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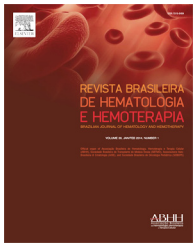

Original article

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.

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 date[2], 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 Clinicas, 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 methods[8] 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]