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SHORT COMMUNICATION

The β-Globin Gene Cluster Haplotypes in Sickle Cell Anemia Patients from Northeast Brazil: A Clinical and Molecular View

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ABSTRACT

The β^S-globin haplotypes were studied in 78 sickle cell Brazilian patients from Bahia, Northeast Brazil, that has a large population of African origin. Hemoglobin (Hb) profiles were developed by high-performance liquid chromatography (HPLC), and β^S-globin gene haplotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) techniques. We identified 44 (55.0%) patients with the CAR/Ben (Central African Republic/Benin) genotype, 16 (20.0%) Ben/Ben, 13 (16.2%) CAR/CAR and seven (8.8%) with other genotypes. Analyses

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of the phenotypes showed clinical differences related only to Hb F levels and blood transfusion therapy; the presence of $-\alpha^{-3.7}$-thalassemia (thal) demonstrated statistical significance when associated with hematocrit ($p = 0.044$), MCV ($p = 0.0007$), MCH ($p = 0.012$) and spleen sequestration events. The haplotype diversity found in the present study can be justified by information about the origin of the slave traffic period in Bahia during the 19th century. The specific characteristics described among the Bahian sickle cell patients could be confirmed by increasing the number of patients with specific genotypes and further studies of genetic markers.

Key Words: β-Globin haplotypes; Sickle cell anemia; Northeast Brazil; Slave trade.

The β^S-globin gene haplotypes are named according to the geographic areas where they predominate and are useful in the definition of African population origins (1). They have been classified as five different types: the Benin (Ben) has been associated with Midwestern Africa; the Bantu or Central African Republic (CAR) with South-Central and Eastern Africa; the Senegal (Sen) with Atlantic West Africa, the Arab–Indian type with the Indian subcontinent and Eastern Arabian Peninsula, and the Cameroon (Cam) along the west coast of Africa (1,2). The Senegal haplotypes have been associated with high Hb F levels and a less severe clinical course, the Benin haplotype with an intermediate clinical course and Hb F levels, and the Central African Republic haplotype with a low Hb F level and a more severe clinical course (2,3). The Arab–Indian haplotype presents the highest Hb F levels with a heterogeneous clinical course (4).

Brazil is the largest country of South America and has a population with a high rate of racial admixture with a strong compound of African genes that were introduced by the slave trade (5). The country has a high prevalence of hemoglobin (Hb) disorders, with the South presenting a frequency of 6.6% sickle cell trait (Hb AS) in a Black population (6), and the Bahia state, located in Northeast Brazil, with a frequency of 7.5% to 15.7%, when different groups of this population were studied (7). In the present study, we investigated the β^S-globin gene haplotypes in 80 sickle cell anemia patients from the Blood Center of Bahia State, in order to confirm the African origin of this population.

The Hb profile was obtained by high-performance liquid chromatography (HPLC) (VARIANT II™; Bio-Rad Laboratories, Hercules, CA, USA) and the DNA was isolated from peripheral blood leukocytes by the GFX™ Genomic Blood DNA Purification KIT (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The β^S-globin gene haplotypes were investigated by polymerase chain reaction (PCR) and the haplotype polymorphic sites identified by restriction fragment length polymorphism analysis (RFLP) as previously described (8).

Among the 160 β^S chromosomes analyzed, 78 (48.8%) were characterized as the Benin type, 74 (46.2%) Central African Republic, three (1.9%) Cameroon, one (0.6%) Arab–Indian, one (0.6%) Senegal and three (1.9%) were characterized as other haplotypes. The genotype characterization showed 44 (55.0%) CAR/Ben, 16 (20.0%) Ben/Ben, 13 (16.2%) CAR/CAR and seven (8.8%) classified as other genotypes. Statistical significance for use of blood transfusion therapy occurred more frequently in

patients with Hb F levels below 10.0% ($p = 0.0015$); statistical differences were observed for hematocrit ($p = 0.044$), MCV ($p = 0.0007$), MCH ($p = 0.012$) and the spleen sequestration events ($p = 0.032$) among $-\alpha^{-3.7}$-thal carriers, according to other studies (9,10).

In this study, the Benin haplotype frequency (48.8%) was slightly higher than the Central African Republic haplotype (46.2%), and the prevalence of the CAR/Ben genotype (54.0%) was consistent with the results described by Gonçalves et al. (11) who studied the β^S-globin gene haplotypes among sickle cell disease patients from Salvador-Bahia, Brazil. The slave trade in Brazil was extensively reported by Curtin (12) who described Brazil as the largest, single importer into the Americas. The information from the British and Brazilian government offices described uncertainty about the Brazilian imports during the 1820s and 1830s, with a total of 8000 slaves of unknown origin imported into Bahia in the 19th century between 1817 and 1843, justifying the presence of the Cameroon, Senegal and Arab–Indian haplotypes found in the present study (12).

These results are different from those observed among other American countries, confirming the diversity of the African influence in Bahia. The United States of America and Jamaica received slaves from Midwestern Africa during the British Atlantic slave trade, where the Benin haplotype is more prevalent. However, in Mexico (Costa Chica region), Colombia, Central-Western regions of Venezuela, Cuban and Puerto Rican regions, there is a predominance of the Central African Republic haplotype, suggesting a different African origin for these populations (13–15).

The results described in Bahia are different from other regions of Brazil, such as the southeast and north, where there is a predominance of the CAR β^S-globin gene haplotypes of 66.2% and 66.7%, respectively, demonstrating the heterogeneity of the African slave trade brought to the country (16,17). These results indicate a contribution of Africans from Congo, Mozambique and Angola, where the CAR haplotype is predominant, that received ships from Bahia with tobacco and returned with slaves; this slave trade was intensified between 1815 and 1824 (18,19). Nevertheless, there is evidence that the northeastern region of Brazil, mainly Bahia State, received Africans from Central West Africa until the middle of the 19th century (Bight of Benin and Bight of Biafra), justifying the frequencies of CAR and Benin haplotypes found in this population (19).

These findings may contribute to the investigation of slave trade routes in Brazil and African origins of the Bahian population that seems to be quite different to other Brazilian states and other world populations An increase in the number of samples analyzed will probably confirm a phenotypical difference among the sickle cell patients from Bahia and other patient groups worldwide.

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