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Heterogeneous Distribution of HbS and HbC Alleles in Afro-derived Brazilian Populations

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KEY WORDS Screening; Afro-derived Brazilian populations; sickle cell diseases; HbS and HbC

ABSTRACT Africans brought to Brazil as slaves established communities known as *Quilombos*. As a consequence of this migration, sickle cell diseases are currently the most frequent hemoglobinopathy in the country. We sampled and analyzed for the presence of HbS and HbC 1182 individuals belonging to 11 remnants of *Quilombo* communities, distributed over three administrative regions of Brazil. Our results were pooled with literature data, resulting in the analysis of 2601 individuals from 24 populations distributed over the five Brazilian administrative regions. There were differences among weighted averages frequencies for the five Brazilian regions, and there were remarkable heterogeneities among all populations and the ones located in the same region. Additionally, we evaluated the utilization of the test of solubility and its efficiency in the field in three communities, reinforcing the reliability of the test as a first screening of hemoglobinopathy. Frequencies of hemoglobin S allele ranged from 0 to 13% and HbC allele was absent in 18 out of the 24 communities. The distribution of HbS and HbC alleles in remnants of *Quilombos* are heterogeneous and independent of geographic location probably due to differences in their history of foundation and miscegenation.

INTRODUCTION

The Brazilian population presents two particularities a) it resulted from the admixture of three distinct genetic groups, i.e., Amerindians, Africans and Europeans (mainly Portuguese), and b) the African contribution came from diverse ethnic groups.

Brazil is constituted of five administrative regions: *Norte* (North), *Nordeste* (Northeast), *Centro-Oeste* (Center-West), *Sudeste* (Southeast) and *Sul* (South). It is estimated that about 45% of the 170 million Brazilians have morphological evidence of African contribution. Percentage of contribution of the three main parental populations varies among the Brazilian regions due to differential migration, the largest African contribution being found in the *Nordeste* region.

Massive migration of Africans to Brazil happened as a consequence of the traffic of slaves towards America. About 40% of African slaves brought to the American continent came to Brazil (Reis and Gomes 1996). Various forms of resistance against slavery were observed in several American countries. In the most typical situation, runaway slaves concentrated in culturally and geographically isolated communities, named *Quilombos* or *Mocambos*. Nowadays, in Brazil as well as in other Latin American countries, there are many small villages whose history and traditions allows them to be identified as *Remnants of Quilombos*.

It has been estimated that about 2 million people live in at least 743 remnants of *Quilombo*s in Brazil, distributed as follows: 62 in the *Norte* Region, 524 in the *Nordeste* region, 15 in the *Centro-Oeste* Region, 128 in the *Sudeste* Region and 14 in the *Sul*. The Brazilian government recognized some of these communities and others are in process of

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recognition. In spite of internal migrations, these communities still have more significant cultural and genetic African contributions than other non-African derived populations.

As a consequence of the extensive forced migration of individuals from Africa to Brazil and the subsequent admixture, sickle cell disease is currently the most frequent hemoglobinopathy in the country. Estimations of sickle cell diseases in Brazil are based mostly in studies of urban populations mainly by hematological centers. It was suggested, in 1996, that almost two million people are heterozygotes HbA HbS and HbA HbC and more than 8,000 are homozygotes HbS HbS (Chamone et al. 1996). Considering that the Brazilian population increased 8.1 % from 1996 to 2000 and that the process of admixture continues, it is reasonable to expect that the number of carriers and homozygotes is increasing at the same rate of the population.

The prevalence of HbS and HbC alleles are heterogeneous when one consider the different regions of the country. The frequency of HbS and HbC is higher in the *Nordeste* region than in *Sudeste*, being more prevalent among Afro-derived individuals (Zago and Costa 1985).

Considering that remnants of *Quilombo* have higher indexes of African contribution when compared to other Brazilian communities, one can question if the establishment of public health programs emphasizing sickle cell diseases for these communities should be preceded by screenings for hemoglobin variants. Thus, the study of the distribution of hemoglobin alleles in remnants of *Quilombo* should provide the scientific knowledge about the real incidence of sickle cell diseases and of carriers of these diseases in this kind of communities. This may provide useful information for the guidance of public health programs concerning populations with higher risks of presenting the disease.

The main goal of our study was to gather information (original and previously published data) on the distribution of mutant hemoglobin alleles (HbC and HbS) in the five Brazilian regions, providing an overview of the allelic frequencies in remnants of *Quilombo* in the country. Additionally, we evaluated the utilization of the test of solubility (Magalhães and Arashiro 1977) and its reliability in field procedures.

SUBJECTS AND METHODS

Population

Figure 1 shows the geographic distribution of 24 remnants of *Quilombos* analyzed to date in Brazil for the presence of hemoglobin variants. We produced original data for eleven of these communities (described below), distributed in three of the five Brazilian regions. Access to these communities occurred usually through an initial contact with local leaders followed by communal meetings, when our goals and medical procedures were explained. Thus we included in the research only people who personally agreed to participate. These communities present the following summarized characteristics:

Nordeste Communities

Barra and Bananal: these communities are located 2 km apart in the municipality of Rio de Contas (13°36'00"S, 41°47'10"W), State of Bahia. They were officially recognized as a remnant of *Quilombo* by the Brazilian government in 1999. We sampled and analyzed blood of 118 of the 179 individuals of Barra, and 69 people of the estimated 82 individuals that inhabit Bananal.

Mocambo: the Mocambo community was officially recognized in 2000. It is located in the State of Sergipe, on the right margin of the São Francisco River (9º 45'S, 37º25'W). Population size at the time of sampling was estimated in approximately 500 individuals, from which we sampled and analyzed 171 individuals. We also collected blood samples from the fingertips of other 80 individuals exclusively for the test of solubility.

Riacho de Sacutiaba: located in the State of Bahia, 97 km North of the city of Wanderley (11°29'S, 43°47'W). We sampled and analyzed 69 individuals out of an estimated population of 209 inhabitants. In this community we also collected blood sample from the fingertips of other 24 individuals exclusively for the test of solubility.

Rio das Rãs: this community was also officially recognized as a remnant of *Quilombo* by the Brazilian government in 2000; it is located near the right margin of the São

Fig. 1. Geographic localization of remnants of *Quilombos* studied to date for hemoglobin. AP = Amapá; PA = Pará; MA = Maranhão; PI = Piauí; AL = Alagoas; SE = Sergipe; BA = Bahia; GO = Goiás; SP = São Paulo; SC = Santa Catarina; RS = Rio Grande do Sul.

Francisco River (13°41'S, 43°20'W to 13°52'S, 43°35'W), in the State of Bahia. We sampled and analyzed 276 individuals, out of a population estimated to be 4,000 people.

São Gonçalo: this community is located at 13°45'S, 41°02'W, near the city of Contendas do Sincorá in the State of Bahia. We sampled and analyzed 53 individuals, out of the 194 of the population.

Centro-Oeste Community

Kalunga: this community occupies an area of approximately 258,000 hectares in the northeast of the State of Goiás, and is located near the cities of Cavalcante, Terezina de Goiás, and Monte Alegre de Goiás. This community was officially recognized in 2000. We sampled and analyzed 76 individuals that inhabit a region known as *Vão de Muleque*, out of a total population of about 5,300 individuals inhabiting the whole *Kalunga* area.

Sudeste Communities

All of the remnants of *Quilombo* communities studied in this region are located close to or on the margins of the Ribeira River in the State of São Paulo, near to the city of Eldorado. These are:

Abobral: a small district located at 24°30'S, 48°00'W. It has not been officially recognized as a remnant of *Quilombo* yet, but its process for recognition has started. We sampled and

analyzed 130 individuals out of its 286 inhabitants at the time of sampling.

Galvão: the community of Galvão is located at 24°30'S, 48°15'W, and the government is still analyzing its recognition as a remnant of *Quilombo*. We sampled and analyzed 50 individuals, out of the 137 people that inhabited the community at the time of sampling.

São Pedro: the community of São Pedro is located in the neighborhoods of the community of Galvão, at 24°30'S, 48°15'W. It was officially recognized as a remnant of *Quilombo* by the Brazilian government in 1998. We sampled and analyzed 51 individuals from the 136 individuals that made up the community at the time of sampling.

Pedro Cubas: the community of Pedro Cubas (24°30'S, 48°15'W) was officially recognized as a remnant of *Quilombo* by the Brazilian government in 1998. We sampled and analyzed 119 individuals, out of an estimated population of 300 individuals at the time of sampling.

Laboratory Analysis

Blood samples were collected in vacutainer tubes containing EDTA as anticoagulant after individual interview. Additionally, we collected blood from children's fingertips in Mocambo and Riacho de Sacutiaba communities, using laboratory lancets.

Population screening with the test of solubility of hemoglobin was performed for Mocambo, Riacho de Sacutiaba and Rio das Rãs communities in the field. In Rio das Rãs the test was performed only for those people that had venous blood collected, while in the other two communities we performed the test both with venous and fingertip blood. This test is a micro method to recognize insoluble hemoglobin in filter paper using a drop of total blood mix with a buffer solution of saponin 1% acting as a reductor solution (Magalhães and Arashiro 1977). Positive test means presence of hemoglobin S but it is not possible to distinguish homozygote HbS/HbS of heterozygotes HbS/HbC or HbA/HbS. Thus, confirmation must be done using conventional electrophoresis or RFLP analysis. Relatives of all the subjects that presented positive results for the test of solubility, as well as the subjects with positive results whose samples had been obtained from fingertips were also solicited to collect venous blood.

After processing, blood samples were analyzed by horizontal starch gel (14%) electrophoresis, according to Naoum (1987). In Barra, Abobral, Galvão, São Pedro and Pedro Cubas communities, the presences of HbC and HbS were analyzed by RFLP. For this purpose, DNA was purified and a fragment of 382 bp was obtained by PCR using specific primers, and then digested with DdeI for detection of HbS and BseRI for HbC. Products of digestion were analyzed in agarose gel 1.5% with ethidium bromide (Akar et al. 1996).

Allelic frequencies were estimated by gene counting. The heterogeneity test inter populations (for all the 24 populations together), intra-region (for four of the regions, since the Centro-Oeste region had only one population), and inter-regions (weighted average for each of the five regions) was performed by Pearson's Chi-square, using BIOSYS software (Swofford and Selander 1981).

RESULTS AND DISCUSSION

Evaluation of the Test of Solubility of Hemoglobin and Its Reliability in Field Procedures

Figure 2 shows a result of a test of solubility of hemoglobin. All subjects from Mocambo, Riacho de Sacutiaba and Rio das Rãs communities were screened for presence of HbS in the field, through the test of solubility of hemoglobin (Magalhães and Arashiro 1977). In Mocambo, there was no evidence of presence of hemoglobin S in blood samples obtained through fingertips, but there was a frequency of 7.1% of it in venous blood samples. In Riacho de Sacutiaba, the presence of hemoglobin S was evidenced in 16.6% of the fingertips samples and 26.1% of the venous blood samples. In Rio das Rãs, the presence of hemoglobin S was evidenced in 5.6% of the venous samples.

All positive results observed in the test of solubility were confirmed by electrophoresis, i.e., no false positives were observed. Thus, the test of solubility of hemoglobin presents many advantages that become it ideal for screening large communities as 1) it can be done with blood drops, 2) it does not require sophisticated

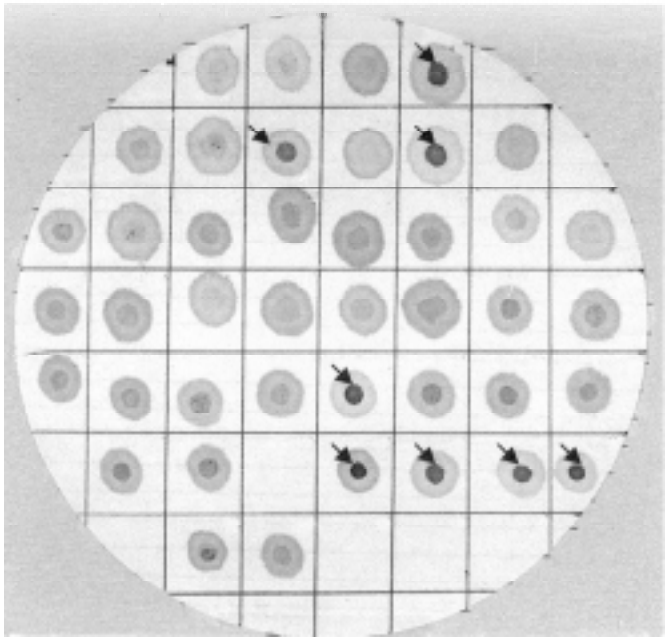

Fig. 2. A result of the test of solubility of hemoglobin. Arrows point to presence of S hemoglobin

equipment, and 3) it is cheap and reliable. However, it must be emphasized that it does not distinguish homozygotes and heterozygotes, and it does not detect the eventual presence of other abnormal alleles of hemoglobin, such as the allele C.

Frequencies of the Alleles HbS and HbC in Afro-Brazilian Populations

Table 1 presents the frequency distribution of the alleles of hemoglobin in the Brazilian remnants of *Quilombos*, according to their geographic distribution. Frequency of HbS ranged from 0 in Itamoari (*Norte* region) and Paredão (*Sul* region) to 13.0% in Riacho de Sacutiaba (*Nordeste* region). The weighted average of this allele for all of the Brazilian remnants of *Quilombos* combined is 3.7% (Table 2).

The complete set of data shows that geographic distance apparently does not influence the distribution of the alleles. In other words, the fact that some communities are located in the same state or geographic region does not implicate neither that there is gene flow between them nor that the foundation occurred in a similar way. For example, despite the communities of São Gonçalo and Riacho de Sacutiaba (*Nordeste* region) are geographically close to each other, they present very distinct frequencies for the allele S (0.9% and 13.0%, respectively). In Riacho de Sacutiaba, the observation of a high frequency of allele S - in fact, the highest not only among the populations studied in this work but also among all of the populations studied so far in Brazil - can be explained by its historic origin, since most of the population is directly related. In *Norte* region, allele S ranged from 0 in Itamoari to 6.0% in Trombetas. In *Sudeste* region, all the analyzed populations are geographically very close to each other. However, the frequency of HbS in Abobral is 8.1%, while in the other three communities it drops to about 1%. Random drift, founder effects and admixture could explain large differences in the frequencies of mutant alleles, even in communities that are geographically close, since many of these communities were usually founded by a few couples and popu-lation sizes are small.

Total absence of the allele C was observed

Table 1: Allelic frequencies of HbA, HbS HbC in Afro-derived Brazilian populations

Population	*State*	*N*	*Allelic frequencies*			*References*
			HbA	*HbS*	*HbC*	
Norte						
Curiaú	Amapá	145	0.893	0.045	0.062	1
Cametá	Pará	93	0.995	0.005	0	2
Trombetas	Pará	201	0.940	0.060	0	3
Pacoval	Pará	166	0.964	0.036	0	1
Itamoari	Pará	88	1.000	0	0	4
Nordeste						
Cajueiro	Maranhão	45	0.912	0.066	0.022	5
Mimbó	Piauí	62	0.968	0.032	0	6
Sítio Velho	Piauí	59	0.975	0.025	0	6
Gaucinha	Piauí	21	0.976	0.024	0	6
Muquém	Alagoas	251	0.924	0.076	0	7
Quilombo	Alagoas	203	0.968	0.032	0	7
Mocambo	Sergipe	171	0.962	0.038	0	Present study
Rio das Rãs	Bahia	276	0.935	0.027	0.038	Present study
Riacho de Sacutiaba	Bahia	69	0.870	0.130	0	Present study
São Gonçalo	Bahia	53	0.972	0.009	0.019	Present study
Barra	Bahia	118	0.996	0.004	0	Present study
Bananal	Bahia	69	0.978	0.022	0	Present study
Centro-Oeste						
Kalunga	Goiás	76	0.980	0.013	0.007	Present study
Sudeste						
São Pedro	São Paulo	51	0.990	0.010	0	Present study
Galvão	São Paulo	50	0.990	0.010	0	Present study
Abobral	São Paulo	130	0.919	0.081	0	Present study
Pedro Cubas	São Paulo	119	0.987	0.013	0	Present study
Sul						
Valongo	Santa Catarina	57	0.992	0.008	0	8
Paredão	Rio Grande do Sul	28	0.964	0	0.036	2

1. Guerreiro et al. 1999; 2. Bortolini et al. 1992; 3. Schneider et al. 1987; 4. Oliveira et al. unpublished; 5. Bortolini et al. 1998; 6. Arpini-Sampaio et al. 1998; 7. Pedrosa 1998; 8. Souza and Culpi 1991

in 18 out of the 24 communities investigated. Among the six that presented the allele C, frequencies ranged from 0.7% in Kalunga (*Centro-Oeste* region) to 6.2% in Curiau (*Norte* region). It is remarkable that this allele is present in frequencies even higher than those observed for the S allele, except in Cajueiro and Kalunga communities. It is also peculiar that allele S is completely absent in Paredão community, where the allele C was observed at a frequency of 3.6%.

Absence of the alleles S and C can be a consequence of allelic loss and/or founder effect. As these populations are generally small, the occurrence of genetic drift can perhaps explain these findings. The same factors can explain the occurrence of high frequencies of the allele C, for example in the populations of Rio das Rãs, Paredão and Curiau, which are geographically distant and present no gene flow among themselves.

About two-thirds of the black slaves that arrived in the ports of Rio de Janeiro (*Sudeste* region) and Pernambuco (*Nordeste* region) came from Angola, having boarded in the ports of Luanda, Bengali and Congo. The other one-third had reached Brazil mainly through the port of Bahia (*Nordeste* region). The weighted averages observed in African populations at countries where Brazilian slaves came from is 8.6% to allele HbS and 2.5% to allele HbC. Globally, the frequency of the allele HbC varies from 0.2% in Libya to 4.7% in Niger (Roychoudhury and Nei 1988). The observation of higher frequencies of these alleles in some of the remnants of *Quilombos* (Table 1), in comparison to the observed in the parental African populations, reinforce the hypothesis of founder effect associate to genetic drift in the establishment of these communities.

We found that the difference relative to the distribution of the HbS and HbC among the five Brazilian regions was statistically significant (Chi-square = 17.7373; D.F. = 8; P = 0.023) at a significance level of 5%. However, some interesting aspects must be highlighted: the frequencies of the *Centro-Oeste* and *Sul* regions were most similar, while the weighted averages frequencies of the other regions are similar among them and different from the first two (Table 2). It must be reminded that the *Centro-Oeste* and *Sul* are the regions where the lowest number of Brazilian remnant of *Quilombos* were sampled and described until this moment.

Table 2: Weighted averages of alleles frequencies of HbA, HbS e HbC in Brazilian Remnants of *Quilombos* (Afro-derived populations) grouped per region.

Region	*N*	*Allelic frequencies (weighted averages)*		
		HbA	*HbS*	*HbC*
Norte	693	0.951	0.036	0.013
Nordeste	1397	0.950	0.041	0.009
Centro-Oeste	76	0.980	0.013	0.007
Sudeste	350	0.963	0.037	0.000
Sul	85	0.983	0.005	0.012
Total	2601	0.954	0.037	0.009

There is also statistical significant difference among the 24 studied populations (Chi-square =306.187; DF = 46; P = 0.000). When the populations are analyzed considering their geographic distribution, there is heterogeneity among the populations from the *Norte* (Chi-square = 88.017; DF = 8; P = 0.000), *Nordeste* (Chi-square =134.697; DF = 22; P = 0.000) and *Sudeste* (Chi-square = 22.035; DF = 3; P = 0.000). The only two populations studied in the *Sul* region were shown to be homogenous (Chi-square = 4.590; DF = 2; P = 0.101) despite clear difference between them: Paredão presents the allele C and does not present the allele S and, on the other hand, Valongo presents the allele S and does not present allele C. It was not possible to make a similar analysis in the *Centro-Oeste* region because only one population was sampled.

The pattern of distribution of the alleles S and C of hemoglobin at the Brazilian remnant of *Quilombos* show neither a cline nor any other geographic trend that could guide health actions or the implementation of a strategy unique for all of the communities. Because of its apparent random distribution patterns, probably determined by internal formation processes (initial population founders, migration and inter-ethnic admixture) of each community, health programs must be formulated and applied in a particular way, according to specific characteristics observed in each population. In conclusion, the distribution of HbS and HbC alleles in remnants of *Quilombo*s are heterogeneous and independent of geographic location and, as a consequence, the incidence of sickle cell diseases are heterogeneous and a population screening, using, for example, the test of solubility, could precede a public health program.

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