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α-Globin genes: thalassemic and structural alterations in a Brazilian population

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Abstract

Seven unrelated patients with hemoglobin (Hb) H disease and 27 individuals with α-chain structural alterations were studied to identify the α-globin gene mutations present in the population of Southeast Brazil. The $-\alpha^{3.7}$, $--^{MED}$ and $-(\alpha)^{20.5}$ deletions were investigated by PCR, whereas non-deletional α-thalassemia ($\alpha^{Hph}\alpha$, $\alpha^{NcoI}\alpha$, $\alpha\alpha^{NcoI}$, $\alpha^{Ic}\alpha$ and $\alpha^{TSaudi}\alpha$) was screened with restriction enzymes and by nested PCR. Structural alterations were identified by direct DNA sequencing. Of the seven patients with Hb H disease, all of Italian descent, two had the $-(\alpha)^{20.5}/-\alpha^{3.7}$ genotype, one had the $--^{MED}/-\alpha^{3.7}$ genotype, one had the $--^{MED}/\alpha^{Hph}\alpha$ genotype and three showed interaction of the $-\alpha^{3.7}$ deletion with an unusual, unidentified form of non-deletional α-thalassemia $[-\alpha^{3.7}/(\alpha\alpha)^{T}]$. Among the 27 patients with structural alterations, 15 (of Italian descent) had Hb Hasharon (α47Asp→His) associated with the $-\alpha^{3.7}$ deletion, 4 (of Italian descent) were heterozygous for Hb J-Rovigo (α53Ala→Asp), 4 (3 Blacks and 1 Caucasian) were heterozygous for Hb Stanleyville-II (α78Asn→Lys) associated with the α^{+}-thalassemia, 1 (Black) was heterozygous for Hb G-Pest (α74Asp→Asn), 1 (Caucasian) was heterozygous for Hb Kurosaki (α7Lys→Glu), 1 (Caucasian) was heterozygous for Hb Westmead (α122His→Gln), and 1 (Caucasian) was the carrier of a novel silent variant (Hb Campinas, α26Ala→Val). Most of the mutations found reflected the Mediterranean and African origins of the population. Hbs G-Pest and Kurosaki, very rare, and Hb Westmead, common in southern China, were initially described in individuals of ethnic origin differing from those of the carriers reported in the present study and are the first cases to be reported in the Brazilian population.

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Introduction

The hemoglobinopathies are a heterogeneous group of genetic disorders caused by mutations affecting the globin-chain genes. Generically, these mutations can be classified as structural alterations which result in the production of abnormal proteins, as alterations in synthesis which modify the normal α/ß globin chain ratio (thalassemias), or as persistent production of fetal hemoglobin (Hb) during adult life (hereditary persistence of fetal Hb) (1,2). The hemoglobinopathies represent a public health problem, particularly in the Mediterranean area, in the Middle East and in parts of India, Africa and South-

east Asia (3,4).

The high degree of racial admixture among native Indians and African and European descendants in the Brazilian population has produced elevated frequencies of Hb alterations, which reflect the diversity of racial origins in each region of the country (5). Clinically, Hb S, Hb C and ß-thalassemia are the most important (6), although α^{+}-thalassemia ($-\alpha^{3.7}$ deletion) is the most frequent alteration, occurring in 20-25% of the Black population (7). Although sporadic cases of Hb H disease and α-chain structural variants have been found (6,8), the α-globin genes have not been systematically investigated. In this study, seven unrelated subjects with Hb H disease and 27 individuals with structural α-globin alterations were investigated in order to identify the mutations present in the population of southeastern Brazil. The Hb H disease patients and the carriers of abnormal Hbs who had hematological alterations were initially screened at the outpatient clinics of the UNICAMP University Hospital and then referred to the Clinical Pathology Laboratory for investigation and diagnosis. Non-symptomatic carriers were detected in a screening program carried out in the same laboratory.

Material and Methods

Peripheral blood samples were collected into Vacutainers (Becton-Dickinson, Corkeysville, MD, USA) with EDTA as anticoagulant and hematological data were obtained with an automated cell counter (Cell Dyn 3500, Abbott Laboratories, Chicago, IL, USA).

Hb analyses were carried out by electrophoresis on cellulose acetate strips at pH 8.9, in agar gels at pH 6.0 (1), and by globin chain electrophoresis on acrylamide gels at acid pH (9). Hb A_2 was measured spectrophotometrically after elution from cellulose acetate strips (1) and Hb F was determined by alkali denaturation (10). The stability of each variant was checked by the n-butanol, isopropanol and heat tests (11). Heinz bodies were investigated by incubation with methyl violet and Hb H was demonstrated by incubation with brilliant cresyl blue (11).

DNA was isolated from peripheral blood leukocytes by organic extraction. Direct sequencing was performed with the Sequenase kit version 2.0 (United States Biochemical Corporation, Cleveland, OH, USA), after selective amplification of the α-globin genes by the polymerase chain reaction (PCR) (12) and single strand separation with magnetic beads (Dynal Inc., Oslo, Norway). Whenever possible, the mutations were confirmed by sequencing the opposite strand and by familial studies and restriction enzyme analyses.

The most common deletions causing α-thalassemia ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{MED}$, $-(\alpha)^{20.5}$, $--^{SEA}$) were screened by PCR (13-15). The five most frequent non-deletional mutations causing α-thalassemia ($\alpha^{Hph}\alpha$, $\alpha^{NcoI}\alpha$, $\alpha\alpha^{NcoI}$, $\alpha^{Ic}\alpha$ and $\alpha^{TSaudi}\alpha$) were investigated with the restriction enzymes *Hph*I, *Nco*I and *Mse*I, respectively (3,16,17) and by specific nested PCR (α^{TSaudi}) (18).

Results

Among the seven Hb H disease patients, all of Italian descent, four had the following genotypes: $-(\alpha)^{20.5}/-\alpha^{3.7}$ (two cases), $--^{MED}/-\alpha^{3.7}$ and $--^{MED}/\alpha^{Hph}\alpha$. The other three had an unusual unidentified form of α-thalassemia which seemed to be non-deletional [$-\alpha^{3.7}/(\alpha\alpha)^{T}$] since both genes were present. No mutation was detected following sequencing from the promoter region to the poly A signal. These results are shown in Table 1.

Among the 27 individuals with structural alterations, 15 (Caucasians of Italian descent) had Hb Hasharon (α47Asp→His), associated with the $-\alpha^{3.7}$ deletion ($-\alpha^{Hasharon}$): one was homozygous ($-\alpha^{Hasharon}/-\alpha^{Hasharon}$) and 14 were heterozygous ($-\alpha^{Hasharon}/\alpha\alpha$). One of

these 14 individuals had a concomitant new ß-globin variant, Hb Rio Claro (ß34Val→Met) (19). Four adults (Caucasians of Italian descent) were heterozygous for Hb J-Rovigo (α53Ala→Asp) and one of them was also a ß-thalassemia carrier. Hb Stanleyville-II (α78 Asn→Lys) was found in four individuals (3 Blacks and 1 Caucasian, all heterozygous), always in association with the $-\alpha^{3.7}$ deletion ($-\alpha^{Stanleyville}$). One Black infant from the north of Brazil had Hb G-Pest (α74Asp→Asn), a very rare Hb described initially in a Hungarian family (20). One boy of Portuguese descent had Hb Kurosaki (α7Lys→Glu), another very rare Hb described only once in a Japanese woman (21). Hb Westmead (α122 His→Gln), a relatively common silent variant in southern China (22,23), was found in a Caucasian adult of Italian descent. Hb Campinas (α26Ala→ Val) was identified in a Caucasian boy whose ethnic origin was unknown. This electrophoretically silent variant, not described previously, results from a base substitution at the 26th codon of the α_2 gene (GCG→GTG) (24). These structural alterations are summarized in Table 2.

Table 1 - Hematological data for the patients with Hb H disease.

RBC, Red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin.

Carrier	BFB	FP	EFF	TCA	CAB	FAF	FVS
Age (year)	40	44	9	7	16	39	6
RBC (x 10^6/ml)	5.64	6.0	5.92	4.63	5.02	6.24	4.67
Hb (g/dl)	9.7	12.1	9.0	8.3	9.4	12.6	7.9
Hematocrit (%)	32.5	43	33	29	31	42	26.5
MCV (fl)	58	69.9	55.5	63	62	67	57
MCH (pg)	17.2	19.8	15.2	17.9	18.6	20.1	16.9
Electrophoretic profile (pH 8.9)	A_2 + A + H	A_2 + A + H	A_2 + A + H + Bart's	A_2 + A + H	A_2 + A + H + Bart's	A_2 + A + H	A_2 + A + H
Hb A_2 (%)	1.0	1.0	1.4	0.7	1.3	1.2	0.9
Hb F (%)	1.3	1.0	1.4	0.9	1.0	1.3	1.0
Hb H (%) (+ Bart's)	4.9	6.1	3.6	26.5	4.5	4.5	14.0
α-Genotype	$-(\alpha)^{20.5}/-\alpha^{3.7}$	$-(\alpha)^{20.5}/-\alpha^{3.7}$	$--^{MED}/-\alpha^{3.7}$	$--^{MED}/\alpha^{Hph}\alpha$	$-\alpha^{3.7}/(\alpha\alpha)^T$	$-\alpha^{3.7}/(\alpha\alpha)^T$	$-\alpha^{3.7}/(\alpha\alpha)^T$

Table 2 - Structural alterations in α-globin genes.

Abnormal hemoglobin	Hasharon (α47Asp→His)	Stanleyville-II (α78Asn→Lys)	J-Rovigo (α53Ala→Asp)	G-Pest (α74Asp→Asn)	Kurosaki (α7Lys→Glu)	Westmead (α122His→Gln)	Campinas (α26Ala→Val)
No. of individuals	15	4	4	1	1	1	1
Race	Caucasians	3 Blacks + 1 Caucasian	Caucasians	Black	Caucasian	Caucasian	Caucasian
Electrophoretic profile (pH 8.9)	A_2',A_2,Hash.,A	A_2',A_2,Stanl,A	A_2,A,J-Rovigo	A_2',A_2,G-Pest,A	A_2,A,Kurosaki	A_2A	A_2A
Globin electrophoresis	$\alpha + \alpha^H$ + ß	α + ß	α + ß	α + ß	$\alpha + \alpha^K$ + ß	α +ß + α^W	$\alpha + \alpha^C$ + ß
α-Genotype	$-\alpha^{3.7}/\alpha\alpha$	$-\alpha^{3.7}/\alpha\alpha$	αα/αα	αα/αα	αα/αα	αα/αα	αα/αα
Mutation	α_1(**G**AC→**C**AC)	α_1(AA**C**→AA**A**)	α_2(G**C**C→G**A**C)	α_2(**G**AC→**A**AC)	α_1(**A**AG→**G**AG)	α_2(CA**C**→CA**G**)	α_2(G**C**G→G**T**G)

Discussion

The thalassemic mutations found in four of the seven Hb H disease patients ($-\alpha^{3.7}$, $--^{MED}$, $-(\alpha)^{20.5}$ and $\alpha^{Hph}\alpha$) are the most frequent in Mediterranean populations: the first three are common deletions which remove 3.7-, 18- and 20.5-kb fragments of DNA from the α-globin gene cluster, respectively, and the latter is a common non-deletional form which removes five nucleotides from the consensus sequence of the splicing donor site of IVS-I (3). All of the Hb H disease patients were of Italian descent. In contrast, the remaining three patients showed the association of the $-\alpha^{3.7}$ deletion with an unusual form of α-thalassemia which leaves both α-genes intact, but without expression. This situation may result from an alteration in the α-major regulatory element (α-MRE or HS-40), a major positive regulatory region located 40 kb upstream of the ξ_2-globin gene cap site (25). A few large deletions are known to remove this locus control region and thereby silence the α-globin genes, which remain structurally intact (3).

Among the structural alterations, Hb Hasharon, the most frequent change, and Hb J-Rovigo, are of Italian origin, whereas Hb Stanleyville-II is of African origin and Hb G-Pest was first described in a Hungarian family in 1972 (20). The last one was found here for the first time in the Brazilian population. Hb Kurosaki was initially described in a 70-year-old Japanese woman (21), being the case described here the second reported in the world and the first one in Brazil. Hb Westmead was discovered in a Chinese female in 1980 (23) and is common in Guangxi, a province in southern China (22). In the case described here, the first in Brazil, the carrier was of Italian descent. Hbs G-Pest, Kurosaki and Westmead were detected in a screening program, but in carriers with ethnic origins different from those of the original descriptions, suggesting *de novo* mutations. Hb Campinas was a novel silent variant encountered in a Caucasian boy of unknown descent (24).

Clinically and hematologically, Hbs J-Rovigo, G-Pest, Kurosaki, Westmead and Campinas caused no abnormalities, since the carriers were asymptomatic. Hb Hasharon and Hb Stanleyville-II carriers had mild microcytic and hypochromic red blood cells, probably because of the association with α-thalassemia.

The present study on 34 persons represents the most extensive analysis to-date of individuals with α-globin gene mutations in Brazil. The results confirmed the strong Italian and African influences in the region of Brazil examined, and reflected the intense immigration that took place in the last century and at the beginning of the twentieth century.

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