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Carta ao Editor / Letter to Editor

The frequency of β-globin gene haplotypes, α-thalassemia and genetic polymorphisms of methylenetetrahydrofolate reductase, factor V Leiden and prothrombin genes in children with sickle cell disease in Rio de Janeiro, Brazil

Frequência dos haplótipos da globina beta, da talassemia alfa e dos polimorfismos genéticos dos genes da metilenotetrahidrofolato redutase, do fator V Leiden e da protrombina em crianças com doença falciforme no Rio de Janeiro, Brasil

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Sr. Editor

The Brazilian population is unique as a result of extreme miscegenation involving several ethnic groups: native Americans, Europeans and Africans (West, Central West and South-Eastern Africa) form the basis of the Brazilian population.[1] Sickle cell anemia (SCA) is the most prevalent inherited disease in Brazil, with an overall occurrence of 1 case per 1000 births.[2] The inclusion of hemoglobin S tests in neonatal screening confirms the magnitude of this disease in our country and has encouraged the implementation of prevention programs and health policies to minimize morbidity and mortality due to sickle cell diseases (SCD). The present study investigated the frequency of β-globin gene haplotypes and co-inheritance with alpha-thalassemia ($-\alpha_2^{3.7kb}$ and $-\alpha_2^{4.2kb}$). The single nucleotide polymorphisms of methylene-tetrahydrofolate reductase (MTHFR-C677T), Factor V Leiden (FV-G1691A) and prothrombin (PT-G20210A) were also studied; these polymorphisms have been implicated as genetic modifiers related to the severity of SCD.

A cross-sectional study was carried out of SCD children identified by the neonatal hemoglobinopathy screening program in the state of Rio de Janeiro, Brazil. The study was approved by the Ethics Research Committee and informed consent was obtained from the children's parents.

One hundred and five children (52 males and 53 females) with a mean age of 29.9 months (range 1-80) were included; in 8 cases β-globin haplotype tests could not be performed. The study showed 81 patients had SCA and 17 had SC hemoglobin. Alpha-thalassemia was investigated in 99 children: 77 with SCA, 16 with SC hemoglobin, 5 with S/β-thalassemia and one case of SD hemoglobin. Polymorphisms of MTHFR, Factor V Leiden and prothrombin genes were investigated in the 81 cases of SCA.

The screening of SCD was carried out by high performance liquid chromatography with positive results being confirmed by studies of the parents. Blood samples were collected and genomic DNA was extracted from peripheral blood leukocytes using a commercial kit.

The β-globin gene cluster was studied by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). A pattern of six polymorphic restriction sites was identified within the beta-globin gene complex (5' γ^G,γ^G, γA,ψβ, 3'ψβ, 5'β).

Alpha ($-\alpha_2^{3.7kb}$ and $-\alpha_2^{4.2kb}$) thalassemia deletions were determined by PCR.

The C677T mutation in the MTHFR gene, G1691A in the Factor V Leiden gene and G21210A in the prothrombin gene were investigated by PCR-RFLP. Of the 105 SCD children, apart from the 81 children with SCA and 17 with SC hemoglobin, 6 had S/β-thalassemia and one had SD hemoglobin.

Alpha thalassemia was investigated in 99 children with 21 (21.2%) presenting the $-\alpha_2^{3.7kb}$ mutation of which 18 children (18.2%) were heterozygous and 3 (3%) were homozygous. No $-\alpha_2^{4.2kb}$ mutation was found. Table 1 shows the distribution of the $-\alpha_2^{3.7kb}$ mutation in respect to the different genotypes.

Polymorphisms linked to Bantu or CAR (Central African Republic) haplotypes were the most common and found in 128 (65.9%) chromosomes and Benin haplotypes in 46 (23.7%) chromosomes (Table 2). Among 80 SCA cases, the distribution

Table 1. Frequency distribution of thalassemia $-\alpha_2^{3.7\,kb}$ mutation according to the genotypes

Genotype	$-\alpha_2^{3.7\,kb}$ mutation N(%)			
	Wild	Heterozygous	Homozygous	N
Hemoglobin SS	59 (76.6)	15 (19.5)	3 (3.9)	77
Hemoglobin SC	14 (87.5)	2 (12.5)		16
Hemoglobin SD	1 (100)			1
Hemoglobin S/ß-Thal*	4 (80)	1 (20)		5
Total	78 (78.8)	18 (18.2)	3 (3)	99

* One not done

Table 2. Frequency of β^S and β^C chromosome in 97 children with sickle cell disease

Haplotypes	SS N (%)	SC N (%)	Total (%)
CAR*/Bantu	117 (73,1)	11 (32,3)	128 (65.9)
Benin	38 (23,8)	8 (23,6)	46 (23.7)
Atypical	5(3,1)	11 (32,3)	16 (8.2)
CI	-	4 (11,8)	4 (2.1)
Total	160	34	194

*Central African Republic

of haplotypes was as follows: 39 (48.8%) were CAR/CAR, 34 (42.5%) CAR/Benin, 5 (6.3%) CAR/Atypical and 2 (2.5%) Benin/Benin. The 17 SC hemoglobin cases have the following haplotypes: 7 (41.2%) CAR/CI, 4 (23.5%) CAR/Atypical, 2 (11.8%) Benin/CI, 2 (11.8%) Benin/ Atypical and 2 (11.8%) CI/Atypical. No Senegal, Cameroon or Arab/Indian haplotypes were found.

Of the 81 SCA cases analyzed, 23.5% presented the MTHFR gene mutation, with 15 (18.5%) being heterozygous and 4 (4.9%) homozygous. No Factor V Leiden or prothrombin mutations were observed.

The frequency of alpha-thalassemia ($-\alpha_2^{3.7kb}$ - 21.2%) was in agreement with a previous study.[3] From the 16th to 19th centuries a massive slave trade occurred with more than 4 millions of Africans being brought to Brazil. The slaves originated from two major regions: West-Central/Southeast Africa, that is, the region of Angola, Congo and Mozambique, and West Africa that covered the region of the Gulf of Guinea (Ghana, Benin and Nigeria). During many years major routes of forced African migration were established from the Bay of Benin in Ghana and Nigeria to the northeast of Brazil, and from Congo and Angola to Rio de Janeiro. The β-globin haplotype distribution represents a profile of the geographical origin of Africans who were brought to Brazil with a high proportion of CAR/Bantu haplotypes being found in all Brazilian regions. The Benin haplotype is mainly found in the northeast,[4] while the Senegal haplotype is more common in the north. The Cameroon and Arab/Indian haplotypes have also been sporadically reported in Brazil.[4] Although Rio de Janeiro is the second largest Brazilian city and its inhabitants reflect the enormous miscegenation that occurred in Brazil, our results are in agreement with historical reports of the slave trade expressed by a high proportion of the CAR/Bantu haplotype, followed by the Benin haplotype.

The polymorphisms of MTHFR C677T, Factor V Leiden and prothrombin G20210A genes have been implicated in the pathogenesis of thromboembolism and thrombophilias 5 in SCD. Thrombosis is an important aspect of the disease clinical spectrum, however, its role as a risk factor for vascular events in SCD patients is not recognized.[5] According to previous reports, Factor V Leiden and prothrombin gene mutations were found at a high frequency in some European regions and were very rare in African descendents.[5,6] In Brazil, reports have described a low frequency in the general population, varying from 1% to 2% and 0.7% to 3.6% for the G1691A and G20210A mutations, respectively.[6-7] The frequency of the 677TT allele (MTHFR) in Brazil is high among Caucasians (10.3%) but low in African descendents (1.4%) and Indians (1.2%).[6-7] Our results are similar to the ones found in the state of Bahia that reported a frequency of 5.3% in the general population and of 5.7% in SCA patients.[7] On the other hand, reports from the State of São Paulo presented very different results for the frequency of the 677TT allele in SCD patients with none in Campinas and 1.8% in São Paulo.[5-6] In respect to the 677CT allele, our findings are very similar to those from the state of Bahia (18.6%) but we report a lower frequency than in São Paulo (34%). The differences in the prevalence of haplotypes as well as of polymorphism frequencies observed in Brazilian regions are probably due to population miscegenation and the number of samples studied. These results are in agreement with the historical reports of the slave trade from Africa to the states of Bahia and Rio de Janeiro.

Key words: *Sickle cell disease; hemoglobinopathies; thalassemia; blood coagulation; Factor V Leiden; prothrombin.*

Resumo

*A freqüência dos haplótipos beta S e beta C do gene da globina e a prevalência de talassemia alfa e de mutações nos genes da metileno-tetrahidrofolato redutase (MTHFR-C677T), do fator V de Leiden e da protrombina (G20210A) foi estudada em crianças com doença falciforme do Rio de Janeiro. O haplótipo Bantu foi o mais freqüente (65,9%), 21,2% das crianças (18% heterozigotas e 3% homozigotas) apresentam talassemia com mutação alfa 3.7kb, ao contrário da mutação alfa 4.2kb que não foi encontrada. Os alelos 677CT e 677TT da MTHFR foram observados em 20,2% e 4,8%, respectivamente. Os haplótipos Camarões, Árabe-Indiano e Senegal não foram detectados na amostra estudada, bem como mutações no gene do fator V de Leiden e da protrombina. Somente o haplótipo beta C CI foi observado. Esse é o primeiro estudo realizado em uma amostra proveniente do Programa de Triagem Neonatal para Hemoglobinopatias do estado do Rio de Janeiro. Apesar do Rio de Janeiro ser a segunda maior cidade brasileira e seus habitantes expressarem o elevado grau de miscigenação ocorrida no país, nossos resultados ainda coincidem com os registros históricos dos fluxos migratórios do gene beta S para o Brasil, bem como refletem a forte influência de indivíduos de origem africana na população do Rio de Janeiro. Rev. Bras. Hematol. Hemoter. 2010;**32**(1):76-78.*

Palavras-chave*: Doença falciforme; hemoglobinopatias; talassemia; coagulação sanguínea; fator V; protombina.*

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