Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

# Duffy blood group genotypes among African-Brazilian communities of the Amazon region

**S.J.Q. Perna, G.L. Cardoso and J.F. Guerreiro**

Laboratório de Genética Humana e Médica, Departamento de Patologia, Centro de Ciências Biológicas, Universidade Federal do Pará, Belém, PA, Brasil
Corresponding author: J.F. Guerreiro
E-mail: joaofg@ufpa.br

Genet. Mol. Res. 6 (1): 166-172 (2007)
Received September 28, 2006
Accepted January 10, 2007
Published March 29, 2007

**ABSTRACT.** Duffy blood group genotype was studied in 95 unrelated subjects from four African-Brazilian communities of the Amazon region: Trombetas, Pitimandeua, Curiaú, and Mazagão Velho. Genotyping was performed using an allele-specific primer polymerase chain reaction technique for determining the three major alleles at FY blood group, and as expected, FY*O allele was the most common one, with frequencies ranging from 56.4% in Mazagão Velho to 72.2% in Pitimandeua, whereas the FY*O/FY*O genotype was found with frequencies between 32.3% in Mazagão Velho and 58.8% in Curiaú. Genotype and allele distributions in the four Amazonian communities are consistent with a predominantly African origin with some degree of local differentiation and admixture with people of Caucasian ancestry and/or Amerindians. These results reveal that the impact of the FY*O/FY*O genotype on the transmission and endemicity of the *vivax* malaria deserves to be investigated in full detail in an attempt to identify the contribution of host biological

 

factors and explain the non-homogeneous prevalence of malaria in the region expressed by its different levels of exposure.

**Key words:** Duffy blood group, DARC, African-Brazilians

## INTRODUCTION

The Duffy blood group locus is characterized by three main alleles: FY*A, FY*B, and FY*O. The FY*A and FY*B alleles are distinguished by a missense mutation, which results in a single amino acid difference (G125A>Gly42Asp) (Chaudhuri et al., 1995; Iwamoto et al., 1995; Mallinson et al., 1995; Tournamille et al., 1995b) and gives the common Fy(a+b-), Fy(a-b+) and Fy(a+b+) phenotypes in European and Asian populations (Issitt and Anstee, 1998). The FY*O allele, which corresponds to the Fy(a-b-) serological phenotype (i.e., the absence of Fy antigen), is due to a T-33C point mutation (Pogo and Chaudhuri, 2000) on the FY*B gene promoter, which abolishes the erythroid gene expression by disrupting a binding site for the GATA-1 erythroid transcription factor and results in the elimination of the transcription of FY mRNA in RBCs, but not in other cell types (Tournamille et al., 1995a). The FY*O allele is at or near fixation in most sub-Saharan African populations. However, it is very rare outside Africa, and the pattern of allele frequencies at FY locus has been attributed to a positive natural selection. This hypothesis is supported by the observation that individuals homozygous for the FY*O allele are completely resistant to *vivax* malaria (Miller et al., 1976), since *Plasmodium vivax* requires the presence of Duffy antigen receptor for chemokines on the RBC surface to be able to invade cells and cause disease. In Brazil, about 99% of the malaria cases are concentrated in the Amazonian area, where socioeconomic and environmental conditions favor the proliferation of Anopheles mosquitoes, the vector of the disease. Thus, malaria represents an important public health concern in the Amazon, being responsible for economic losses and contributing to the precarious health condition of the exposed populations, which are generally considered highly susceptible to infection by *Plasmodium*. Therefore, it is important to investigate biological parameters that may be related to malaria, such as the Duffy blood group, in view of the marked contribution of African blacks for the formation of the present-day Amazonian populations, as well as the existence of many remaining old quilombo or mocambo communities, settlements of fugitive slaves, mainly in the State of Pará.

## MATERAL AND METHODS

### Study population

The study was performed with 95 unrelated subjects from four African-Brazilian communities of the Amazon region: Trombetas (N = 29), Pitimandeua (N = 18), Curiaú (N = 17), and Mazagão Velho (N = 31). A brief description of these populations follows.

*Curiaú*

This semi-isolated community made up of African slave descendants is located in the metropolitan region of Macapá city, capital of the State of Amapá, in the northern region of Brazil, at about 0°; 51° W. From 1770 on, this *mocambo* constituted the point of convergence of runaway slaves of the region, including slaves from Dutch Guyana (modern-day Surinam) and French Guyana (Muggiati, 1990).

*Trombetas*

The blacks of the Trombetas River live in a rural area of the municipality of Oriximiná, in the northwestern region of Pará State (1° 8' S; 55° 51' W). They are scattered along the banks of the Trombetas and Cuminá Rivers. This *mocambo* was created in 1820 under the leadership of the slave Atanásio, and by 1823 it had a population of about 2,000 runaway slaves, when it was destroyed by the Portuguese (Klein, 1986).

*Mazagão Velho*

Located on the banks of the Mutuacá River, in the State of Amapá (0° 07' 02" S; 51° 17' 04" W), the town of Mazagão Velho was founded in 1770 under orders of the Portuguese Crown to shelter 163 families of Christian Portuguese settlers of the Castle of Mazagran (nowadays El Djadidá) in Morocco from political-religious conflicts between Portuguese and Muslims. The blacks of Mazagão Velho descend from 103 African slaves who arrived in 1771 to the newly founded village accompanying the first Portuguese families.

*Pitimandeua*

The community of Pitimandeua is located on the right bank of the Inhangapi River in the municipal district of Inhangapi (01° 25' 45" S; 47° 54' 54" W), metropolitan Belém, capital of the State of Pará. The present-day inhabitants of Pitimandeua are descendants of African slaves who received the lands as a donation after the landlady's death, soon after the abolition of slavery in Brazil.

## Genotyping

DNA was extracted from whole blood with phenol-chloroform (Old and Higgs, 1993). Duffy blood group genotyping was performed using an allele-specific primer polymerase chain reaction (PCR) technique described by Olsson et al. (1998). Amplification was performed for each subject with sense primers corresponding to normal and GATA-1-mutated promoter sequence combined with antisense primers that discriminate the *FY*A* and *FY*B* alleles in four different combinations of primers. PCR mixtures included 100 ng genomic DNA, 0.2 µM of each primer, 100 µM of each dNTP, 1.5 µM $MgCl_2$, and 0.5 U AmpliTaq Gold polymerase (Perkin Elmer, USA) in the AmpliTaq Gold buffer supplied by Perkin Elmer in a reaction volume of 25 µL. Mixtures were incubated for 8 min at 95°C, followed by 10 cycles of 94°C for 1 min and 69°C for 1 min, 25 cycles of 94°C for 1 min, 64°C for 1 min and 72°C for 1 min, and a final

incubation at 72°C for 10 min. PCR products were separated electrophoretically using 1.5% agarose gel containing ethidium bromide at 150 V for 30 min and visualized under UV irradiation. Allele frequencies and expected Hardy-Weinberg values were estimated by the maximum likelihood method using the PopGene program version 1 (Yeh and Boyle, 1997). Comparisons of allele frequencies between populations were based on two-way contingency tables of the same program.

## RESULTS AND DISCUSSION

The genotype and allele frequency distributions recorded for Duffy blood group are presented in Table 1. Genotype distributions were consistent with the Hardy-Weinberg expectations in the four communities, and allele frequencies were similar in the four populations (P = 0.314).

FY*O was the most common allele with frequencies ranging from 56.4% in Mazagão Velho to 72.2% in the Pitimandeua, and the FY*O/FY*O genotype was found with frequencies between 32.3% in Mazagão Velho and 58.8% in Curiaú. Genotype FY*O/FY*O and FY*O mutation frequencies found in the Amazonian African-descending communities were high when compared to those observed in miscegenational Brazilian populations, but are lower than those found in sub-Saharan African populations, whose mutation is virtually fixed, as well as those described by Moulds et al. (1998) for African North Americans (92%) and by Estalote et al. (2005) for people of African ancestry in the population of Ribeirão Preto, State of São Paulo (86%). Genotype and allele distributions in the Amazonian communities are consistent with a predominantly African origin, with some degree of local differentiation and admixture with people of Caucasian ancestry and/or Amerindians, a finding that is corroborated by previous estimates of racial mixture for these communities (Trombetas and Curiaú) from uniparental genetic markers (mtDNA or Y-DNA) by Ribeiro-dos-Santos et al. (2002), autosomal hypervariable loci (Cayres-Vallinoto et al., 2003) and protein loci (Schneider et al., 1987; Guerreiro et al., 1999). On the other hand, the genotype and allelic frequencies found in Mazagão Velho, the lowest among the communities studied, can be also due to the fact that the African slaves who founded that community came from Morocco, North Africa, a region where the mutation frequencies are usually lower than those of African Sub-Sahara (Aireche and Benabadji, 1988; Fernandez-Santander et al., 1999). Incidentally, considering that *FY*O* allele homozygosity confers complete resistance to *vivax* malaria, the impact of the FY*O/FY*O genotype on the transmission and endemicity of *vivax* malaria deserves to be investigated in full detail, together with other human host genetic factors that confer resistance to malaria already identified at different levels of epidemic analysis, such as HbS, HbC, alpha-thalassemia, beta-thalassemia, G6PD deficiency, etc. In addition, Duffy blood group genotyping is a requisite in determining the prevalence of *P. vivax* infection among heterozygotes for the FY*O allele, because standard serology assigns the RBC phenotype Fy(a+b-) to individuals with genotypes FY*A/FY*A and FY*A/FY*O, and phenotype Fy(a-b+) to those with genotypes FY*B/FY*B and FY*B/FY*O, despite the 2-fold difference in FY antigen expression between homozygotes and heterozygotes for wild-type promoter. Populations of the Amazonian region are generally considered as showing a high susceptibility level to malaria and variable levels of acquired immunity that do not have a protective effect (PNCM, 2003). Thus, it is important to investigate the potential relationship between the human host genetic polymorphisms and the clinical severity of malaria, as well as the preva-

**Table 1.** Allele and genotype frequencies of the Duffy blood group in African-Brazilian communities of the Amazon region.

| Population | N | Genotype frequency | | | | | | Allele frequency | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | FY*A/FY*A | FY*A/FY*O | FY*B/FY*B | FY*B/FY*O | FY*A/FY*B | FY*O/FY*O | FY*A | FY*B | FY*O |
| Trombetas | 29 | 0.172 | 0.241 | 0.000 | 0.103 | 0.034 | 0.448 | 0.310 | 0.069 | 0.621 |
| Mazagão Velho | 31 | 0.129 | 0.290 | 0.000 | 0.194 | 0.065 | 0.323 | 0.307 | 0.129 | 0.564 |
| Pitimandeua | 18 | 0.000 | 0.333 | 0.000 | 0.111 | 0.056 | 0.500 | 0.194 | 0.083 | 0.722 |
| Curiaú | 17 | 0.000 | 0.176 | 0.059 | 0.059 | 0.118 | 0.588 | 0.147 | 0.147 | 0.706 |
| Overall | 95 | 0.095 | 0.263 | 0.011 | 0.126 | 0.063 | 0.442 | 0.258 | 0.105 | 0.637 |

lence and the profile of asymptomatic malaria in the Amazon region in an attempt to investigate the contribution of host biological factors to the non-homogeneous prevalence of the disease in the region expressed by different risk levels for contracting malaria (measured by the Annual Parasitic Incidence). This may elucidate how the differences in ethnic composition of the Amazonian populations contribute to this heterogeneity.

## ACKNOWLEDGMENTS

Research supported by the National Council for Scientific and Technological Development (CNPq) and the Federal University of Pará, Brazil.

## REFERENCES

Aireche H and Benabadji M (1988). Rh and Duffy gene frequencies in Algeria. *Gene Geogr.* 2: 1-8.

Cayres-Vallinoto IMV, Vallinoto ACR, Valente CMD and Guerreiro JF (2003). Allele frequency distributions of six hypervariable loci (D1S80, APOB, D4S43, vW1, F13A and DYS19) in two African-Brazilian communities of the Amazon region. *Genet. Mol. Biol.* 26: 235-240.

Chaudhuri A, Polyakova J, Zbrzezna V and Pogo AO (1995). The coding sequence of Duffy blood group gene in humans and simians: restriction fragment length polymorphism, antibody and malarial parasite specificities, and expression in nonerythroid tissues in Duffy-negative individuals. *Blood* 85: 615-621.

Estalote AC, Proto-Siqueira R, Silva WA Jr, Zago MA, et al. (2005). The mutation G298A→Ala100Thr on the coding sequence of the Duffy antigen/chemokine receptor gene in non-caucasian Brazilians. *Genet. Mol. Res.* 4: 166-173.

Fernandez-Santander A, Kandil M, Luna F, Esteban E, et al. (1999). Genetic relationships between southeastern Spain and Morocco: new data on ABO, RH, MNSs, and Duffy polymorphisms. *Am. J. Hum. Biol.* 11: 745-752.

Guerreiro JF, Ribeiro-dos-Santos AKC, Santos EJM, Vallinoto ACR, et al. (1999). Genetic-demographic data from two Amazonian populations composed of descendants of African slaves: Pacoval and Curiaú. *Genet. Mol. Biol.* 22: 163-167.

Issitt PD and Anstee DJ (1998). The Duffy blood group system. In: Applied blood group serology (Issitt PD and Anstee DJ, eds.). Montgomery, Miami, 439-458.

Iwamoto S, Omi T, Kajii E and Ikemoto S (1995). Genomic organization of the glycoprotein D gene: Duffy blood group Fya/Fyb alloantigen system is associated with a polymorphism at the 44-amino acid residue. *Blood* 85: 622-626.

Klein H (1986). African slavery in Latin America and the Caribbean. Oxford University Press, New York.

Mallinson G, Soo KS, Schall TJ, Pisacka M, et al. (1995). Mutations in the erythrocyte chemokine receptor (Duffy) gene: the molecular basis of the Fya/Fyb antigens and identification of a deletion in the Duffy gene of an apparently healthy individual with the Fy(a-b-) phenotype. *Br. J. Haematol.* 90: 823-829.

Miller LH, Mason SJ, Clyde DF and McGinniss MH (1976). The resistance factor to *Plasmodium vivax* in blacks. The Duffy-blood-group genotype, FyFy. *N. Engl. J. Med.* 295: 302-304.

Moulds JM, Hayes S and Wells TD (1998). DNA analysis of Duffy genes in American blacks. *Vox Sang.* 74: 248-252.

Muggiati A (1990). O último quilombo. *Rev. Manchete* 1971: 648-657.

Old JM and Higgs DR (1993). Gene analysis. In: The thalassemias: methods in hematology (Weatherall DJ, ed.). Churchill Livingstone, Edinburgh, 74-102.

Olsson ML, Hansson C, Avent ND, Akesson IE, et al. (1998). A clinically applicable method for determining the three major alleles at the Duffy (FY) blood group locus using polymerase chain reaction with allele-specific primers. *Transfusion* 38: 168-173.

PNCM. Programa Nacional de Prevenção e Controle da Malária/Ministério da Saúde, Secretaria de Vigilância em Saúde, Brasília: Ministério da Saúde, 2003.

Pogo AO and Chaudhuri A (2000). The Duffy protein: a malarial and chemokine receptor. *Semin. Hematol.* 37: 122-129.

Ribeiro-dos-Santos AK, Pereira JM, Lobato MR, Carvalho BM, et al. (2002). Dissimilarities in the process

of formation of Curiau, a semi-isolated Afro-Brazilian population of the Amazon region. *Am. J. Hum. Biol.* 14: 440-447.

Schneider H, Guerreiro JF, Santos SEB, Weimer TA, et al. (1987). Isolate breakdown in Amazonia: the blacks of the Trombetas River. *Braz. J. Genet.* 10: 565-574.

Tournamille C, Colin Y, Cartron JP and Le Van KC (1995a). Disruption of a GATA motif in the Duffy gene promoter abolishes erythroid gene expression in Duffy-negative individuals. *Nat. Genet.* 10: 224-228.

Tournamille C, Le Van KC, Gane P, Cartron JP, et al. (1995b). Molecular basis and PCR-DNA typing of the Fya/fyb blood group polymorphism. *Hum. Genet.* 95: 407-410.

Yeh FC and Boyle TJB (1997). Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belg. J. Bot.* 129: 157.