Three new structural variants of fetal hemoglobin: Hb F-Campinas [^{A}g121(GH4)Glu→Gln], Hb F-Paulinia [^{G}γ80(EF4)Asp→Tyr] and Hb F-Joanopolis [^{G}γ73(E17) Asp→Ala]

Three new structural variants of fetal hemoglobin were detected in newborns during a neonatal screening for Hb S in the southeast of Brazil: Hb F-Campinas [^{A}γ121 (GH4)Glu→Gln], Hb F-Paulinia [^{G}γ80(EF4)Asp→Tyr] and Hb F-Joanopolis [^{G}γ73(E17) Asp→Ala]. These variants were not related to clinical abnormalities.

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Most of the hemoglobin (Hb) structural variants are known to be caused by single amino acid substitutions in the globin molecule. Many of them are not related to clinical or hematolologic manifestations because the alterations do not affect either the stability or the function of the molecule,[1] although they can contribute to a better understanding of the correlation between structure and function of this protein.[1] We describe, herein, three novel fetal hemoglobin (Hb F) variants, which we nominated Hb F-Campinas, Hb F-Paulinia and Hb F-Joanopolis. These variants were detected during a neonatal screening program for Hb S, performed at CIPOI-UNICAMP, and their names correspond to the cities, in the southeast of Brazil, from where the respective families originated. Hemoglobin F-Campinas [^{A}γ121 (GH4) Glu⟶Gln] was found in a Caucasian male newborn; it migrated between Hb S and Hb C on cellulose acetate electrophoresis, at alkaline pH2. Hemoglobin F-Paulinia [^{G}γ80 (EF4) Asp⟶Tyr] was detected in a baby of African descent and demonstrated a Hb S-like electrophoretic band. Hemoglobin F-Joanopolis [^{G}γ73 (E17) Asp⟶Ala] was found in two Caucasian siblings, at different times, and showed an electrophoretic behavior similar to that of Hb F-Campinas. At acid pH, the three variants migrated as normal Hb F. The globin-chain electrophoreses on acrylamide gel, at acid pH, did not show any abnormal chains. Solubility and stability tests[2] were normal.

The molecular analyses involved selective amplification of the γ-globin genes by polymerase chain reaction (PCR),[3] followed by automated DNA sequencing[4] by an ABI Prism DNA Automated Sequencer, model 377 (Applied Biosystems, Foster City, CA, USA), using the *ABI Prism Big Dye Terminator Cycle Sequencing* kit. Hemoglobin F-Campinas is caused by a single base substitution at codon 121 of the ^{A}γ-globin gene (GAA⟶CAA), which replaces glutamic acid with glutamine in

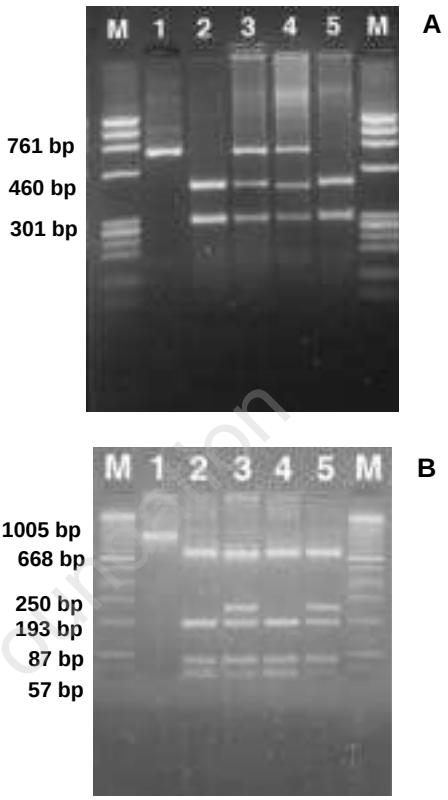

Figure 2. Restriction analyzes. (A) Restriction analysis with Eco RI enzyme: 1- undigested normal control (one fragment of 761 bp); 2- digested normal control (fragments of 460 and 301 bp); 3 and 4- Hb F-Campinas neonate and mother, respectively (fragments of 761, 460 and 301 bp, showing the lack of the Eco RI site in heterozygosis); and 5- father (normal); (B) Restriction analysis with MboI enzyme: 1- undigested control (one fragment of 1005 bp); 2- digested normal control (fragments of 668, 193, 87 and 57 bp); 3 and 5- Hb F-Paulinia neonate and father, respectively, showing the 250 bp extra fragment corresponding to the lack of the MboI site between the 193 and 57 bp fragments; and 4- mother (normal).

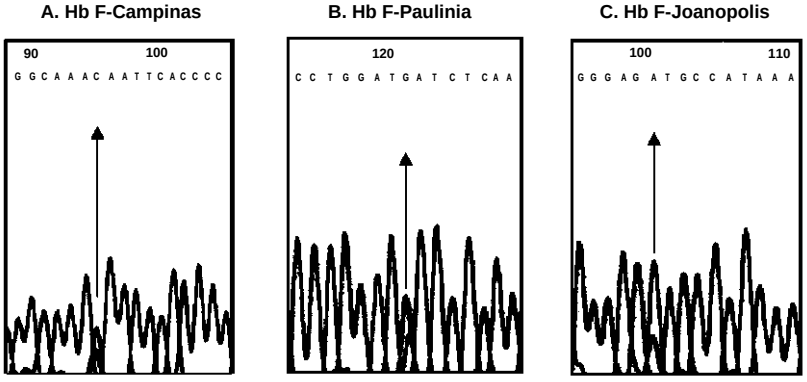

Figure 1. Electropherograms. A) Hb F-Campinas, sequencing of the ^{A}γ-gene showing the mutation at codon 121 (GAA→CAA); B) Hb F-Paulínia, sequencing of the ^{G}γ-gene showing the mutation at codon 80 (GAT→TAT); C) Hb F-Joanópolis, sequencing of the ^{A}γ-gene showing the mutation at codon 73 (GAT→GCT).

the $^{A}\gamma$ chain (Figure 1A). This mutation results in the lack of the Eco RI restriction site, normally occurring at this codon (Figure 2A). Familial analysis revealed that the mother was also a carrier. This is the third description of an alteration at codon 121 of the $^{A}\gamma$ gene; the other two previously described variants are Hb F-Siena ($^{A}\gamma^{T}$ 121 Glu⟶Lys) and Hb F-Hull ($^{A}\gamma$121 Glu⟶Lys), detected in Italians and English babies, respectively. Both these variations are clinically silent.[5,6] Hemoglobin F-Campinas is the $^{A}\gamma$ counterpart of Hb D-Punjab (β121 Glu⟶Gln), which co-polymerizes with Hb S but has no clinical consequences in the presence of Hb A[1].

Hemoglobin F-Paulinia is due to a base substitution at codon 80 (GAT⟶TAT) of the $^{G}\gamma$ gene (Figure 1B), causing the replacement of aspartic acid by tyrosine. This substitution was confirmed by MboI digestion (the site normally present was abolished) (Figure 2B). The carrier's father was also a heterozygote. This is the second description of mutation at codon 80 of the $^{G}\gamma$ gene; the previously described variant, Hb F-Marietta ($^{G}\gamma$ 80 Asp⟶Asn), was identified in a healthy Caucasian neonate.[7] In the β-globin, there is only one described variant with a replacement at this position, normally occupied by asparagine, Hb Szuhu (β80 Asn⟶Lys). Although the replacement is located on the 2,3 DPG binding site, functional studies and the clinical presentation of the heterozygotes were normal.[8]

Hemoglobin F-Joanópolis is the result of an alteration at codon 73 (GAT⟶GCT) of the $^{G}\gamma$ gene (Figure 1C), causing the replacement of aspartic acid by alanine in the corresponding chain; the mutation was confirmed by analysis of the family (the mother was also a carrier). This is the first description of mutation at codon 73 of the $^{G}\gamma$ gene. Other substitutions at residue 73 that have been described in the $^{A}\gamma$ chain are Hb F-Xin-Su [$^{A}\gamma^{I}$ 73 (E17) Asp⟶His] and Hb F-Forest Park [$^{A}\gamma^{T}$ (E17) Asp⟶Asn], found in a healthy Chinese neonate and in normal Caucasian babies, respectively.[9,10] Hemoglobin F-Joanopolis has four analogous mutations in the β-globin gene: Hb Korle-Bu (β 73 Asp⟶Asn), Hb Vancouver (β 73 Asp⟶Tyr), Hb Tilburg (β 73 Asp⟶Gly) and Hb Mobile (β 73 Asp⟶Val), all with reduced O_2 affinity, but none associated with clinical manifestations in heterozygotes.[8]

The carriers of the three variants described here did not show any clinical abnormalities, and their hematologic data were all normal, suggesting that the residue replacements did not compromise the stability or the function of the molecule. However, as functional studies could not be performed, the apparent normality may also be because the low proportion of the variant in the total Hb was insufficient to cause significant alterations.

Regarding globin-chain synthesis, these mutations seem not modify the expression of the affected γ-genes, since Hb F levels below were below 1% in all the adult family carriers.

Denise Faustino Duarte, Dulcinéia Martins Albuquerque,° Vitoria Regia Pereira Pinheiro ,# Fernando Ferreira Costa,° Maria de Fátima Sonati**

**Dept. of Clinical Pathology and °Dept. of Clinical Medicine, School of Medical Sciences, State University of Campinas, UNICAMP; #Integrated Center for Childhood Oncology-Hematology Investigation, CIPOI, State University of Campinas, UNICAMP, Brazil*

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Correspondence: Dr. Maria de Fátima Sonati, Department of Clinical Pathology, School of Medical Sciences, State University of Campinas, UNICAMP, P.O.Box 6111, Zip Code 13083-970 , Campinas, São Paulo, Brazil.
Phone: international +55.19.3788-9453. Fax: international +55.19.37889434. E-mail: sonati@fcm.unicamp.br

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Cell lineage assignment of cytogenetic findings in acute lymphoblastic leukemia using combined immunomagnetic cell separation and chromosome preparation

In acute lymphoblastic leukemia (ALL) abnormal karyotypes frequently constitute a minor part of the dividing cells, and the origin of metaphases in normal diploid cases remains obscure. We used a combination of immunomagnetic cell separation and chromosome preparation (ICSCP) to focus on the metaphases of interest and to assign the chromosome findings to $CD19^{+}$ or $CD7^{+}$ leukemia cells.

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In order to select $CD19^{+}$ cells, we followed the manufacturers' instructions to prepare immunomagnetic microspheres coated with a monoclonal anti-CD19 antibody (clone AB-1; Dynabeads®, M-450 CD19, DYNAL, Hamburg, Germany). For