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The mutation G298A→Ala100Thr on the coding sequence of the *Duffy* antigen/ chemokine receptor gene in non-caucasian Brazilians

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ABSTRACT. Ala100Thr has been suggested to be a Caucasian genetic marker on the *FY*B* allele. As the Brazilian population has arisen from miscegenation among Portuguese, Africans, and Indians, this mutation could possibly be found in Euro- and Afro-Brazilians, or in Brazilian Indians. Fifty-three related individuals and a random sample of 100 subjects from the Brazilian population were investigated using the polymerase chain reaction and four restriction fragment length polymorphisms. Confirming the working hypothesis, among the related individuals three Afro-Brazilians (two of them a mother and daughter) and a woman of Amerindian descent had the Ala100Thr mutation on the *FY*B* allele. Five non-related Euro-Brazilians also carried the mutation. All nine indi-

viduals presented the Fy(a-b+) phenotype. We conclude that the Ala100Thr mutation can occur in populations other than Caucasians and that this mutation does not affect Duffy expression on red blood cells. Gene frequencies for this allele in the non-related individuals were in agreement with those of other populations. The *Duffy* frequencies of two Amerindian tribes were also investigated.

Key words: *Duffy* blood group, DARC, DNA polymorphisms, Genetic polymorphisms, Amerindians, Brazilian population

INTRODUCTION

Duffy (FY) glycoprotein or *Duffy* antigen/chemokine receptor (DARC) functions as a receptor for *Plasmodium vivax* merozoites and binds members of the C-C and C-X-C classes of chemokines. It is postulated to be a scavenger for chemokines. DARC is expressed in human erythrocytes, on endothelial cell lining postcapillary venules throughout the body, and in vascular endothelial and epithelial cells in some nonerythroid organs (Hadley and Peiper, 1997).

The polymorphic human FY blood group system includes six antigenic determinants, and it is clinically significant in transfusion medicine since antibodies against antigens of this system are involved in hemolytic reactions and hemolytic disease of newborns (Issitt and Anstee, 1998). The *FY*A* and *FY*B* alleles are distinguished by a missense mutation, which conditions a single amino acid difference (G125A→Gly42Asp) (Chaudhuri et al., 1995; Iwamoto et al., 1995; Mallinson et al., 1995; Tournamille et al., 1995b). This substitution defines two antithetical antigens, Fy^a and Fy^b, giving the common Fy(a+b-), Fy(a-b+) and Fy(a+b+) phenotypes in Caucasian populations (Issitt and Anstee, 1998).

The Fy(a-b-) phenotype, common in Blacks, is due to a T-33C point mutation (Pogo and Chaudhuri, 2000) [previously described as T-46C (Tournamille et al., 1995a)] on the *DARC*B* gene promoter. This mutation, further designated as $FY*B^{ES}$ [*ES* stands for "erythroid silent" (Pogo and Chaudhuri, 2000)], abolishes the erythroid gene expression by disrupting a binding site for the GATA-1 erythroid transcription factor, resulting in a silent expression of the *FY* gene only in the erythroid lineage (Tournamille et al., 1995a).

The *FY*X* allele is characterized by a weak anti-Fy^b reaction. Some authors (Olsson et al., 1998; Tournamille et al., 1998; Pogo and Chaudhuri, 2000; Yazdanbakhsh et al., 2000) have implicated it with a single polymorphism of the *FY*B* allele (C265T→Arg89Cys), while others have indicated two polymorphisms (C265T and G298A→Ala100Thr) (Parasol et al., 1998; Gassner et al., 1998; Reid et al., 1998). Both mutations are associated with the absence of a restriction site (*Aci*I and *Mwo*I, respectively). The Ala100Thr change alone, without weakening of the FY expression, was observed in 33% of Swedes but not in Blacks from Pinetown, South Africa (Olsson et al., 1998). Intermixture between Euro- and Afro-Brazilians and/or Brazilian Indians could have introduced this mutation, which until now has only been described in Caucasians. Based on this working hypothesis a screening of several Brazilian populations was performed.

MATERIAL AND METHODS

Family studies

Approval from each individual was obtained prior to the collection of samples, as requested by the Institutional Ethics Committee (registration number: 138/99 UFRJ). Venous blood from 53 individuals from Rio de Janeiro, RJ, Brazil, belonging to 15 families, was drawn for FY erythrocyte phenotyping and genotype determination.

The ethnic stocks were designated as Euro- and Afro-Brazilians, following the nomenclature presently used by most Brazilian geneticists (Araújo da Silva Jr. et al., 1999; Palatnik et al., 2002), which includes several categories of the traditional classification (Palatnik, 1984). Thus, the term Euro-Brazilian indicates people from European descent whose phenotype does not show signs of admixture. Afro-Brazilians are the Brazilian descendants of Africans, who include light and dark Mulattoes and Blacks.

The ethnic classification was uniformly determined by just one of the authors; it was based on skin color, eye color, hair color and type, nose form and breadth, lip form and width. Fifty-one individuals were classified as Afro-Brazilians (35 without any sign of admixture, 11 with predominant Black features, and 5 with predominant Caucasian features). One person was classified as Euro-Brazilian and another was a woman of probably mixed Amazonian Indian descent (tribe not known).

Population studies

Three Brazilian urban groups from Ribeirão Preto, a city in northwestern São Paulo State [25 Euro-, 25 Afro-, and 25 Asian-Brazilians or Asian-derived (Japanese) subjects (Araújo da Silva Jr. et al., 1999)] were analyzed. Only individuals who reported absence of any other ethnic group in all of the four grandparents were included. Twenty-five DNA samples of unrelated individuals from two Brazilian Amazon tribes were also analyzed: 12 Cayapo and 13 Yanomama. These Indians had been studied for other genetic markers by Zago et al. (1996), Santos et al. (1998), and Araújo da Silva et al. (1999), and previous permission had been obtained from the Fundação Nacional do Índio (FUNAI) and from local leaders.

The Cayapo Indians speak a Ge language and formerly occupied a vast region in Central and Northern Brazil. Fifty years ago they were still hostile to non-Indians. Presently they live in at least 13 semi-independent communities in the States of Pará and Mato Grosso, roughly distributed from latitude 4° to 11°S and longitude 51° to 55°W. The village studied in this investigation was Kubenkokre, situated in the region bathed by the Iriri River (8°43'S; 52° 24'W). The Yanomama Indians speak an isolated language (Ninam). They live in the Amazonian forest, being one of the least affected by Neo-Brazilian influences. Information about their history, exact location and demographic characteristics can be found in Neel and Weiss (1975) and Salzano and Callegari-Jacques (1988).

Phenotyping of erythrocyte *Duffy* antigens

The antithetical Fy^a and Fy^b antigens were determined by agglutination tests on washed erythrocytes with anti-Fy^a and anti-Fy^b human polyclonal antibodies (Gamma Biologicals, Hous-

ton, and DiaMed, Switzerland) by the indirect antiglobulin test, after incubation (1 h at 37°C).

DNA extraction/amplification

DNA was extracted from whole blood using the "super quik gene-DNA isolation kit" (AGTC, Denver, CO, USA), according to the manufacturer's instructions. Two polymerase chain reactions (PCR) were performed, using 200 ng genomic DNA each, in a total volume of 50 µl. The PCR buffer consisted of 1.5 mM Tris-HCl, pH 8.5, 0.1 M KCl, 1.5 mM $MgCl_2$, 1 µg/µl bovine serum albumin, 0.1 µM of each primer, 100 mM dNTPs, and two units of Biotools DNA polymerase (B&M Labs, Madrid, Spain). The amplification profile and the primers encompassing the *FY*A/FY*B* and 265/298 polymorphisms were carried out as previously reported by Parasol et al. (1998). For the second PCR, essential to distinguish between functional and nonfunctional GATA-1 box mutations (T-33C), the primers were already described by Rios et al. (1999). The PCR reactions were performed in an automatic thermal cycler (Perkin Elmer 9700, Norwalk, CA, USA).

PCR product analysis for genotype determinations

The amplified products from the first PCR were submitted to the B*an*I, A*ci*I, and M*wo*I restriction enzymes (New England Biolabs, MA, USA). The amplified products for the GATA-1 box mutation, critical for the expression of Fy^b on red blood cells (Tournamille et al., 1995a), were submitted to S*ty*I enzyme (Gibco BRL, MD, USA). Digestion incubations were as follows: a) overnight at 37°C for *Ban*I and *Sty*I, b) 4 h, also at 37°C for A*ci*I, and c) 1 h at 60°C for M*wo*I. The B*an*I-restriction fragment length polymorphism (RFLP) was detected on 2% agarose gel with ethidium bromide, while the other RFLPs were identified on 6% acrylamide gels, all silver stained (Sanguinetti et al., 1994).

RESULTS AND DISCUSSION

The family studies are synthesized in Table 1. The Amerindian parentage woman and three Afro-Brazilians with predominantly Black features (mother and her daughter, and an unrelated individual) had the Ala100Thr point mutation. Since this child necessarily inherited her $FY*B^{ES}$ allele from her father, who was homozygous for both the *FY*B* and the GATA mutations ($FY*B^{ES}/FY*B^{ES}$), FY expression on her red cells depended exclusively on the Ala100Thr allele. The four individuals with the Ala100Thr mutation were typed as Fy(a-b+), with strong reactions against anti-Fy^b (Estalote et al., 2000). These findings indicate that the Ala100Thr mutation can occur in non-Caucasian populations, and confirmed the finding that it does not affect FY expression on red blood cells (Olsson et al., 1998). Additionally, we concluded that the Brazilian population exhibits considerable biological diversity (see also Araújo da Silva Jr. et al., 1999; Proto-Siqueira et al., 2000).

Among the non-related individuals, the Ala100Thr mutation was present in 5/25 Euro-Brazilian samples (one in the homozygous state, Table 2). They were of Italian, German, and Spanish parentage. All these samples were typed as Fy(a-b+), showing strong reactions with anti-Fy^b reagents, and *FY*B/FY*B*. Thus, 20% of the Caucasians carried the allele, corresponding to an allele frequency of 6%, similar to that found in the Swedish population (Olsson et al.,

Table 1. Family studies: phenotypes and genotypes for the *Duffy* blood group system.

Ethnic stock	N	Erythrocyte phenotype	PCR-RFLP				*Duffy* genotype
			*Sty*I T-33C	*Ban*I G125A	*Aci*I C265T	*Mwo*I G298A	
Euro-Brazilian	1	Fy(a+b+)	T/T	G/A	C/C	G/G	*FY*A/FY*B*
Amerindian parentage	1	Fy(a-b+)	T/T	A/A	C/C	**G/A**	*FY*B/FY*B*
Afro-Brazilians	51						
Blacks (partial N = 35)							
	1	Fy(a+b-)	T/C	G/A	C/C	G/G	*FY*A/FY*B^{ES}*
	1	Fy(a-b+)	T/C	A/A	C/C	G/G	*FY*B/FY*B^{ES}*
	33	Fy(a-b-)	C/C	A/A	C/C	G/G	*FY*B^{ES}/FY*B^{ES}*
Dark and light Mulattoes (partial N = 16)							
	1	Fy(a+b+)	T/T	G/A	C/C	G/G	*FY*A/FY*B*
	2	Fy(a+b-)	T/T	G/G	C/C	G/G	*FY*A/FY*A*
	2	Fy(a+b-)	T/C	G/A	C/C	G/G	*FY*A/FY*B^{ES}*
	1	Fy(a-b+)	T/T	A/A	C/C	**G/A**	*FY*B/FY*B*
	3	Fy(a-b+)	T/C	A/A	C/C	G/G	*FY*B/FY*B^{ES}*
	1	Fy(a-b+)	T/C	A/A	C/C	G/G	*FY*B/FY*B^{ES}*
	2*	Fy(a-b+)	T/C	A/A	C/C	**G/A**	*FY*B/FY*B^{ES}*
	4	Fy(a-b-)	C/C	A/A	C/C	G/G	*FY*B^{ES}/FY*B^{ES}*

*Related individuals (mother and daughter). In the *Mwo*I-RFLP column, the letters G/A in bold indicate the presence of the G298A→Ala100Thr mutation.

1998). Seventy-six percent of the Afro-Brazilians were *FY*B/FY*B* and homozygous for the GATA-1 box mutation; i.e., 96% of the Blacks carried the silent allele, and the *FY*B^{ES}* allele frequency was 86%. These results are in agreement with data on: a) other Afro-Brazilians, from the North and Northeast regions [*FY*B^{ES}* allele frequency = 76.25% (Proto-Siqueira et al., 2000)], b) Afro-Americans (Moulds et al., 1998), and c) Afro-Caribbeans and Colombians (Nickel et al., 1999) in unrelated subjects. None of the Afro-Brazilians had the two missense mutations on the *FY*B* allele.

Among the Asian-Brazilians, only three were *FY*A/FY*B*, and none of them had the GATA-1 box mutation. The *FY*A* allele was present in 100% of the subjects and its allele frequency was 94% (Proto-Siqueira et al., 2001), similar to that found for other Mongoloid populations (Shimizu et al., 1997, 2000; Peng et al., 2000).

Among the Amerindians, neither C265T nor G298A was found. All samples had the *FY*B* allele, but none of them had the GATA-1 box mutation. The frequencies that we obtained by molecular biology, *FY*A*: Cayapo = 0.79; Yanomama = 0.58, show good agreement with the previous known frequencies based on erythrocyte phenotyping [(*FY*A*: Cayapo = 0.68, n = 1,979; Yanomama = 0.56, n = 3,753) (Salzano and Callegari-Jacques, 1988)]. For the Cayapo, the estimated admixture with Caucasoids was 0.002 [number of markers (nm) = 10; number of genes examined (ng) = 8,162] and with Blacks, 0.012 (nm = 12; ng = 10,652). For the Yanomama, the admixture was 0.000 with both Caucasians and Blacks (nm = 12; ng = 52,682 and 58,794; respectively) as estimated by Salzano and Callegari-Jacques (1988). Therefore, it is possible

Table 2. Phenotypes and genotypes for the *Duffy* blood group system in Brazilian populations.

Ethnic stock	N	PCR-RFLP				*Duffy* genotype
		*Sty*I T-33C	*Ban*I G125A	*Aci*I C265T	*Mwo*I G298A	
Euro-Brazilians						
	5	T/T	G/G	C/C	G/G	*FY*A/FY*A*
	7	T/T	A/A	C/C	G/G	*FY*B/FY*B*
	4	T/T	A/A	C/C	**G/A**	*FY*B/FY*B*
	1	T/T	A/A	C/C	**A/A**	*FY*B/FY*B*
	8	T/T	G/A	C/C	G/G	*FY*A/FY*B*
Afro-Brazilians						
	19	C/C	A/A	C/C	G/G	$FY*B^{ES}/FY*B^{ES}$
	1	T/T	G/A	C/C	G/G	*FY*A/FY*B*
	5	T/C	G/A	C/C	G/G	$FY*A/FY*\ B^{ES}$
Asian-Brazilians [or Asian-derived (Japanese) individuals]						
	22	T/T	G/G	C/C	G/G	*FY*A/FY*A*
	3	T/T	G/A	C/C	G/G	*FY*A/FY*B*
Amerindians (Cayapo/Yanomama)						
	7/5	T/T	G/G	C/C	G/G	*FY*A/FY*A*
	0/3	T/T	A/A	C/C	G/G	*FY*B/FY*B*
	6/4	T/T	G/A	C/C	G/G	*FY*A/FY*B*

Allele frequencies: Euro-Brazilians: *FY*A*: 0.36; *FY*B*: 0.64 ($\chi^2 = 2.3$); Afro-Brazilians: *FY*A*: 0.119998; *FY*B*: 0.02; $FY*B^{ES}$: 0.86 ($\chi^2 = 0.5$); Asian-Brazilians: *FY*A*: 0.94; *FY*B*: 0.06; Cayapo Amerindians: *FY*A*: 0.79; *FY*B*: 0.21; Yanomama Amerindians: *FY*A*: 0.58; *FY*B*: 0.42. In Euro-Brazilians, the allele frequency of the missense mutation G298A was 0.06. The red blood cells of the five individuals carrying the G298A mutation were typed as Fy(a-b+), showing strong reactions with anti-Fy^b reagents. In the *Mwo*I-RFLP column, the letters G/A and A/A in bold indicate the presence of the G298A→Ala100Thr mutation in hetero- and homozygous state, respectively.

that in essentially unmixed Amerindians the T-33C GATA box or the C265T and/or G298A mutations are absent. On the other hand, previous data have demonstrated that the study of a small number of Indian tribes (even if a large number of individuals are studied) may not be representative of the variability of Amerindians as a whole, probably because genetic drift acting on small subpopulations and founder effects can produce striking differences in allele distribution (Zago et al., 1996). Additional interpopulational studies are necessary to determine the whole range of genetic variability in the *Duffy* genes of Brazilian Indians.

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