



## Inherited hemoglobin disorders in an Afro-Amazonian community: Saracura

Greice Lemos Cardoso, Silvania Yukiko Lins Takanashi and João Farias Guerreiro

*Laboratório de Genética Humana e Médica, Instituto de Ciências Biológicas,  
Universidade Federal do Pará, Belém, PA, Brazil.*

### Abstract

The most common hemoglobinopathies, viz, hemoglobins S and C, and  $\alpha$ - and  $\beta$ -thalassemias, were investigated through the molecular screening of 116 subjects from the community of Saracura, comprising fugitive African slaves from farms of the municipality of Santarém, in the west of Pará State, Brazilian Amazon. The observed frequency of the *HBB\* $\beta$ S* gene (0.9%) was significantly lower than that encountered in other Afro-derived communities in the region. Concomitantly, the absence of the *HBB\* $\beta$ C* allele has been reported for most of the Afro-Amazonian communities thus far studied. As remnant populations of *quilombos* are generally small, the heterogeneous distribution of *HBB\* $\beta$ S* and *HBB\* $\beta$ C* alleles among them is probably due to genetic drift and/or founder effect. The observed frequency of 3.7 kb deletion in Saracura (8.5%) was consistent with the African origin of the population, with a certain degree of local differentiation and admixture with individuals of Caucasian ancestry, placed in evidence by the occurrence of  $\beta$ -( $\beta$ -MED) deletion (1.2%), a common mutation in Mediterranean regions. As regards  $\beta$ -thalassemia, among the seven different mutations found in Saracura, three  $\beta^0$  and two  $\beta^+$  mutations were of Mediterranean origin, and two  $\beta^+$  of African. Thus, only 28% of the local  $\beta$ -thalassemia mutations found in Saracura were of African origin.

*Key words:* sickle cell disease, thalassemias, Afro-Amazonian.

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### Introduction

Inherited hemoglobin disorders fall into two main groups, namely structural hemoglobin variants and thalassemias. The structural hemoglobin variants mostly result from single amino-acid substitutions in the  $\alpha$  or  $\beta$  chains. The thalassemias are classified according to the particular globin chains that are ineffectively synthesized into the  $\alpha$ ,  $\beta$ ,  $\delta\beta$ , and  $\epsilon\delta\beta$  thalassemias. However, from a public health point of view, only  $\alpha$  and  $\beta$  thalassemias are sufficiently common to be of importance (Weatherall and Clegg, 2001).

The most common structural hemoglobin variants are hemoglobin S (Hb S beta 6(A3) Glu > Val), hemoglobin C (Hb C beta 6(A3) Glu > Lys) and hemoglobin E (Hb E beta 26(B8) Glu > Lys). Although encountered worldwide, Hb S reaches its highest frequencies in sub-Saharan Africa, the Middle East and parts of the Indian sub-continent, where carrier frequencies range from 5% to 40%, or more, of the population. Whereas Hb C reaches its peak in West Africa, Hb E is found in the eastern half of the Indian sub-continent and throughout South-East Asia, where, in some areas, carrier rates may exceed 60% of the population (Flint *et al.*, 1998; Weatherall and Clegg, 2001).

$\beta$ -thalassemia results from mutations in  $\beta$ -globin genes, which lead to a reduction in, or absence of, beta-globin chain synthesis. Most known cases of  $\beta$ -thalassemia are caused by a large number of point mutations of various types, which affect either the quality or the quantity of RNA produced. Almost all kinds of mutations that reduce synthesis of mRNA have been identified as causing  $\beta$ -thalassemia. Deletions of the beta-globin gene are uncommon. The spectrum of mutations, which differs among ethnic groups, includes a few common mutations and a variable number of rare ones.  $\beta$ -thalassemia is prevalent in Mediterranean countries, the Middle East, Central Asia, India, South China and the Far East, as well as countries along the north coast of Africa and in South America. The highest carrier frequency is reported in Cyprus (14%), Sardinia (10.3%) and Southeast Asia. The high gene frequency of beta-thalassemia in these regions is most likely related to selective pressure from *Plasmodium falciparum* malaria (for a review see Galanello and Origa, 2010).

$\alpha$ -thalassemia, the most common single-gene disease in the world, is characterized by the reduction or complete absence of  $\alpha$ -globin gene expression. Two different deletions - 3.7 kb and 4.2 kb - in the  $\alpha$ -globin gene on chromosome 16p13.3, which originate from homologous recombination between misaligned chromosomes, are the most common causes of  $\alpha$ -thalassemia (Higgs *et al.*, 1989; Harteveld and Higgs, 2010). Although 3.7 kb deletion alpha-2

( $-\alpha^{3.7}$ ), a deletion of 3804 nts from the alpha2 gene to the alpha1, occurs worldwide, it is most prevalent in some African, Indian, Nepalese, Sardinian, several other Mediterranean, Chinese, and East Asian populations. 4.2 kb deletion ( $-\alpha^{4.2}$ ), involving the alpha2 gene alpha-Thal-2, also spread worldwide, it is more commonly found in Indian, Melanesian, Thai, and some other southeast Asian populations (Harteveld and Higgs, 2010). Like all common globin gene disorders (sickle-cell trait and  $\beta$ -thalassemia),  $\beta$ -thalassemia occurrence is highly frequent throughout all the tropical and subtropical regions of the world, whereby the inference that all of these globin-gene disorders (including  $\alpha$  thalassemia) are selective, since in some way they protect carriers from the ravages of falciparum malaria. However, prevalence has increased in previously non-endemic areas as a consequence of historical and recent immigration flows, the slave-trade, trading activities and colonization (Harteveld and Higgs, 2010).

Only in the second half of the 18<sup>th</sup> century were Africans, as slaves, brought to the Amazon region on a steady basis, the estimated number arriving between 1755 and 1820 reaching about 53,000. Their regional distribution was not uniform. Although the concentration in Belém, close to the mouth of the Amazon river, and in the nearby villages was high, there was a gradual reduction in upstream-towns, where commercial activities did not justify the extensive employment of this form of hand-labor (Santos *et al.*, 1999). Evidently about 90% of the slaves imported into northern Brazil were from Angola, the Congo and Mozambique, and the remainder from Senegambia, Guinea-Bissau, and Cape Verde, along Atlantic-West-Africa. There are records of a small number from French Guyana and Surinam entering the region, some through the illegal slave trade and others as fugitives (Curtin, 1969; Vergolino-Henry and Figueiredo, 1990).

In accordance with what occurred in other Brazilian regions and South American countries, several communities, the so-called *mocambos* or *quilombos*, were founded in the Brazilian Amazon by fugitive slaves, especially in the state of Pará, point of entry of African slaves into the region. As, to date, many of these communities remain relatively isolated, much of their identity is still preserved. From genetic studies undertaken in populations of African descent, the level of isolation and mobility has been evaluated, and the gene contribution from other sources quantified. Briefly, the genetic variability observed in these communities is consistent with their African origin, with some degree of local differentiation and admixture with individuals of Caucasian and/or Amerindian ancestry (Bortolini *et al.*, 1987; Guerreiro *et al.*, 1999; Oliveira *et al.*, 2002).

In this study we report the prevalence of sickle cell hemoglobin (Hb S), alpha-thalassemia deletion alleles (3.7 kb deletion, 4.2 kb deletion,  $-(\alpha)20.5$ , and  $-(MED)$  and beta-thalassemia mutations ( $-88 (C > T)$ ,  $-87$

$(C > G)$ ,  $-29 (A > G)$ , Codon 6 ( $-A$ ), Codon 15 ( $G > A$ ), Codon 24 ( $T > A$ ), Codon 39 ( $C > T$ ), IVS-I-1 ( $G > A$ ), IVS-I-6 ( $T > C$ ) and IVS-I-110 ( $G > A$ )) in Saracura, a community founded by fugitive African slaves in the municipality of Santarém, the mid-Amazon region, Pará State, Brazil.

## Subjects and Methods

The community of Saracura, formed by African slaves, fugitives from farms of the municipality of Santarém, is located in the rural area, along the left and right margins of the Amazon River, in the western region of the State of Pará, at about 2° 25' 59" S; 54° 36' 38" W. Blood samples from 116 individuals, representing 50% of the total population, were collected. 56% were females with ages varying from nine to 78 years. All the participants gave their written consent.

The presence of Hb S and Hb C in the blood was first determined by electrophoresis in cellulose acetate pH 8.0-9.0, as well as agar electrophoresis pH 6.2, and then confirmed by *DdeI* and *BseRI* digestion of a 382 bp amplified globin gene fragment, according to Old *et al.* (1996) and Akar *et al.* (1996).

The  $\alpha$ -globin genotype was determined by identification of 3.7 kb, 4.2 kb,  $-(\alpha)20.5$  ( $-\alpha^{20.5}$ ) and  $-(MED)$  ( $-\text{MED}$ ) deletions by means of multiplex gap-PCR (Liu *et al.*, 2000; Chong *et al.*, 2000).

Characterization of  $\beta$ -thalassemia mutations was by direct genomic sequencing of the  $\beta$ -globin gene amplified through PCR. A 1.433-kb gene fragment containing exons 1 and 2, as well as 0.802 kb of flanking sequence, was PCR amplified with primers corresponding to positions 2802 to 2775 and 604 to 631 of the human  $\beta$ -globin. After purification, PCR products were sequenced, using internal primers to cover those gene regions where common  $\beta$ -thal mutations are found in African and Mediterranean populations, viz.,  $-88 (C > T)$ ,  $-87 (C > G)$ ,  $-29 (A > G)$ , Codon 6 ( $-A$ ), Codon 15 ( $G > A$ ), Codon 24 ( $T > A$ ), Codon 39 ( $C > T$ ), IVS-I-1 ( $G > A$ ), IVS-I-6 ( $T > C$ ) and IVS-I-110 ( $G > A$ , with an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA). Electrophoresis of reaction products was on an automated ABI Prism 3130 sequencer (Applied Biosystems). Allele and genotype frequencies were estimated by gene counting.

## Results

### Hemoglobin S (Hb S) and hemoglobin C (Hb C)

Of the 116 individuals analyzed, two presented the sickle-cell trait (1.7%), thereby corresponding to a frequency of 0.9% for the *HBB*\*S gene. Hemoglobin C was not encountered.

### $\alpha$ -thalassemia

Among the 116 patients analyzed, 24 (20.7%) presented  $\alpha$ -thalassemia. Of these, 20 (17.2%) were heterozygotes ( $-\alpha^{3.7}/\alpha\alpha$ ), one (0.9%) a homozygote ( $-\alpha^{3.7}/-\alpha^{3.7}$ ), and 3 (2.6%) carriers of the - - (MED) deletion ( $-\alpha^{MED}/\alpha\alpha$ ). The frequencies of  $-\alpha^{3.7}$  and  $-\alpha^{MED}$  deletions were 9.5% and 1.3%, respectively. Neither 4.2 kb nor - (alpha) 20.5 deletions were observed.

### $\beta$ -thalassemia

$\beta$ -thal mutations were observed in 19 out of the 116 individuals (16.4%). This corresponded to a spectrum of seven different mutations, three of which,  $\beta^0$  (IVS-I-1 (G- > A), Codon 39 (C- > T) and Codon 15 (G- > A)), and four  $\beta^+$  (-88 (C- > T), Codon 24 (T- > A), IVS-I-110 (G- > A) and IVS-I-6 (T > C)). Mutations -87 (C- > G), -29 (A- > G) and Codon 6 (-A) were not found. Allele frequencies are shown in Table 1.

### Discussion

The low frequency of HBB\*S genes observed in Saracura (0.9%), although similar to that in the Afro-descendants (quilombo remnants) of Cametá (0.5%) and Itamoari (0%) counties, Pará State, is significantly lower than in the other Afro-derived communities in Trombetas (6.0%) and Pacoval (3.6%), Pará State, and Curiau (4.5%), Amapá State (Schneider *et al.*, 1987; Bortolini *et al.*, 1992; Guerreiro *et al.*, 1999; Oliveira *et al.*, 2002). The distribution of hemoglobin S in Afro-Brazilian communities from other regions in the northeast, midwest, southeast and south, is also heterogeneous. In some communities, the HBB \* S allele is either absent or only at low frequencies, whereas in others, frequencies are high, thus in agreement with expectancies for populations of predominantly African origin. On the other hand, even though absence of the HBB\*C allele has also been reported in most of the Afro-Amazonian communities so far studied, in Curiau frequency was 6.2%. Although this particular variant is also

absent in most of the Afro-Brazilian communities from other regions, it occurred in at least one in each (Oliveira *et al.*, 2002; Auricchio *et al.*, 2007). As remnant *quilombo* populations are generally small, the occurrence of genetic drift and/or founder effect, as well as admixture with individuals of European and Amerindian ancestry could explain this heterogeneous distribution of the HBB\*S and HBB\*C alleles among these populations.

Even though the paucity of data on thalassemia genotyping at the molecular level in Brazilian populations, particularly Afro-Brazilians, hindered the comparison of results, it was possible to consider that the variability observed in Saracura is consistent with the basic African origin of this population, with a certain degree of local differentiation and admixture with individuals of Caucasian ancestry. Although the frequency of 3.7 kb deletion (8.5%) was similar to that observed in Afro-newborns from Salvador, Bahia (11.0%) (Adorno *et al.*, 2008), as well as Afro-descendants from Campinas, São Paulo (13.0%) (Sonati *et al.*, 1991) and Porto Alegre, Rio Grande do Sul (12.0%) (Wagner *et al.*, 2010), it was higher than the frequency found in Euro-descendants from Porto Alegre (2.0%) (Wagner *et al.*, 2010) and individuals from the urban area of Santarém, PA (3.3%) (Souza *et al.*, 2009). Gene flow from local non-Afro-individuals was placed in evidence by the occurrence of - - (MED) deletion (1.2%), a common mutation in Mediterraneans (Higgs and Weatherall, 2009). This is the first report of the prevalence of - - (MED) deletion in Brazilians.

As to  $\beta$ -thalassemia, among the seven different mutations found in Saracura, the three  $\beta^0$ , (IVS-I-1 (G- > A), Codon 39 (C- > T) and Codon 15 (G- > A), and two of the  $\beta^+$ , (IVS-I-110 (G- > A) and IVS-I-6 (T > C)), were of Mediterranean origin. The other two  $\beta^+$  mutations (-88 (C- > T) and Codon 24 (T- > A) *i.e.*, 28%, were really of African origin.

The European contribution to the local population, as revealed by distribution of inherited hemoglobin disorders, is in accordance with estimates of interethnic admixture based on Y-DNA and mtDNA polymorphisms. These show that the most important non-African contributions to the structure of South-American *quilombos* were, as a whole, from European males and Amerindian females. In this specific case, the European male contribution was estimated at 57% (Palha *et al.*, 2011).

Based on historical genetic data, plausibly the Mediterranean thalassaemic mutations found in Saracura are predominantly of Portuguese origin, since these alleles are relatively common in Portugal, as a result of occupation of the country over past centuries by peoples of the Mediterranean region, especially Phoenicians, Greeks, Romans and Arabs (Tamagnini *et al.*, 1993). Gene flow from Portuguese men to African and Amerindian groups, is a well-known social fact in the process of colonization of the Amazon, and has been well documented through estimates of inte-

**Table 1** - Allele frequencies of  $\beta$ -thalassemia in Saracura.

Mutation	Phenotype	N	%	Origin
-88 C > T	$\beta^{++}$	4	1.7	Africa
codon 15 G > A	$\beta^0$	3	1.3	Mediterranean
codon 24 T > A	$\beta^+$	1	0.4	Africa
codon 39 C > T	$\beta^0$	4	1.7	Mediterranean
IVS1-nt1 G > A	$\beta^0$	2	0.8	Mediterranean
IVS1-nt6 T > C	$\beta^{+/++}$	3	1.3	Mediterranean
IVS1-nt110 G > A	$\beta^+$	4	1.7	Mediterranean

$\beta^0$ : complete absence of beta globin on the affected allele;  $\beta^+$ : residual production of beta globin (around 10%);  $\beta^{++}$ : very mild reduction in beta globin production.

rethnic admixture based on Y-DNA and mtDNA polymorphisms (Palha *et al.*, 2011). Furthermore, historical records reveal the introduction of American immigrants, as well as English and German, these to a lesser extent, in a region inhabited by Afro-Amazonian communities in the municipality of Santarém. These immigrants arrived at the end of 1867, thereby establishing Afro-Amazonian relations, with evident inter-ethnic mixing (Funes EA, 1995 Doctoral thesis, University of São Paulo), thereby constituting an additional local source of non-African genes. Finally, historical data also reveal that immigrants from the northeast, especially Ceará State, who went to the Amazon for rubber-tapping or fleeing from the prolonged droughts between 1872 and 1910, were another important factor in the formation of local populations.

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