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Research Article

α-thalassemia, HbS, and β-globin gene cluster haplotypes in two Afro-Uruguayan sub-populations from northern and southern Uruguay

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Abstract

Hemoglobinopathies are the most common monogenic disorders worldwide; however, they have never been systematically studied from a genetic perspective in Uruguay. In this study, we determined the frequencies of hemoglobin variants in Afro-Uruguayans. A sample of 52 healthy unrelated Afro-Uruguayans from the northern (N = 28) and southern (N = 24) regions of the country was analyzed. Eight individuals (15.4%) were heterozygous for $-\alpha^{3.7}$thalassemia; seven of them (29.2%) were originally from the southern region, whereas one of them (3.6%) was from the northern region; the differences between both regions were statistically significant (p = 0.016 +/-0.003). The only structural mutation detected was β^S, which is typical of African populations. Four individuals (10%) were heterozygous for β^S, three of them (13.6%) from the South, and one (5.6%) from the North. The β^S haplotypes were analyzed in eight individuals: two were homozygous β^S/β^S, two were heterozygous β^S/β^{thal}, and four were heterozygous β^S/β^A. This haplotype distribution (60% Bantu, 20% Benin, and 20% Bantu A2) is in agreement with historical records reporting a predominantly Bantu origin for the enslaved Africans brought to Uruguay. Even though this is a preliminary study, due to the small sample size, our results are suggestive of a relatively high incidence of hemoglobinopathies in the Afro-Uruguayan population.

Key words: hemoglobinopathies, α-thalassemias, HbS, haplotypes, Afro-derived populations.

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Introduction

The hemoglobinopathies are the most common monogenic diseases in the world, as a result of the heterozygote advantage in front of malaria (Steinberg *et al*., 2001; Weatherall and Clegg, 2001). These diseases reach high frequencies in tropical regions of Africa and Asia, as well as in the Mediterranean basin. Yet, hemoglobinopathies are found in practically all American countries as a result of the African slave trade.

The hemoglobinopathies fall into two main categories: the structural hemoglobin variants and the thalassemias. According to the World Health Organization (Weatherall and Clegg., 2001) nearly 7% of the world population carry a hemoglobinopathy, and roughly 370,000 homozygotes or compound heterozygotes are born each year.

In America, hemoglobins S (HbS) and C (HbC), both typical of African and African-derived populations, are the most common structural variants. HbS is the product of one mutation (β^S) in the second position of the sixth codon of the β-globin gene (A → T), which results in a single amino acid substitution (glutamic acid for valine) in the β-globin chain. Similarly, HbC is produced by one mutation (β^C) in the second position of the sixth codon of the β-globin gene (A → C), which also results in a single amino acid substitution (glutamic acid for lysine) in the same position (Steinberg *et al*., 2001). Based on the analysis of the β^S haplotypes, it has been proposed that the β^S mutation has at least five different ethnic and geographic origins. Four of these haplotypes originated in Africa and are known as Senegal, Benin, Central African Republic (CAR) or Bantu, and

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Cameroon haplotypes. The fifth haplotype emerged in Asia and is referred to as Arab-Indian haplotype (Pagnier *et al*., 1984; Kulozik *et al*., 1986; Lapoumeroulie *et al*., 1992). Consequently, the haplotypes found in a population of African descent could point to its place of origin within Africa. Moreover, the clinical severity and the hematological characteristics of the sickle cell disease are influenced by these haplotypes. (Powars *et al*., 1994).

Thalassemias are distributed worldwide and are characterized by the decrease (+) or absence (-) of the synthesis of one or more globin chains. Depending on which globin chain is ineffectively synthesized, thalassemias are classified into α, β, δβ and εδβ thalassemias. Only α and β-thalassemias reach high frequencies in human populations; α-thalassemias are found in Africa, Asia and the Mediterranean basin, whereas β-thalassemias are found mainly in Asia and in the Mediterranean basin.

The population of Uruguay has received unequal contributions of people of European, African and Native American origin. According to historical and demographic data, in the first decade of the 19th century more than 30% of the population of the city of Montevideo (southern Uruguay) was of African origin, mainly belonging to Bantu-speaking groups from Angola, Congo, and Mozambique, with minor contributions from Senegambia and Benin; a similar percentage was reported in the city of Melo (northeastern Uruguay) for the same period (Isola, 1975; Sans and Barreto, 1998). The African-derived populations of northern Uruguay have two different origins: some slaves arrived through the port of Montevideo and subsequently migrated North, whereas others entered Uruguay from southern Brazil pursuing freedom, since slavery was abolished earlier in Uruguay (1842) than in Brazil (1888) (Sans, 1994). In the last population census (I.N.E., 1996) the Uruguayan population reached 3,163,763 individuals, who were classified as 93.3% White, 5.9% Black or Mulatto, 0.4% Native American (actually, their descendants), and 0.4% Asian, based on self-identification. Populations from different regions of the country exhibit remarkable heterogeneity with respect to admixture components. For example, Sans *et al*. (1997), using protein and blood group markers, showed that in Montevideo the contribution of populations of European, African, and Native American origin were 92%, 7%, and 1%, respectively, while in the northeastern city of Tacuarembó, the respective contributions were 65%, 15%, and 20%. Among self-identified Afro-Uruguayans from Melo, the contribution of African genes determined by autosomal loci markers was around 50% (Sans *et al*., 2002).

In spite of historical, demographic and genetic data that demonstrate the significant African contribution to the Uruguayan population, no epidemiological studies of the incidence and prevalence of hemoglobinopathies have been attempted so far, either in the population as a whole or among individuals of African origin. Moreover, considering that the Uruguayan population is mainly of Spanish and Italian origin and both countries have high frequencies of the β-thalassemia determinants, the risk of disease associated with the β^S allele can result in a relevant health problem. Therefore, our purpose was to determine the frequencies of HbS, HbC, α-thalassemias and β-thalassemias, as well as to determine the β^S haplotypes in two sub-populations of African descent from southern and northern Uruguay.

Subjects and Methods

Blood samples were collected from a random sample of 52 apparently healthy unrelated Afro-Uruguayan individuals. Each of them gave written informed consent to participate in this research. The main criteria for inclusion in the Afro-Uruguayan category relied on phenotypic traits of the individual and his/her ancestors, as well as on self-identification. The individuals were assigned to the Northern or Southern subgroups according to the predominance of grandparents from one of these regions. Twenty-four individuals from the South and twenty-eight from the North were tested. Additionally, six patients clinically and biochemically diagnosed with hemoglobinopathies were included in the study: three patients had sickle cell disease (β^S/β^S), one was heterozygous for sickle cell disease (β^S/β^A), one was heterozygous for β-thalassemia (β^{thal}/β^A), and the last had major β-thalassemia ($\beta^{thal}/\beta^{thal}$), as revealed by hemoglobin electrophoresis.

DNA samples were obtained from peripheral blood leukocytes by the salting-out method (Miller *et al*., 1988). The α-thalassemias were investigated by PCR-based methods (Saiki *et al*., 1988). The allele for -$\alpha^{3,7}$ thalassemia was detected by the method of Dode *et al*. (1993), alleles for -α^{Med} and -$\alpha^{4,2}$ thalassemias were analyzed as described by Oron-Karni *et al*., (1998), and -$\alpha^{20,5}$ was examined according to the method of Bowden *et al*. (1992).

In order to ascertain the presence of structural hemoglobinopathies and β-thalassemias, a β-globin gene DNA segment of 770 bp, located between positions -161 in the promoter and +565 in intron 2, was amplified and automatically sequenced with a Big Dye Terminator Version II Sequencing Kit (Applied Biosystems), using an internal primer located in the +2 position relative to the CAP site (Miranda *et al*., 1997).We also analyzed eight polymorphic sites in the β-globin gene cluster: 1) *HincII* 5'ε; 2) *HindIII*-^{G}γ; 3) *HindIII* -^{A}γ; 4) *HincII*-ψβ; 5) *HincII*-3'ψβ; 6) *HinfI*-5'β; 7) *AvaII*-β; and 8) *HinfI*-3β. Fragments containing each of these sites were amplified by PCR, using primers and conditions previously described (Saiki *et al*., 1988; Sutton *et al*., 1989; Guerreiro *et al*., 1992). The haplotypes of individuals with the β^S mutation were built assuming that the presence of two common haplotypes was more probable than the combination of one common and one rare haplotype, or of two rare haplotypes (Kulozik *et*

al., 1986; Long *et al*., 1990; Castro de Guerra *et al*., 1997; Vivenes de Lugo *et al*., 2003).

Haplotype frequencies were estimated by the counting method, while the frequencies of those alleles that cause hemoglobinopathies were estimated by the maximum likelihood method, using the MAXLIK program (Reed and Schull, 1968). Hardy-Weinberg equilibrium and heterogeneity among the samples were determined by the exact test for population differentiation, using the Arlequin software version 1.1 (Schneider *et al*., 1997).

Results

α-thalassemias

We detected only one of the three mutations investigated, the $-\alpha^{3,7}$ deletion that is characteristic of African populations. The genotype frequencies were in Hardy-Weinberg equilibrium within the northern and southern populations and in both sub-populations combined. In the South, the genotype frequency of carriers of the $-\alpha^{3,7}$ deletion was significantly higher than in the North (0.292 vs. 0.036, $p = 0.016 \pm 0.003$), as determined by the exact differentiation test (Table 1).

Structural hemoglobinopathies and β-thalassemias

Only one of the structural hemoglobinopathies mutations was detected in our study, namely β^S, which is typical of African populations. The genotype frequencies were in Hardy-Weinberg equilibrium in both the northern and the southern population, and in both populations combined. The genotypic frequency of β^S/β^A carriers observed in the South (0.136) was not significantly different from that of the North (0.056), as shown by the exact differentiation test. The frequency of the heterozygous genotype β^S/β^A in the combined sample was 0.10 (Table 2).

We further performed DNA tests in a group of patients previously diagnosed with hemoglobinopathies by clinical and biochemical approaches. We confirmed the diagnosis of sickle cell disease in two of the three patients tested; the third patient was a compound heterozygote, with the β^S mutation and the β^0 thalassemic mutation in codon 39 (C → T). The patient originally diagnosed as heterozygote (β^S/β^A) also carried the thalassemic mutation β^+ IVS-I-110 (G → A), being therefore a compound heterozygote. The two patients considered β-thalassemic presented the β^0 thalassemic mutation at codon 39 (C → T), one in heterozygosis (β^0/β^A) and the other in homozygosis (β^0/β^0).

β^s haplotypes

We determined haplotypes in ten chromosomes that harbored the β^S mutation. Four chromosomes belonged to heterozygous individuals, while six chromosomes came from patients with hemoglobinopathies (two homozygous and two heterozygous). Six chromosomes (60%) showed the Central African Republic haplotype (CAR), two the Benin haplotype (BEN), and two an atypical haplotype that could be the Bantu A2 haplotype (Table 3) (Pagnier *et al*., 1984; Kulozik *et al*., 1986; Srnivas *et al*., 1988). The homozygous patients had genotypes CAR/CAR and CAR/BEN, while among the six heterozygous individuals (β^S/β^A and β^S/β^{thal}) three carried the CAR haplotype, two the Bantu A2 haplotype, and one the BEN haplotype.

Discussion

These are the first data on hemoglobin variant frequencies in the Afro-Uruguayan population. The high frequency of the $-\alpha^{3,7}$ deletion (0.292) observed in the South is similar to that seen in black populations of Brazil, Jamaica,

Table 1 - Genotype and allele frequencies of the $-\alpha^{3,7}$ deletion and results of the differentiation test between the Northern and Southern sub-populations.

		Genotype			Alelles		Exact differentiation test
	N	αα/αα	αα/$-\alpha^{3,7}$	$-\alpha^{3,7}/-\alpha^{3,7}$	αα	$-\alpha^{3,7}$	
Northern	28	27(0.964)	1(0.036)	0(0.000)	0.982	0.018	$0.01583^* \pm 0.0027$
Southern	24	17(0.708)	7(0.292)	0(0.000)	0.887	0.113	
Grouped	52	44(0.846)	8(0.154)	0(0.000)	0.923	0,077	

* = significant ($p < 0.05$).

Table 2 - Genotype and allele frequencies of the β^S mutation and results of the differentiation test between the Northern and Southern sub-populations.

		Genotype			Alleles		Exact differentiation test
	N	β^A/β^A	$\beta^S\beta^A$	β^S/β^S	β^A	β^S	
Northern	18	17(0.944)	1(0.056)	0(0.000)	0.972	0.028	0.62320 ± 0.0027
Southern	22	19(0.864)	3(0.136)	0(0.000)	0.932	0.068	
Grouped	40	36(0.846)	4(0.100)	0(0,000)	0.950	0.050	

Table 3 - β^S haplotypes in individuals heterozygous and homozygous for sickle cell anemia.

			Haplotype 5'		Haplotype 3'					
	5'ε	$^{G}\gamma$	$^{A}\gamma$	5'ψβ	3'ψβ	5'β	β	3'β	N	%
Haplotype	*HincII*	*HindIII*	*HindIII*	*HincII*	*HincII*	*HinfI*	*AvaII*	*HinfI*		
CAR	-	+	-	-	-	-	+	+	6	60
BEN	-	-	-	-	+	-	+	+	2	20
Atypical (Bantu A2?)	+	-	-	-	-	-	+	+	2	20
Total									10	100

N = number of chromosomes.

United States, and Cuba. (Sonati and Costa, 1990; Sonati *et al*.; 1991, Cabrera *et al*., 1995). Despite the limited sample size of our study, the significant difference in frequency (0.292 vs. 0.036, p = 0.016) between the southern and northern regions is worthy of note. This difference may be explained by the greater contribution of genes from Native American populations to the gene pool of the northern population (Sans *et al*., 1997, 2002). Furthermore, the European contribution in the northern region is predominantly of Spanish and Portuguese origin, and in these countries the frequency of α-thalassemias is comparatively lower that in other Mediterranean countries (Villegas *et al*., 2001). The low frequencies observed in the North may be also explained by the action of genetic drift, favored by a low demographic density in the region, bottlenecks, or founder effects.

The frequency of HbS carriers (10%) detected in this study is higher than that observed in the black population of Southern Brazil. In fact, in the black population of Porto Alegre, Salzano *et al*. (1968) found a 6.8% frequency of HbS carriers. The frequency found in Uruguay is similar to that of the State of Bahia, located in the Brazilian Northeast, where the frequency of carriers varies from 7.4% to 15.9% (Azevedo *et al*., 1980). In that region, Adorno *et al*. (2005) found a 9.4% frequency of HbS carriers in black and mulatto newborns, similar to the one found in our study among black adults. Although the difference in the frequency of HbS carriers between the North and the South is not significant and the sample is small, the similarity in carrier frequency between northern Uruguay (5.6%) and Porto Alegre in southern Brazil (6.8%) is remarkable. This finding suggests that an important influx of slaves took place from southern Brazil to northern Uruguay during the 19th century. The similarity in frequencies between southern Uruguay (13.6%) and northeastern Brazil (7-15%) could be explained by a common origin in Africa and/or microevolutionary factors.

Additionally, the frequencies of HbS carriers found in Uruguay are higher than or similar to those present in populations with a higher proportion of genes of African origin. For example, Afro-Amazonian populations, like Ganga in Venezuela and Curiau in Brazil, show 76% and 73.6% of African genetic contribution, respectively, whereas the corresponding HbS carrier frequencies amount to 4.8% and 8.4%, respectively (Castro de Guerra, 1993; Guerreiro *et al*., 1999). In an Afro-Uruguayan population from the Northeast with similar characteristics to our study population, the African genetic contribution was estimated as being 50% (Sans *et al*., 2002). The inconsistency between the African genetic contribution and the HbS carrier frequency may be attributed to microevolutionary factors such as genetic drift and/or founder effects, and/or to differences in the origin of the slaves brought to Uruguay.

Both β-thalassemia alleles found among our patients are characteristic of Mediterranean populations. The β^0 codon 39 (C → T) mutation is the most frequently observed in Spain and in some regions of Italy, whereas the β^+ IVS-I-110 (G → A) mutation is fifth in frequency in Spain, and among the five most frequent mutations in Italy (Ferrara *et al*., 2001; Villegas *et al*., 2001). These two mutations are also the first and second most commonly found in Greece, although in reverse order (Georgiou *et al*., 2003). Our results revealed the presence of genes of European origin in both samples, a contribution previously estimated at around 32% in the compound sample from northern and southern Uruguay (da Luz, 2004). These mutations are also useful to uncover the origin of the European immigrants, who were mainly from Spain and Italy (Sans, 1994). However, it should be noted that the two most frequent mutations in Equatorial Africa and in African Americans, -88 (C → T) and -29 (A → G) (Huisman 1997, Hardison *et al*., 1998), located in the promoter region of the β-globin gene, were not analyzed in this preliminary study. Consequently, it is not possible to assert which are the most common β-thalassemia mutations present in Afro-Uruguayans.

Although the number of chromosomes carrying the allele β^S that were analyzed is small, the high frequency of the CAR haplotype (60%) is indicative of an influx of slaves mainly from Central Africa. The presence of the Benin haplotype at 20% suggests a lower yet significant contribution from West Africa. These results are in agreement with historical data on the slave trade to Uruguay and southern Brazil. Specifically, historical sources indicate

that most of the slaves taken to Uruguay and southern Brazil were from Angola, Mozambique and Congo, where the Bantu haplotype predominated (Curtin, 1969; Isola, 1975; Sans and Barreto, 1998).

The frequency of the atypical haplotype Bantu A2 (20%) is the highest reported in American populations so far. This haplotype may arise as the result of recombination events between the 5' regions of the Bantu typical haplotype and other haplotypes present in β^A chromosomes (Srinivas *et al*., 1988). However, this figure may be overestimated due to the small number of chromosomes analyzed.

In summary, the frequency of hemoglobinopathies in the Afro-Uruguayan population, essentially those caused by HbS and by the $-\alpha^{3,7}$ deletion, is consistent with the origins of this population. Nevertheless, some differences are found between the Northern and the Southern sub-populations in the $-\alpha^{3,7}$ deletion frequency.

Our data allow us to estimate the incidence of sickle cell disease in newborns, by taking into account the frequency of the β^S mutation (0.05), the frequency of individuals of African descent in the general population (0.06), and the annual birth rate (approximately 54,000 for the total population). Therefore, the number of newborns with this disease is estimated to be between 1 and 8 per year, depending on the sub-structure of the population, as well as on its marriage patterns. If we assume that the Afro-Uruguayan population is completely endogamic, the expected number of newborns with sickle cell disease will be around the estimated maximum of 8 per year. On the other hand, if we assume random mating in the population as a whole, the frequency of ^{S} will descend to 0.003, therefore the expected number of newborns with sickle cell disease will drop to around the estimated minimum of 1 per year.

The results obtained so far show the need for public health policies to assist the affected families, notwithstanding the importance of conducting further hemoglobinopathy studies using larger samples and including other ethnic groups. Finally, our results also contribute towards a better understanding of the way Uruguay was populated and link population structure analysis to anthropological and historical approaches.

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