower neutrophil counts [34] and some studies have associated Duffy polymorphisms with susceptibility or resistance to diseases [2].

Considering the importance of the Duffy blood group system in several study fields, a number of studies have described the Duffy phenotype, genotype and allele frequencies in different regions of the world [14]. These studies includes groups of patients with malaria, periodontitis, chronic kidney disease, sickle cell disease and others [4,25,39,44]. While a number of studies have been conducted in healthy individuals, few studies have described Duffy frequencies in the Brazilian populations, which are characterised as highly admixed [4,8,30], and no study has considered the population from Minas Gerais (Southeast Brazil). Minas Gerais is localised in Brazilian extra-Amazonian region, which is considered a non-endemic area for malaria. The extra-Amazonian region consists of 18 states, and comprises more than 40% of the Brazilian territory and 86.6% of the country's population [21].

At the same time, although several molecular methods have been developed for Duffy polymorphism genotyping, not all of them have been sufficiently described and validated, and all present limitations [45].

In the present study we describe a new approach for Duffy polymorphisms genotyping based on real time PCR (TaqMan assay) and report Duffy alleles and genotypes frequencies in Minas Gerais blood donors with different proportions of African, European and Amerindian ancestry, and in transfusion-dependent patients. We also report Duffy phenotype frequencies in a large blood donor cohort taking into account participants' self-reported skin colour (ethnoracial classification adopted by the Brazilian Institute of Geography and Statistics). Furthermore, a discussion about Duffy system nomenclature is presented.

2. Methods

2.1. Population and samples

To standardise Duffy genotyping by real-time PCR, we analysed individual samples obtained from subjects previously phenotyped for antigens Fy^a/Fy^b and genotyped by PCR-restriction fragment length polymorphisms (PCR-RFLP) [22]. The polymorphism rs12075 was evaluated in 16 samples with the genotype *FY*A/FY*A* (G/G), 16 with *FY*A/FY*B* (G/A) and 16 *FY*B/FY*B* (A/A). The polymorphism rs2814778, was evaluated in 16 samples with the genotype *FY*B/B* (T/T), 16 with *FY*B/B^{ES}* (T/C) and 16 *FY*B^{ES}/FY*B^{ES}* (C/C). The assays' precisions were calculated as the coefficients of variation for cycle thresholds (Ct) from duplicate determinations for 8 samples that were positive for each genotype. After validation, the real-time PCR genotyping technique was used to analyse samples from 195 random blood donors.

Duffy alleles and genotypes frequencies were estimated from 388 transfusion-dependent patients. All these subjects were genotyped by PCR-RFLP at Fundação Hemominas/Minas Gerais-Brazil as part of pre-transfusion testing between 2005 and 2016 and the frequencies were calculated specifically for the present study. Sickle cell disease was the most frequent clinical diagnosis among these patients (34.28%), followed by myelodisplasic syndrome (9.79%) and onco-haematologic diseases (6.7%).

Also, Duffy phenotype frequencies were estimated in 33,195 voluntary blood donors who attended the Minas Gerais blood bank (Fundação Hemominas). Subjects were clustered by self-defined pre-established skin colour categories: 'Branco' (white), 'Pardo' (brown), 'Preto' (black), 'Amarelo' (yellow) and 'Indígena' (indigenous), which are the ethnoracial categories used by the Brazilian Institute of Geography and Statistics. The cohort of phenotyped blood donors is composed of repeat donors, since this is an inclusion criterion of Fundação Hemominas for extended blood group phenotyping. The ancestry of healthy blood donors from Minas Gerais, who represent the general population reasonably well, has previously been described as 33.8% African, 57.7% European and 8,4% Amerindian [38].

This study was approved by the Fundação Hemominas Ethics Committee.

2.2. Phenotyping of Duffy antigens

Duffy phenotype data were obtained from the database of blood donors at Fundação Hemominas and exported to SPSS (version 17) for statistical analysis. These phenotypes had been previously determined by indirect antiglobulin tests using anti-Fy^a and anti-Fy^b reagents and LISS/Coombs gel cards from 1996 to 2011 during routine pre-transfusion tests of the blood bank.

2.3. Genotyping of FY*A, FY*B and FY*B^{ES} alleles

Genotyping of SNPs rs12075 and rs2814778 by PCR-RFLP was performed according to previously described methods [3,22].

Two new assays were standardised for the detection of the SNPs rs12075 (A > G; *FY*B > FY*A*) and rs2814778 (−46T > C; *FY*B > FY*B^{ES}*) by real-time PCR in 20 μL reactions using the primers and probes described in Table 1. The assays used 1X PCR Genotyping Master Mix Kit (Applied Biosystems, Foster City, CA), 100 ng of DNA, 600 nM of primers, and 200 nM of each of the fluorescent minor groove binder probes specific for the alleles. Both reactions were performed in a real-time PCR system, model ABI 7500 Fast (Applied Biosystems, Foster City, CA) under the following conditions: a pre-amplification step of 60 °C for 30 min and 95 °C for 10 min, followed by 50 cycles at 92 °C for 15 s and 60 °C for 1.5 min, and a post-amplification step of 60 °C for 30 s. The analysis was performed using the Allelic Discrimination Plot software.

2.4. Estimation of individual ancestry

The ancestry analysis for a subset of 95 blood donors was done using a panel of 192 Ancestry Informative Markers developed for the Brazilian population [36]. The genotyping was performed by OpenArray Real-Time PCR (Applied Biosystems). The evaluation of genomic ancestry was conducted using the Admixture program [1], using a supervised approach for ancestry determination. The

Table 1
Primers and probes used for Duffy genotyping.

Oligos	Sequence	Region*/Allele
FY1	5′-CTGAGAACTCAAGTCAGCTG-3′	6468–6487
FY2	5′-AGGATGAAGAAGGGCAGTGC-3′	6608–6627
FYA-P	5′-VIC-CAGATGGAGACTATGGTG-3′	6437–6454
FYB-P	5′-FAM-ATGGAGACTATGATGCCA-3′	6440–6457
GATA-1	5′-CGTGGGGTAAGGCTTCCTGA–3′	5808–5827
GATA-2	5′–CTGTGCAGACAGTTCCCCAT–3′	5948–5967
GATA-W	5′-VIC-TTGGCTCTTATCTTGGA-3′	5871–5887
GATA-M	5′-FAM-TTGGCTCTTACCTTGG-3′	5871–5887

*NCBI Reference Sequence: NG_011626.3.
The primers were described by Tanaka et al. [42]. The probes were designed using the Primer Express Software (Applied Biosystems, Foster City, CA).