Hemoglobin Variant Profiles among Brazilian Quilombola Communities


ABSTRACT
Brazilian Quilombolas are communities composed of African-derived populations that have their territories guaranteed by the Brazilian Constitution. The present study investigated the hemoglobin (Hb) variants among these population groups. This study was conducted in a total of 2843 individuals of Brazilian Quilombola communities of the Bahia, Pará, and Piauí states. All the participants had their Hb profiles evaluated. The Hb S (HBB: c.20A>T) variant was described in all the studied localities. However, the individuals in Bahia State had the highest frequency of the Hb C (HBB: c.19G>A) variant; individuals from Piaui State had a higher frequency of the Hb D-Punjab (HBB: c.364G>C) variant compared to the other states, and individuals from Pará State only carried the Hb S variant. The present study revealed a specific distribution of Hb variants that could represent different waves of African influence in these Brazilian populations.

Introduction
Historically, Brazil was colonized by Portugal for over three centuries, and its economy was based on the exploitation of cheap slave labor brought from Africa; these slaves had no political or social rights. The Portuguese colonization in Brazil was maintained by the Africans and their descendants in poor conditions. Africans and their descendants in Brazil were associated with the Bantu people who were organized and took refuge in places called Quilombos, where they lived according to their culture and custom. The term Quilombo (Kilombo) is of African origin from the Bantu language and means ‘village.’ The presence of Quilombos in Brazil was associated with the Bantu people who were brought from Africa and enslaved during the Brazilian colonization [1].

The Brazilian Constitution guarantees the human rights of African origin communities in its territory, provides public policies for Quilombola regularization, and ensures that the land belongs to the remnants of Quilombo communities. According to the Brazilian Ministry of Culture, there are 3524 identified Quilombola communities in Brazil, and of these, 1342 are legally recognized. The federal government seeks to improve the quality of life in these communities through public policies aimed at the Quilombola population [2].

Since 2004, the Brazilian Quilombola Program has coordinated governmental actions inside these communities, which have important socioeconomic deficiencies, as shown by the results of the Brazilian population census of 2010 [2,3]. Some of these actions are directed to assist the most prevalent pathologies, including hemoglobinopathies, a group of genetic diseases that have a high incidence and prevalence in African countries and, most likely, in their descendant groups such as the Quilombola communities [4].

Hemoglobinopathies are classified by structural or synthetic changes in genes associated with the synthesis of globins, the protein portion of the hemoglobin (Hb) molecule. These diseases include structural Hb variations and thalassemias. Sickle cell disease is a group of diseases associated with the presence of Hb S (HBB: c.20A>T); the most severe form is called sickle cell anemia, which has the Hb SS (ββ) homozygous phenotype. However, Hb S can be found in association with other Hb variants or with thalassemia, such as in Hb SC disease (HBB: c.19G>A), and Hb S-β-thalassemia (Hb S-β-thal). Notably, patients with sickle cell disease have a high morbidity and mortality and a heterogeneous clinical picture, requiring frequent hospitalization and specific vaccines, therapies, and medical follow-up; a high cost of public resources is spent on monitoring these patients [5].

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Supplemental data for this article can be accessed here.

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Hemoglobinopathies are among the most common monogenetic diseases worldwide [5]. According to the World Health Organization (WHO), approximately 5.0% of the world’s population carries gene alterations related to hemoglobinopathies; the genes associated with sickle cell disease and thalassemia represent the most common types. In addition, over 300,000 infants with the most severe form of hemoglobinopathies are born each year worldwide, and the majority of these births are in sub-Saharan Africa [4]. These diseases occur at a high prevalence in the tropics, but with the occurrence of population migrations, the gene alterations have spread to several countries worldwide [6].

Brazil has received a large migration of individuals, resulting in a population with a high cultural, social, and ethnic diversity [7]. This is corroborated by the heterogeneous distribution of hemoglobinopathies in Brazil, including a high prevalence of thalassemia in the south and southeast regions, and of Hb variants, such as Hb S and Hb C, in the north and northeast regions [8,9]. Because of the large interbred component of the Brazilian population, which is compounded by Indians (the original natives), Europeans, particularly Portuguese and Spaniards, and Africans from different African countries, Brazil has a population with unique ethnic characteristics. These populations are mixed in different proportions in its 27 federal units, with 26 states and a federal district that includes the capital of the country [10]. Despite the high number of Quilombola communities in Brazil, few studies have investigated the prevalence of Hb variants of this population. The present study aimed to investigate Hb variant frequencies among several Quilombola communities in Brazil and to determine whether sickle cell disease is predominant.

Materials and methods

We studied the prevalence of the Hb variants in Quilombola communities in São Francisco do Conde (SFC) (Bahia), Laje dos Negros (Bahia), Queimada Nova, Amarante and Paulistana (Piauí), and Saracura and Arapemá (Pará). Locations included in this study were chosen based on their histories of colonization and their stratifications as Quilombola communities [2].

We conducted a cross-sectional study, and 2843 permanent residents of these specific Quilombola Brazilian communities were included in this study. All of the participants were informed about the purpose of the research and signed an informed consent form.

The human research board of the Centro de Pesquisas Gonçalo Moniz of Fundação Oswaldo Cruz, Salvador, Bahia, approved the studies that were conducted at SFC and Laje dos Negros. The Piauí Quilombola study was approved by the human research board of the Universidade Federal do Piauí, Terezina, Piauí, Brasil, and the Saracura and Arapemá study was approved by the human research board of Fundação Oswaldo Cruz in Amazonia, Amazonas, Manaus, Brasil. All of the studies were performed in accordance with ethics principles and with the Declaration of Helsinki and its revisions (1975).

The state of Bahia is located in the northeast of Brazil and houses 638 Quilombola communities, which are certified and regulated by specific law. This state has 417 municipalities, including the SFC and Campo Formoso [11]. The municipality of SFC is located in the Recôncavo of Bahia and displays a strong colonial Brazilian heritage. In the past, the city’s economy was based on sugar cane plantations. Currently, extracting, refining and processing oil are the main economic activities in the region, which gives this city the largest national gross domestic product per capita; however, the municipality of SFC has a poverty rate of 55.02% [12]. This study included 1246 children aged 6–12 years from elementary schools of SFC. The data were collected from October 2010 to March 2011.

The Saliter River Basin is a sub-basin of the São Francisco River located in North-Central Bahia and has an area of 13,467.93 km². This basin is located in the city of Campo Formoso, which has a population of approximately 72,271 inhabitants [13]. The Laje dos Negros, a remnant of the Quilombo, are located in Campo Formoso, Bahia, Brasil. The Laje dos Negros community has significant natural wealth, large mountains, several minerals (chrome, crystal), and caves; however, few houses have adequate sanitation. The sample from Laje dos Negros included 98 individuals living in the vicinity of the river.

The state of Piauí is located in the northwest of Northeastern Brazil; it occupies an area of 251,529 km² and has 3,195,000 inhabitants [14]. There are several Quilombola communities in the rural areas in the state of Piauí. Samples from these Quilombola communities included 1240 individuals aged 1–89 years.

The state of Pará is located in the east of the northern region of Brazil; it covers an area of 1,247,950.003 km² and has 8,074,000 inhabitants [15]. Located in Santarém, Pará has Quilombola communities in Saracura and Arapemá. These were the first Quilombola communities in western Pará to receive recognition and delineation in July 2010. Saracura and Arapemá have territories of 2,889 and 3,828 hectares, respectively. The sample communities of Saracura and Arapemá included 259 individuals aged 10–88 years.

Blood samples were collected and sent to the Department of Clinical Analyses and Toxicology located at Faculdade de Farmácia, at Universidade Federal da Bahia (UFBA). The hematological parameters were obtained using a Coulter Counter hematology analyzer (Coulter Corporation, Miami, FL, USA).

The Hb profiles were analyzed by high performance liquid chromatography (HPLC) using the VARIANT II™ Hb analyzer system (Bio-Rad Laboratories, Hercules, CA, USA). The integrated peaks are assigned to manufacturer-defined windows derived from the retention time (RT). All individuals with Hb S showed a peak in the S-window with RTs ranging from 4.30 to 4.70 min. In addition, all individuals with Hb C showed a peak in the C-window with RTs ranging from 4.90 to 5.30 min. Likewise, all individuals with Hb D showed a peak in the D-window with RTs ranging from 4.06 to 4.13 min., indicating presence of Hb D-Punjab (HBB: c.364G>C).
All the individuals of this study presented Hb A\textsubscript{2} levels within the reference value with RTs in the A\textsubscript{2}-window, ranging from 3.30 to 3.90 min. Since all of them had Hb A\textsubscript{2} within the reference value, we are able to exclude the possibility of individuals heterozygous for Hb C and \(\beta\)-thal, and also for Hb S and \(\beta\)-thal. In addition, we did not find individual carriers of higher levels of Hb variant compared to the levels of Hb A, a characteristic exhibited by compound heterozygotes for association of these types of Hb and \(\beta\)-thal, escaping the pattern of an individual with the trait, which presents a balance between the amount of both Hbs.

The Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 17.0 was used to construct the database and to process the data using descriptive statistics to characterize the studied populations. To evaluate the association of Hb variants in the different communities, we used the \(\chi^2\) test in the program GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) version 5.02. A \(p\) value of <0.05 was considered to be statistically significant.

**Results**

The present study evaluated Brazilian individuals of Quilombola communities, and according to the Hb pattern analyzes, the Hb AA phenotype or the normal Hb profile was found in 89.57\% (1116/1246) of the individuals from SFC. In Laje dos Negros, the Hb AA phenotype was found in 88.77\% (87/98) of the individuals. In Piauí, the Hb AA phenotype was found in 92.90\% (1152/1240) of the individuals in the SFC and Arapemà communities in Pará, 94.60\% (245/259) of the studied individuals had normal Hb profiles (Table 1).

The prevalence of sickle cell disease among the studied individuals from SFC was 0.24\% (Hb SC and Hb SS), of whom 0.08\% (1/1246) had the Hb SS phenotype and 0.16\% (2/1246) had the Hb SC phenotype. In Laje dos Negros, 2.04\% (2/98) had the Hb SC phenotype. Sickle cell disease was found in 0.89\% (11/1240) of the studied individuals in Piauí, of whom 0.32\% (4/1240) had the Hb SC phenotype and 0.57\% (7/1240) had the Hb SS phenotype. In the communities of Saracura and Arapemà, 0.4\% (1/259) had the Hb SS phenotype (Table 1).

The homozygous phenotype of Hb C disease (\(\beta^C/\beta^C\)) was only found in SFC and Laje dos Negros. In SFC, the Hb CC disease was found in 0.08\% (1/1246) individuals; in Laje dos Negros, this disease was found in 1.02\% (1/98) individuals. The heterozygous phenotypes of Hb D-Punjab (\(\beta^A/\beta^D\)-Punjab) and Hb D-Punjab disease (\(\beta^D\)-Punjab/\(\beta^D\)-Punjab) were only found in Piauí, 0.57\% (7/1240) of whom carried the Hb AD phenotype and 0.08\% (1/1240) carried the Hb D-Punjab disease phenotype. The heterozygous phenotype of Hb S (Hb AS) was found in three of the studied localities with a frequency of 4.89\% (61/1246) in SFC, 5.40\% (67/1240) in Piauí, and 5.00\% (13/259) in Pará (Table 1).

The heterozygous Hb AC phenotype was found in SFC, Laje dos Negros and Piauí. In SFC, 5.22\% (65/1246) had the heterozygous Hb AC phenotype. In Laje dos Negros, 8.17\% (8/98) had the heterozygous Hb AC phenotype, and in Piauí, 0.16\% (2/1240) also had the heterozygous Hb AC phenotype (Table 1).

Of the 259 individuals in Saracura and Arapemà, 52.5\% (136/259) were female and 47.5\% (123/259) were male. The Hb AA phenotype was found in 95.6\% (130/136) of the females and in 93.5\% (115/123) of the males. The Hb AS phenotype was found in the total of 13 individuals, corresponding to a rate of 3.6\% (5/136) in females and 6.5\% (8/123) in males. The Hb SS phenotype was found in 0.8\% of the females (1/136) (Table 2).

In the 1344 people of SFC and Laje dos Negros, 49.9\% (671/1344) of the hemoglobinopathies occurred in females and 50.1\% (673/1344) occurred in males. The Hb AA phenotype was found in 1203 individuals [corresponding to rates of 88.1\% (591/671) in females and 91.0\% (612/673) in males]. The Hb AS phenotype was found in 61 individuals [5.4\% (36/671) of females and 3.7\% (25/673) of males]. The Hb AC phenotype was found in 73 individuals [5.5\% (37/671) of females and 5.3\% (36/673) of males]. The Hb SC phenotype was found in four females [0.6\% (4/671)]. The Hb SS phenotype was found in one female [0.1\% (1/671)], and the Hb CC phenotype was found in two females [0.3\% (2/671)] (Table 2).

Of the 1,240 individuals in Piauí, 54.8\% (680/1,240) were females and 45.2\% (560/1240) were males. The Hb AA phenotype was found in 1,152 individuals [92.0\% (626/680) of females and 94.0\% (526/560) of males]. The Hb AS phenotype was found in 44 (6.6\%) individuals among the 680 females, and 23 (4.1\%) individuals among the 560 males.

The Hb AC phenotype was found in two females (0.3\%, 2/680). The Hb SC phenotype was found in four individuals [0.1\% (1/680) of females and 0.5\% (3/560) of males]. The Hb SS phenotype was found in seven individuals [0.7\% (5/680) of females and 0.3\% (2/560) of males]. The Hb AD phenotype was found in seven individuals [0.3\% (2/680) of females and 0.9\% (5/560) of males]. The Hb DD phenotype was found in one male (0.2\%, 1/560). Table 2 shows the Hb variant patterns and their distribution between sexes in the Quilombola groups in the Brazilian states of Bahia, Piauí and Pará.

When comparing the individuals of the Quilombola communities, we found that the SFC community had a higher rate of Hb variants than the Piauí and Pará communities [odds ratio (OR) = 1.542; 95\% confidence interval (95\% CI) = 1.161 to 2.049 and OR = 2.039; 95\% CI = 1.155 to

**Table 1. Distribution of hemoglobin variant profiles among the studied Brazilian Quilombola communities.**

<table>
<thead>
<tr>
<th>Communities (n)</th>
<th>Hb AA (%)</th>
<th>Hb AS (%)</th>
<th>Hb AC (%)</th>
<th>Hb SC (%)</th>
<th>Hb SS (%)</th>
<th>Hb CC (%)</th>
<th>Hb AD (%)</th>
<th>Hb DD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laje dos Negros (98)</td>
<td>87 (88.77)</td>
<td>–</td>
<td>8 (8.17)</td>
<td>2 (2.04)</td>
<td>–</td>
<td>1 (1.02)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>São Francisco do Conde (1,246)</td>
<td>1,116 (89.57)</td>
<td>61 (4.89)</td>
<td>65 (5.22)</td>
<td>2 (0.16)</td>
<td>1 (0.08)</td>
<td>1 (0.08)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Piauí (1,240)</td>
<td>1,152 (92.90)</td>
<td>67 (5.40)</td>
<td>2 (0.16)</td>
<td>4 (0.32)</td>
<td>7 (0.57)</td>
<td>–</td>
<td>7 (0.57)</td>
<td>1 (0.08)</td>
</tr>
<tr>
<td>Pará (259)</td>
<td>245 (94.60)</td>
<td>13 (5.00)</td>
<td>–</td>
<td>–</td>
<td>1 (0.40)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Hemoglobin variants are endemic in Africa and play a strong selective pressure in combination with malaria infections; carriers of these Hb profiles were brought to Brazil during slave trafficking times [18]. The distribution of Hb variants was heterogeneous among the Brazilian states, and Bahia has the highest incidence and prevalence of sickle cell disease and other Hb variants [8,19,20].

Interestingly, we found different frequencies of Hb variants in the Quilombola groups in our study, in which SFC had similar frequencies of Hb S and Hb C; there were also two individuals with Hb SC, one case of Hb SS and one case of Hb CC disease. We identified two Hb SC individuals in Laje dos Negros and only Hb S in Pará; moreover, Piauí had both sickle cell disease and Hb D-Punjab individuals, which were not found in the other Quilombola communities in this study. Thus, we found a distinct Hb variant distribution among the studied Quilombola communities, as observed in the general population of Brazil. This may represent different waves of influence of African populations in Brazilian regions.

The origin of Hb S possibly occurred independently in three African regions: Atlantic Africa (Senegal), West Africa, and Bantu-speaking Africa [21]. In our country, the Hb S distribution was heterogeneous and present in all Brazilian regions. The high Hb S frequency may be associated with African ancestry in Brazil, as a result of the illegal slave trade of African people. The Bahia State received slaves from

Table 2. Distribution of sex and hemoglobin profile among the studied Quilombola populations.

<table>
<thead>
<tr>
<th>Brazilian states (n)</th>
<th>Sex n (%)</th>
<th>Hb AA</th>
<th>Hb AS</th>
<th>Hb AC</th>
<th>Hb SC</th>
<th>Hb SS</th>
<th>Hb CC</th>
<th>Hb AD</th>
<th>Hb DD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bahia (1,344)</td>
<td>Females: 671 (49.9)</td>
<td>591 (88.1)</td>
<td>36 (5.4)</td>
<td>37 (5.5)</td>
<td>37 (5.5)</td>
<td>4 (0.6)</td>
<td>1 (0.1)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Males: 673 (50.1)</td>
<td>612 (91.0)</td>
<td>25 (3.7)</td>
<td>36 (5.3)</td>
<td>36 (5.3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Piauí (1,240)</td>
<td>Females: 680 (54.8)</td>
<td>626 (92.0)</td>
<td>33 (6.6)</td>
<td>2 (0.3)</td>
<td>1 (0.1)</td>
<td>5 (0.7)</td>
<td>–</td>
<td>2 (0.3)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Males: 560 (45.2)</td>
<td>526 (94.0)</td>
<td>23 (4.1)</td>
<td>–</td>
<td>3 (0.5)</td>
<td>2 (0.3)</td>
<td>–</td>
<td>5 (0.9)</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Pará (259)</td>
<td>Females: 136 (52.5)</td>
<td>130 (95.6)</td>
<td>5 (3.6)</td>
<td>–</td>
<td>–</td>
<td>1 (0.8)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Males: 123 (47.5)</td>
<td>115 (93.5)</td>
<td>8 (6.5)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
</tr>
</tbody>
</table>

Table 3. Mean corpuscular volume and hemoglobin variant levels of the studied Quilombola populations.

<table>
<thead>
<tr>
<th>Laboratory data</th>
<th>Communities (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LaJE dos Negros</td>
</tr>
<tr>
<td>Hb AS</td>
<td>–</td>
</tr>
<tr>
<td>Hb S (%)</td>
<td>34.65 ± 4.36</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>80.45 ± 1.90</td>
</tr>
<tr>
<td>Hb AC</td>
<td>–</td>
</tr>
<tr>
<td>Hb C (%)</td>
<td>32.22 ± 2.20</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>78.23 ± 4.36</td>
</tr>
<tr>
<td>Hb SC</td>
<td>–</td>
</tr>
<tr>
<td>Hb S (%)</td>
<td>48.00 ± 2.40</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>38.35 ± 1.62</td>
</tr>
<tr>
<td>Hb AD</td>
<td>–</td>
</tr>
<tr>
<td>Hb D (%)</td>
<td>38.95 ± 2.15</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>–</td>
</tr>
<tr>
<td>Hb DD</td>
<td>–</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>88.65 ± 0.00</td>
</tr>
</tbody>
</table>

SD: standard deviation; Hb: hemoglobin; MCV: mean corpuscular volume.

Figure 1. Hemoglobin patterns were searched to establish the Hb phenotypes of Quilombola population groups in Brazil. Analyses of individuals from SFC vs. the studied group of Piauí (A) and Pará (B) showed that SFC had the highest prevalence in the individuals with Hb variants (OR = 1.542; 95% CI = 1.161 to 2.049 and OR = 2.039; 95% CI = 1.155 to 3.599, respectively).
different African regions that speak Bantu (Congo and Angola), Gbè (Benin and Nigeria), Oirubá-nagó (Benin) and Haussá (Nigeria and Republic of Cameroon). Para State received slaves who speak Bantu and Gbè, and in Piauí State only Bantu-speakers were found [22].

Hb C is common in West Africa, especially in Burkina Faso and is associated with a protective role against severe malaria [23]. Hb C has high frequency in Central West Africa and has its frequency reduced concentrically from this place. This Hb is found in Niger River west and in places where Hb S is present [24]. In our study, this Hb was found in Bahia and Piauí states and both received Bantu African slaves, demonstrating the influence of this ethnic group in Hb profile of two different Brazilian states.

The Hb D-Punjab was first described by Itano in 1951 [25] and is the third most common Hb variant in Brazil. It is prevalent in India, more specifically in the Gujarat region, however, it can also be found in Italy, Belgium and Austria [26]. This Hb is polymorphic in Africa and in South Asia, and has the highest frequency in West Africa that can be considered an origin center [23]. In our study, we only found Hb D-Punjab in Piauí State, which, according to Anjos and Cypriono [22], received slaves only from the Bantu African region. Therefore, this data is not in accord with the location of this Hb in Africa [22].

Our study did not identify different distributions related to sex and gender. This result differed from Carneiro et al. [27] who described differences in the distribution of Hb variants between the sexes.

Importantly, based on our results, we found a high frequency of Hb variants, with description of sickle cell disease and other hemoglobinopathies. These results demonstrate that in these Brazilian Quilombola communities, clinical follow-up of individuals with sickle cell disease and hemoglobinopathies is required, and also genetic counseling for individuals with the Hb variants.

We found a specific distribution of Hb variants in all of the Quilombola communities investigated in this study; this could represent different waves of African influence in these Brazilian populations (Figure S1). We cannot forget the importance of conducting genetic studies in these population groups with the aim to clarifying the African origins and their interactions with other ethnic groups. This will allow us to identify their genetic origins and the contribution of different people who have participated in the Brazilian population history and the pattern of disease they have exhibited.

Acknowledgements

We thank the Quilombola communities of São Francisco do Conde, Laje dos Negros, Saracura, Arapemã, Queimada Nova, Amarante and Paulistana for their participation, because without these communities, this study would not have been conducted. We would like to thank to the editors of American Journal Experts for the great improvement on the English language, grammar, spelling, and overall style of this manuscript. RPS, RMO and LFS performed the sample collection, analyzed the data and co-wrote the manuscript; CVBF, JPMN, DOS, LMF and AFHG performed the experiments; RPS, RMO, LFS, JFM, EVA and CGB co-wrote and critically reviewed the manuscript; and MSG idealized the project, contributed intellectually, analyzed the data and co-wrote the manuscript.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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