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SHORT COMMUNICATION

Hb ALESHA [β67(E11)Val→Met, *G*TG→*A*TG] IN AN ARGENTINEAN GIRL

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□ *Hb Alesha is caused by a* G*TG*→A*TG mutation at codon 67 of the β-globin gene, resulting in abnormal β-globin chains in which the normal β67(E11) valine is changed to methionine. This hemoglobin (Hb) is also known as Hb Bristol, the first unstable Hb described, since in a fraction of the variant the methionine is modified into an aspartic acid by a posttranslational modification. This replacement disrupts the apolar bonds between the valine and the heme group, producing an unstable Hb and severe hemolysis. We have identified this rare hemoglobinopathy in an Argentinean girl with severe hemolytic anemia, splenomegaly and frequent requirement for red blood cell transfusions.*

Keywords Unstable hemoglobin (Hb), Abnormal Hb, Hemolytic anemia

The structural hemoglobin (Hb) variants mostly result from single amino acid substitutions in the α or β chains. In many cases, these are innocuous but in others they may alter the stability or functional properties of the Hb and lead to clinical disorder (1). Unstable Hb Alesha [β67(E11)Val→Met, *G*TG→*A*TG] was reported by Molchanova

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et al. (2), and in a patient with a Moyamoya syndrome by Brockmann *et al.* (3).

In 1952, the first unstable Hb was found, and its structural study showed that the valine at β67 was replaced by an aspartic acid, and this variant was named Hb Bristol. In 1996, Rees *et al.* (4) restudied these patients, previously diagnosed as carriers of Hb Bristol, finding the *G*TG→*A*TG mutation at codon 67 in the β-globin gene, predicting a valine to methionine substitution similar to Hb Alesha. Further analysis with electrospray ionization mass spectrometry (ESI-MS) and globin change biosynthesis, suggested that this anomaly is due to a novel posttranslational mechanism with conversion of the translated methionine into an aspartate residue.

We recently detected a hemoglobinopathy in a 4-year-old girl with severe hemolytic anemia, splenomegaly and frequent requirements for red blood cell transfusions. The patient was born at term, after a normal pregnancy, by Cesarean section. During the neonatal period, she developed hyperbilirubinemia requiring phototherapy. Rhesus and ABO isoimmune hemolysis were ruled out. Urine culture was positive for *E. coli* and parenteral antibiotics were given. She had a history of multiple episodes of pallor and jaundice related to infections and has received several red blood cell transfusions since the age of 6 months. A splenectomy was performed 3 years later in an attempt to reduce the transfusion requirements. On admission to our Institution her physical examination revealed growth delay, peculiar facial appearance with bossing of the skull, hypertrophy of the maxilla, prominent malar eminences and mongoloid slant of the eyes, generalized icterus, pallor and marked hepatosplenomegaly.

Hematological parameters were measured with a Coulter Counter Model JT (Coulter Corporation, Hialeah, FL, USA). Hemolysates were analyzed by electrophoresis on cellulose acetate at pH 8.4 and on agar citrate at pH 6.0 (5). Hb A_2 was measured by anion exchange chromatography (6) and Hb F according to the method described by Betke *et al.* (7). Reticulocytes, Heinz body formation, heat denaturation and isopropanol instability tests were performed using standard methods (5). Venous blood samples, under anaerobic conditions, were collected with heparin as anticoagulant for the P_{50} determination. Measurements were performed on the Radiometer ABL 520 analyzer (Radiometer A/S, Copenhagen, Denmark). Actual P_{50} was calculated by the simplified oxygen status algorithm studies (8–10). DNA was extracted from peripheral blood samples by standard methods after previous written informed consent. The coding regions of the β-globin gene were amplified in two segments, and polymerase chain reaction (PCR) amplification was accomplished according to conditions already described (11).

The patient showed the following hematological data: Hb 8.4 g/dL, RBC 2.16×10^{12}/L, PCV 0.28 L/L, MCV 128.9 fL, MCH 30.8 pg, MCHC

34.0 g/dL and reticulocytes 25%. The peripheral blood smear showed severe anisocytosis with macrocytosis, pronounced basophilic stippling and polychromatophilia. Blood chemistry showed: unconjugated bilirubin 544 μmol/L (normal range 51–170 μmol/L); total bilirubin 969 μmol/L (normal range 68–238 μmol/L); haptoglobin <5 mg/dL (normal range 70–378 mg/dL); LDH 1951 UI/L (normal range 137–415 UI/L). Both instability tests were positive and Heinz bodies were present in the red cells after incubation with brilliant cresyl blue. Hb A_2 was 2.9% and Hb F 5.6%. The oxygen dissociation curve (ODC) showed a shift to the right (P_{50} 33.62 mm Hg, normal value: 27 ± 2 mm Hg).

Electrophoretic cellulose acetate and citrate agar were normal, however, sequencing of the β- globin gene showed a substitution of *G*TG→*A*TG at codon 67, corresponding to Hb Alesha. This Hb is characterized by a substitution of valine to methionine at position 67(E11) of the β chain. The introduction of the larger methionine residue into the heme pocket, and the loss of the bonds between valine at β67 and the heme group, adequately account for the severe instability of Hb Alesha and the clinical condition found in this patient. Hb Alesha is electrophoretically silent with standard procedures, although partial separation of Hb X and Hb A by cation exchange high performance liquid chromatography (HPLC) has been reported (12,13).

It is important to emphasize the results found by Rees *et al.* (4) when they restudied the patients with Hb Bristol. Their findings, using DNA and protein analyses, show that the original characterization of Hb Bristol was partly incorrect, in that a silent posttranslational modification of Met→Asp was mistaken for the primary mutation, which is in fact β67(E11)Val→Met. According to these studies, both Hbs (Hb Alesha and Hb Bristol) are the same entity. In our patient, this unstable Hb is very likely a *de novo* mutation, since both parents proved to be normal.

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