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Short Communication

Polycythemia and Hb Coimbra [beta 99 (G1) Asp → Glu] in Brazil

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Abstract

We report the clinical and laboratory findings concerning three unrelated Brazilian patients investigated for polycythemia, whose definitive diagnosis could only be established after the presence of Hb Coimbra (β99 Asp → Glu) was demonstrated. This illustrates the importance of properly investigating hereditary hemoglobinopathies in cases of erythrocytosis because in some populations variants with high oxygen affinity may be more frequent than expected but go undetected when conventional electrophoresis is used as the sole detection procedure.

Key words: polycythemia, erythrocytosis, Hb Coimbra, beta-globin gene.

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Some rare structural hemoglobin (Hb) variants have been reported to be associated with functional abnormalities such as high oxygen affinity and a left shift in the Hb-oxygen dissociation curve, leading to tissue hypoxia and compensatory erythrocytosis (Bunn and Forget, 1986). At present, 84 Hb variants with high oxygen affinity have been documented, 77 of which contain β-chain residue replacements (Globin Gene Server Web Site, http://globin.cse.psu.edu). The majority of these variants have substitutions at one of the three crucial regions for Hb stability and function, the $\alpha_1\beta_2$ interface, the β chain C-terminal end and the 2,3-DPG binding site (Bunn and Forget, 1986; http://globin.cse.psu.edu). A particular mutation at codon 99 of the β-globin gene (GA<u>T</u> → GA<u>A</u>) results in the replacement of aspartic acid for glutamic acid in the β-chain and has been associated with polycythemia, this variant having been named Hb Coimbra from the town of Coimbra in Portugal where it was first described by Tamagnini *et al.* (1991).

In this paper we report the clinical and laboratory findings for three unrelated patients investigated for polycythemia at the University of Campinas (UNICAMP) University Hospital, in Campinas, São Paulo state, southeastern Brazil. The definitive diagnosis for these patients could only be established after the presence of Hb Coimbra was demonstrated.

The patients were as follows: *Patient 1.* G.P.P., a 68-year-old female of German descent, was advised to undergo examination after routine laboratory tests revealed a high hematocrit (Hct) value. The patient had no health complaints and no other previously diagnosed diseases. At physical examination, only a ruddy facial color was observed and the cause of the elevated Hct value could not be determined. Hemoglobin electrophoresis and solubility and stability tests were all normal. However, Hb analysis by HPLC (Variant II, BioRad, USA) showed an abnormally enlarged peak in the Hb A position and the same HPLC profile was observed in the patient's 48-year-old son, who was asymptomatic. *Patient 2.* A.T., a 49-year-old male of Portuguese ancestry sought health care because of pruritus, visual disturbances and an unusual red coloration of his cheeks, ears, nose and neck. He had been a regular blood donor for the previous 13 years, since phlebotomy made him feel better. Blood tests showed a high Hct and Hb investigation revealed a normal electrophoretic profile and a HPLC chromatogram similar to that observed in patient 1. *Patient 3.* R.H.C, a 13-year-old male of German descent who was initially referred to the Pediatrics Department presenting with impaired neuropsychomotor development due

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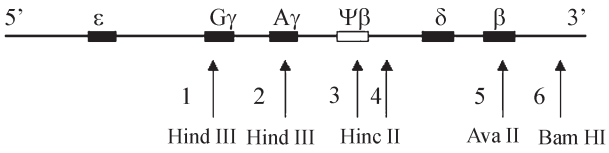
to congenital toxoplasmosis, and microcytic and hypochromic anemia (ferritin = 9.6 ng mL^{-1}). The HPLC chromatogram of this patient and his mother was similar to those of patients 1 and 2. The hematological data on all these individuals are shown in Table 1.

Hemoglobin functional analyses were performed according to Rossi-Fanelli and Antonini, (1958). Since the variant could not be separated from normal Hb A, total hemolysate (in the stripped form and in the presence of organic polyphosphates) was analyzed. In a preliminary test, oxygen affinity, measured in the absence of anionic cofactors, was higher than that of stripped Hb A: the P_{50} value was 0.76 mm Hg, while the P_{50} of Hb A was 2.52 mm Hg, when studied under the same conditions (pH 7.0, 20 °C). The Hill coefficient (*n*) was reduced to about 1.5 at pH 7.0; however, this *n* value did not reflect cooperativity, since total hemolysate was used, *i.e.*, a mixture of two hemoglobins of widely different oxygen affinities. Upon addition of organic phosphates (inositol hexaphosphate - IHP), the P_{50} value increased to 2.35 mm Hg at pH 7.0 (HbA P_{50} = 32.0 mmHg), demonstrating a small change in oxygen affinity of the total hemolysate.

For molecular genetic analyses, genomic DNA was extracted from peripheral blood using the Blood Purification Kit (Invitrogen, Carlsbad, CA, USA). A 661 bp fragment of the β-globin gene (from position -140, relative to the Cap site, to position +521, at the beginning of IVS-II) was amplified by the polymerase chain reaction (PCR) using the primers described by Miranda *et al.* (1997). Direct sequencing of the PCR products was performed using the BigDye™ Terminator Cycle sequencing Kit and the ABI PRISM™ 377 Genetic Analyser (PE Applied Biosystems, Foster City-CA, USA). A single base substitution at codon 99 of the β-globin gene, corresponding to Hb Coimbra (GAT → GAA), was detected in heterozygosis in all individuals investigated (Patient 1 and her son, Patient 2, Patient 3 and his mother).

The β-globin gene cluster haplotypes were determined, by genotyping restriction fragment length polymorphisms (RFLPs) in and around the β-globin-like genes: *Hind* III - 5' $^{G}\gamma$, *Hind* III - 5' $^{A}\gamma$, *Hinc* II - 5'ψβ, *Hinc* II - 3'ψβ, *Ava* II - 3'β and *BamH* I - 3'β (Antonarakis *et al.*, 1982; Orkin *et al.*, 1982; Sutton *et al.*, 1989; Lee *et al.*, 2002). The results are shown in Figure 1. The association between the β-haplotype (- - - - - +) and the Hb Coimbra mutation could be established in Patient 3. Data on Patients 1 and 2 also make this association a possibility.

The nucleotide substitution (GAT → GAA) at the 99th codon of the β-globin gene, detected in our patients is responsible for the previously described Hb Coimbra. This point mutation was first described in a Portuguese family (Tamagnini *et al.*, 1991), and later in several members of a



Sites	Patient 1	Patient 1' son	Patient 2	Patient 3	Patient 3' s mother
1	-/+	-/-	-/-	-/-	-/+
2	-/+	-/-	-/-	-/-	-/+
3	-/-	-/-	-/-	-/-	-/-
4	-/+	-/-	-/-	-/-	-/-
5	-/+	-/+	-/+	-/-	-/-
6	+/-	+/-	+/-	+/-	+/+

Figure 1 - Restriction sites at the β-globin gene cluster and the restriction fragment length polymorphism (RFLP) haplotypes found in the Hb Coimbra carriers.

Table 1 - Hematological data.

	Patient				
Gender, age and hematological data	Patient 1*	Son of patient 1	Patient 2	Patient 3**	Mother of patient 3
Gender	Female	Male	Male	Male	Female
Age (years)	65	48	49	13	35
Red blood cells (x 10^6 cells μL^{-1})	5.72	7.31	6.46	6.39	5.73
Hemoglobin (g dL^{-1})	17.2	22.4	19.2	16.0	15.4
Hematocrit (%)	51	64.7	59.9	48.5	46.5
Mean cell volume (fL)	89.2	88.5	92.7	75.9	81.2
Mean cell hemoglobin (pg)	30.1	30.6	29.8	25.0	26.9
Red cell distribution width	14.2	14.9	17.1	13.6	15.1
Reticulocytes (%)	1.16	-	1.26	-	-
White blood cells (x 10^3cells μL^{-1})	5.44	-	6.20	8.87	8.39
Platelets (x 10^3cells μL^{-1})	154	-	211	340	231

*Blood sample collected during treatment for phlebotomy.
**Blood sample collected during treatment for iron deficiency anemia.

German family and in an American-German family in Michigan, USA (http://globin.cse.psu.edu). It is noteworthy that our Patients 1 and 3 have German ancestry, while Patient 2 was of Portuguese descent. The β-haplotype (- - - - - +), common in normal Mediterranean populations (Orkin *et al.*, 1982), was found to be associated with the mutation in Patient 3. Since it may also be the case in the other two patients, a common origin for this mutation in the three Brazilian families is a possibility. Data on the association of the β-haplotype and Hb-Coimbra both in our population and in European families are not available.

Human Hb normally interacts with organic polyphosphates (Chanutin and Curnish, 1967). The physiological implication of the Asp → Glu in Hb Coimbra is the weakening of the interaction of anionic cofactor, leading to increased Hb synthesis in order to compensate for low tissues oxygenation (Reed *et al.*, 1968; Nagai *et al.*, 1982). Currently, seven mutations at the β99Asp are known: Hb Kempsey (Asp → Asn), Hb Yakima (Asp → His), Hb Radcliffe (Asp → Ala), Hb Ypsilanti (Asp → Tyr), Hb Hotel-Dieu (Asp → Gly), Hb Chemilly (Asp → Val) and Hb Coimbra (Asp → Glu) (http://globin.cse.psu.edu; Tamagnini *et al.*, 1991). Variable degrees of oxygen affinity demonstrate a 25 to 60 percent decrease in the Bohr effect, and all cases were characterized clinically by erythrocytosis. Interestingly, despite the fact that Patient 3 and his mother had high erythrocyte counts they did not present high Hct levels or clinical complaints.

Our findings demonstrate the importance of investigating Hb variants when erythrocytosis is detected. Although conventional Hb electrophoresis did not show a distinctive profile for Hb Coimbra, the HPLC methodology allowed its detection. Functional studies and molecular analyses were also effective.

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