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Investigating alpha-globin structural variants: a retrospective review of 135,000 Brazilian individuals



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ABSTRACT

Background: Brazil has a multiethnic population with a high diversity of hemoglobinopathies. While screenings for beta-globin mutations are far more common, alterations affecting alpha-globin genes are usually more silent and less well known. The aim of this study was to describe the results of a screening program for alpha-globin gene mutations in a representative sample of the Southeastern Brazilian population.

Methods: A total of 135,000 individuals, including patients with clinical suspicion of hemoglobinopathies and their family members, randomly chosen individuals submitted to blood tests and blood donors who were abnormal hemoglobin carriers were analyzed. The variants were screened by alkaline and acid electrophoreses, isoelectric focusing and cation-exchange high performance liquid chromatography (HPLC) and the abnormal chains were investigated by reverse-phase high performance liquid chromatography (RP-HPLC). Mutations were identified by molecular analyses, and the oxygen affinity, heme-heme cooperativity and Bohr effect of the variants were evaluated by functional tests.

Results: Four new and 22 rare variants were detected in 98 families. Some of these variants were found in co-inheritance with other hemoglobinopathies. Of the rare hemoglobins, Hasharon, Stanleyville II and J-Rovigo were the most common, the first two being S-like and associated with alpha-thalassemia.

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Conclusion: The variability of alpha-globin alterations reflects the high degree of racial miscegenation and an intense internal migratory flow between different Brazilian regions. This diversity highlights the importance of programs for diagnosing hemoglobinopathies and preventing combinations that may lead to important clinical manifestations in multiethnic populations.

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Introduction

Alpha-globin chain variants are the result of point mutations (single nucleotide polymorphisms – SNPs) or small base insertions or deletions that affect the region encoding the α_1 and α_2 genes, thus resulting in amino acid substitutions in the protein chains or even elongated chains.¹

More than 400 structural changes in the alpha-chain have been described to date², most of which are caused by simple DNA base substitutions with the corresponding substitutions of amino acid residues in the protein. Many of these alterations do not result in clinical symptoms; however, some can affect the function of the hemoglobin (Hb) molecule, resulting in erythrocytosis or cyanosis, and its stability, causing hemolytic anemia.³ In addition, there are elongated and extremely unstable variants that lead to thalassemia phenotypes.⁴

The Hemoglobinopathy Laboratory of the Hospital das Clínicas, Universidade Estadual de Campinas (UNICAMP), São Paulo, Southeastern Brazil is a referral laboratory for the diagnosis of hemoglobinopathies. This paper summarizes the alpha-chain structural variants identified in the laboratory during its 30 years of existence.

Methods

To date, 135,000 cases have been investigated in this laboratory. This population sample includes patients with clinical suspicion of hemoglobinopathies and their family members, randomly chosen individuals who had blood tests and blood donors who were carriers of hemoglobin variants. The local ethics committee approved this study and the subjects or their legal guardians gave informed consent for participation. Structural changes in the alpha-chain were identified in 124 individuals from 98 different families (approximately 0.1% of the analyzed cases).

Peripheral blood samples were collected in tubes containing EDTA, and hematological analyses were carried out using an automated counter (Sysmex XE 2100, Sysmex, Kobe, Japan). Hb variants were identified and characterized by electrophoresis on cellulose acetate at pH 8.9 and agar gel at pH 6.0, isoelectric focusing (RESOLVE Neonatal Hemoglobin Test Kit, PerkinElmer Wallace, Akron, OH, USA) and cation exchange high performance liquid chromatography (HPLC) (VARIANT II, Bio-Rad Laboratories, Hercules, CA, USA). Abnormal globin chains were identified by reverse phase HPLC (RP-HPLC) (Waters Alliance HPLC System, Waters, Milford, MA,

USA). Stability tests (heat stability and stability in n-butanol and in isopropanol) and solubility tests were also carried out, as well as an investigation of Heinz bodies and Hb H inclusion bodies in red cells.⁵

Functional studies were carried out by plotting the oxygen-hemoglobin dissociation curve at different pHs to measure the Bohr effect and evaluate heme-heme cooperativity of the globin chains in the presence and absence of allosteric effectors.^{1,5–7}

Genomic DNA samples were extracted from peripheral blood leukocytes, initially by organic methods⁸ and, more recently using a specific kit (Blood Genomic Prep Mini Spin, GE Healthcare, UK). Alpha-globin genes were selectively amplified by polymerase chain reaction (PCR) according to the method described by Dodé et al.⁹ and sequenced using an automated technique (ABI PRISM 377 DNA Automated Sequencer, Applied BioSystems, Foster City, CA, USA). Mutations were confirmed by sequencing the opposite DNA strand, family analysis and enzyme restriction analysis, whenever possible. The presence of concomitant deletional alpha-thalassemia was investigated by multiplex PCR and gap PCR.^{10,11} The most common non-deletional alpha-thalassemic mutations were also investigated when these were suspected.^{12,13}

Results

Four new alpha-chain variants (Table 1) and 22 rare variants (Table 2) were detected, five of the latter concomitantly with other structural or thalassemic mutations.

New variants

The four new variants were identified in 13 individuals belonging to six different families (Table 1). None resulted in significant hematological or clinical abnormalities in their carriers.

Hb Campinas [HBA2:c.80C>T p.Ala26Val] was first described in a nine-year-old boy and his mother and later was observed in three related individuals during a hemoglobinopathy screening program.¹⁴

Hb Boa Esperança [HBA2:c.50A>C p.Lys16Thr] was identified in two unrelated individuals. Functional studies showed that the stripped hemolysate of this Hb had less affinity for oxygen than Hb A but that the addition of inositol hexaphosphate (IHP) to the stripped hemolysate resulted in increased affinity. This abnormal function, however, may be compensated *in vivo* by a higher proportion of normal Hb.¹⁵

Table 1 – New alpha-globin variants identified.

New variant	No. of families/No. of cases	Mutation	α -genotype	Clinical manifestations/hematological alterations
Hb Boa Esperança	2/2	HBA2:c.50A>C p.Lys16Thr	$\alpha^{\text{Boa Esperança}}/\alpha\alpha$	No
Hb Campinas	2/5	HBA2:c.80C>T p.Ala26Val	$\alpha^{\text{Campinas}}/\alpha\alpha$	No
Hb Bom Jesus da Lapa	1/3	HBA1:c.92A>C p.Glu30Ala	$\alpha\alpha^{\text{B Jesus da Lapa}}/\alpha\alpha$	No
Hb Itapira	1/3	HBA3:c.92A>T p.Glu30Val	$\alpha\alpha^{\text{Itapira}}/\alpha\alpha$	No
Total	6/13			

Two new mutations resulting in substitutions of the same amino acid residue (glutamic acid at position 30) by chemically similar residues were also identified: Hb Bom Jesus da Lapa [HBA1:c.92A>C p.Glu30Ala] and Hb Itapira [HBA1:c.92A>T p.Glu30Val]. In both, a polar amino acid residue was replaced by a nonpolar one, but no clinical manifestations were observed in the carriers. Hb Bom Jesus da Lapa was detected in three related heterozygous individuals of indigenous descent. Hb Itapira, for which the mutation was found in a triplicate alpha allele ($\alpha\alpha\alpha^{\text{anti-3.7}}$) and which accounted for only 5.5% of total Hb production, was found in three related individuals.¹⁵

Rare variants

Twenty-two rare alpha-chain variants were detected in 111 individuals from 92 different families. Of these, the most prevalent was Hb Hasharon (41 families, 45 cases) (Table 2), corresponding to 44.6% of the families with rare alpha-chain structural variants. The second most prevalent variant was Hb Stanleyville II (15 families, 17 cases – Table 2), corresponding to 16.3% of the carrier families. In third place was Hb J-Rovigo, with nine affected families (10 cases) (Table 2) and corresponding to 9.8% of the carrier families with rare alpha-globin variants. Hb Hasharon and Hb Stanleyville II resulted in slightly altered hematological findings (hypochromia and microcytosis), which may be explained by the fact that they were in association with the $-\alpha^{3.7}$ deletion.

Hb Hasharon was found in heterozygosis (38 families, 41 individuals), in homozygosis (1 case) and in association with other mutations of globin genes. In one family, two individuals had both Hb Hasharon and Hb Rio Claro [HBA2:c.142G>C p.Asp47His; HBB:c.100G>A p.Val33Met], a beta-chain variant newly described in this laboratory.¹⁶ Both individuals had microcytosis and hypochromia (Table 2). In another family, Hb Hasharon was detected with one β^+ (IVS-I-110) and one β^0 (CD39) allele in the same individual, who presented with a typical clinical course of thalassemia intermedia (Table 2).¹⁷

Like Hb Hasharon, Hb Stanleyville II was found concomitantly with other structural variants in two unrelated individuals (Table 2). In the first, it was present with Hb S, the most common structural variant in the Brazilian population. In the second case, there was a combination of Hb Stanleyville II and Hb Campinas [HBA1:c.237C>G p.Asn78Lys; HBA2:c.80C>T p.Ala26Val] and the individual also had microcytosis and hypochromia (Table 2).¹⁸ In both cases, the red blood cells of the carriers showed microcytosis and hypochromia as the Hb Stanleyville II mutation was associated with alpha-thalassemia ($-\alpha^{3.7}$ deletion).

Hb G-Norfolk was identified in combination with β^0 (CD39) thalassemia [HBA2:c.256G>A p.Asp85Asn; HBB:c.118C>T p.Gln39Stop] in two related individuals resulting in microcytosis and hypochromia (Table 2).

Hb Chelsea [HBA2:c.116C>T p.Thr38Ile] was found in two related individuals, one with erythrocytosis and the other without any abnormal hematological findings (Table 2).

Conversely, Hb Icaria [HBA2:c.427T>A p.Stop142Lys] was identified in combination with a common α^0 thalassemia determinant, the (α)^{20.5} allele. Hb Icaria (X142K) is an elongated chain with 31 extra residues and a change in the C-terminal region of the protein [(142)Lys-Ala-Gly-Ala-Ser-Val-Ala-Val-Pro-Pro-Ala-Arg-Trp-Ala-Ser-Gln-Arg-Ala-Leu-Leu-Pro-Ser-Leu-His-Arg-Pro-Phe-Leu-Val-Phe-(172)Glu-COOH]. In this case, the elongated and unstable alpha-chain in association with the $-(\alpha)^{20.5}$ deletion, resulted in Hb H disease with high levels of Hb H and Hb Bart's (14.7% and 19.0%, respectively) (Table 2).⁴

The other rare alpha-globin variants (Table 2) were found in blood donors and asymptomatic individuals participating in a hemoglobinopathy screening program, which also identified most of the cases of Hb Hasharon, Hb Stanleyville and Hb J-Rovigo.

Discussion

Hb variants and thalassemias have a diverse geographic distribution in Brazil as they are very closely related to the ethnic origins of the population and the colonization process that took place between the 16th and 19th centuries. The largest ethnical influence during the colonization of Brazil was due to Portuguese and African immigrants.

Brazil has the largest Black population in South America, primarily as a result of the African slave trade which was responsible for some 3.6 million black people entering the country from the 16th century onwards, particularly to the Northeast and Southeast. In the 19th century, other populations began to arrive, most notably Italian immigrants, who came in large numbers to settle in the South and Southeast. This miscegenation was also influenced by various migrations from other European (Spain, Germany, Holland, Russia and Poland), Asian (China, Japan and Korea) and Middle Eastern countries, all of which contributed to the formation of the Brazilian population. In addition, internal migratory flows between the populations of different Brazilian states led to miscegenation between all these population groups.¹⁹

This miscegenation has contributed to the high prevalence of hemoglobinopathies, reflecting the racial diversity

Table 2 – Rare alpha-globin variants detected.

Hb variant	Co-inheritance	No. of families/No. of cases	Mutation	α -Genotype	Clinical manifestations/hematological alterations
Hb J-Paris-I		2/2	HBA2:c.38C>A p.Ala12Asp	$\alpha^{\text{J-Paris-I}}/\alpha\alpha$	No
Hb I-Interlaken		1/1	HBA2:c.47G>C p.Gly15Asp	$\alpha^{\text{I-Interlaken}}/\alpha\alpha$	No
Hb Chelsea		1/2	HBA2:c.116C>T p.Thr38Ile	$\alpha^{\text{Chelsea}}/\alpha\alpha$	1 of the carriers with polycythemia 1 asymptomatic carrier
Hb Hasharon		38/41	HBA2:c.142G>C p.Asp47His	$-\alpha^{\text{Hasharon}}/\alpha\alpha$	Microcytosis and hypochromia
Hb Hasharon		1/1	HBA2:c.142G>C p.Asp47His	$-\alpha^{\text{Hasharon}}/-\alpha^{\text{Hasharon}}$	Microcytosis, hypochromia and polycythemia
	Hb Hasharon + Hb Rio Claro	1/2	HBA2:c.142G>C p.Asp47His; HBB:c.100G>A p.Val33Met	$-\alpha^{\text{Hasharon}}/\alpha\alpha$	Microcytosis and hypochromia
	Hb Hasharon + β^+ IVS-I-110 + β^0 CD39	1/1	HBA2:c.142G>C p.Asp47His; HBB:c.93-21G>A; HBB:c.118C>T p.Gln39Stop	$-\alpha^{\text{Hasharon}}/\alpha\alpha$	Thalassemia intermedia clinical feature
Hb J-Rovigo		9/10	HBA2:c.161C>A p.Ala53Asp	$\alpha^{\text{J-Rovigo}}/\alpha\alpha$	No
Hb Shaare Zedek		1/1	HBA2:c.169A>G p.Lys56Glu	$\alpha^{\text{Shaare Zedek}}/\alpha\alpha$	No
Hb Pontoise		1/2	HBA2:c.191C>A p.Ala63Asp	$\alpha^{\text{Pontoise}}/\alpha\alpha$	No
Hb Daneshgah.Tehran		4/5	HBA2:c.218A>G p.His72Arg	$\alpha^{\text{Daneshgah.Tehran}}/\alpha\alpha$	No
	Hb G-Norfolk + β^0 CD39	1/2	HBA2:c.256G>A p.Asp85Asn; HBB:c.118C>T p.Gln39Stop	$\alpha^{\text{G-Norfolk}}/\alpha\alpha$	Microcytosis and hypochromia
Hb Cemenelum		1/2	HBA2:c.277C>T p.Arg92Trp	$\alpha^{\text{Cemenelum}}/\alpha\alpha$	No
Hb Setif		1/5	HBA2:c.283G>T p.Asp94Tyr	$\alpha^{\text{Setif}}/\alpha\alpha$	No
Hb Westmead		1/1	HBA2:c.369C>G p.His122Gln	$\alpha^{\text{Westmead}}/\alpha\alpha$	No
Hb Jackson		1/1	HBA2:c.384G>C p.Lys127Asn	$\alpha^{\text{Jackson}}/\alpha\alpha$	No
Hb Icaria		1/1*	HBA2:c.427T>A p.Stop142Lys	$\alpha^{\text{Icaria}}/\alpha\alpha$	No
	Hb Icaria + $-(\alpha)^{20.5}$ hetero	1/1*	HBA2:c.427T>A p.Stop142Lys	$\alpha^{\text{Icaria}}/\alpha/-(\alpha)^{20.5}$	Hb H Disease
Hb Douala		1/2	HBA1:c.11C>T p.Ser3Phe	$\alpha\alpha^{\text{Douala}}/\alpha\alpha$	No
Hb Kurosaki		3/3	HBA1:c.22A>G p.Lys7Glu	$\alpha\alpha^{\text{Kurosaki}}/\alpha\alpha$	No
Hb Ube-2		1/1	HBA1:c.205A>G p.Asn68Asp	$\alpha\alpha^{\text{Ube-2}}/\alpha\alpha$	No
Hb G-Pest		1/1	HBA1:c.223G>A p.Asp74Asn	$\alpha\alpha^{\text{G-Pest}}/\alpha\alpha$	No
Hb Stanleyville-II		13/15	HBA1:c.237C>G p.Asn78Lys	$-\alpha^{\text{Stanleyville-II}}/\alpha\alpha$	Microcytosis and hypochromia
	Hb Stanleyville-II + Hb S	1/1	HBA1:c.237C>G p.Asn78Lys; HBB:c.20A>T p.Glu6Val	$-\alpha^{\text{Stanleyville-II}}/\alpha\alpha$	Microcytosis and hypochromia
	Hb Stanleyville-II + Hb Campinas	1/1	HBA1:c.237C>G p.Asn78Lys; HBA2:c.80C>T p.Ala26Val	$-\alpha^{\text{Stanleyville-II}}/\alpha^{\text{Campinas}}/\alpha$	Microcytosis and hypochromia
Hb Iwata		1/1	HBA1:c.263A>G p.His87Arg	$\alpha\alpha^{\text{Iwata}}/\alpha\alpha$	No
Hb Tamano		2/4	HBA1:c.269A>G p.His89Arg	$\alpha\alpha^{\text{Tamano}}/\alpha\alpha$	No
Hb Sunshine Seth		1/1	HBA1:c.283G>C p.Asp94His	$\alpha\alpha^{\text{Sunshine Seth}}/\alpha\alpha$	No
Total	22	92/111			

* Related individuals.

in each region of the country.^{19,20} Hence, any investigation into the prevalence of hemoglobinopathies in Brazil must take into account the historical processes involved in the ethnic make-up of the population. To date, no evidence of the existence of hemoglobinopathies in pre-Columbian America has been found, suggesting that these were brought to the Americas and to Brazil by the various migratory flows.^{20,21}

Because it has the most developed economy in the country and is responsible for over half of the country's output, the southeast of Brazil is the main destination for migrants from other countries and Brazilian regions. The State of São Paulo in particular attracts the most immigrants as, in addition to its economic strength, it has relatively good social indicators. Indeed, the metropolitan region of Campinas, where UNICAMP is located, has the highest human development

index in Brazil according to United Nations Development Program (UNDP) data.²² The population in this part of Brazil is very mixed, mainly made up of individuals of Italian, Portuguese, Amerindian, African, Arabic, German, Spanish, Japanese and Chinese descent.

This miscegenation is reflected in the great variety of unusual hemoglobins identified in the cases referred to our laboratory: Hb Stanleyville II, Hb Douala and Hb Chelsea are from the African continent^{2,23,24}; Hb Jackson is found in Black Americans from Southwestern United States²⁵; Hb Westmead, Hb Kurosaki, Hb Ube-2, Hb Iwata and Hb Tamano are of Asian origin^{2,26-29}; Hb Hasharon, Hb Shaare Zedek, Hb Daneshgah-Tehran and Hb Setif are from the Middle East^{2,30-32}; Hb J-Paris, Hb I-Interlaken, Hb J-Rovigo, Hb Pontoise, Hb Cemenelum, Hb G-Norfolk and Hb Sunshine Seth are found in European populations³³⁻³⁹; and Hb Icaria and Hb G-Pest are from Eastern Europe.^{40,41} In this respect, different alpha-globin variants have been found in other Brazilian regions, such as in the state of Minas Gerais, where Hb Etobicoke, Hb Ottawa and Hb St. Luke's were detected for the first time in Brazil.⁴²

Thus, despite the individuals analyzed here belonging to a hospital sample and not reflecting the general population, the variety of hemoglobins identified is the result of the contributions made by the different population groups to the ethnic make-up of the Brazilian population and highlights the importance of programs for diagnosing hemoglobinopathies in Brazil, particularly because of the large number of immigrants from countries with high risk for hemoglobinopathies. The combination of alpha-chain variants with other structural or thalassemic mutations can result in clinically relevant phenotypes, highlighting the importance of characterizing hemoglobins.

Conclusion

The findings presented here corroborate the high degree of diversity among Hb variants in the Brazilian population, in which miscegenation has increased the likelihood of rare hemoglobinopathies. Identification of Hb variants can therefore make a significant contribution to anthropological studies of the different groups that make up the Brazilian population and to an assessment of the incidence of Hb variants, which, in combination with other common hemoglobinopathies, such as Hb S, Hb C and alpha- and beta-thalassemia, represent a public health problem in Brazil. This information could be used by individuals and their families in genetic counseling. In addition, identification and characterization of new hemoglobin structural variants will contribute to a better understanding on the relation between structure and function of this and other important proteins.

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Conflicts of interest

The authors declare no conflicts of interest.

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