CORRESPONDENCE

A SPONTANEOUS MUTATION PRODUCED A NOVEL ELONGATED β-GLOBIN CHAIN STRUCTURAL VARIANT (Hb Agnana) WITH A THALASSEMA-LIKE PHENOTYPE

To the Editor:

A number of β-chain structural variants, producing in the heterozygous state the clinical manifestations typical of β-thalassemia, have been described. In this report, we describe a novel variant in a patient with a thalassemia intermedia-like phenotype born from normal parents. This variant eluded identification by conventional hemoglobin analysis and was detected solely by DNA sequencing. Hematologic analysis showed marked thalassemia-like gous red cell abnormalities. On microscopic examination, large inclusion bodies were detected in peripheral red blood cells. DNA sequencing was performed by the dideoxy chain termination method on amplified single strand DNA using the enzyme T7 DNA polymerase (Sequenase-USB). The proband, a girl of Southern Italian descent, presented at 3 years of age with anemia (Hb levels, 6.6 g/dL) and splenomegaly (3 cm below the costal margin). She received sporadic blood transfusions until 6 years of age, when a regular transfusion program was started with packed red blood cells given every 2 to 3 months. She was splenectomized at 14 years of age and died at 27 years of age due to hepatic coma. Her Hb values, before starting a transfusion therapy, were in the range of 6 g/dL. Blood film examination showed marked thalassemia-like red cell abnormalities. On incubation with brilliant cresyl blue, large inclusion bodies were detected in peripheral red blood cells. The HbF and HbA2, levels were 10% and 2.2%, respectively. The α/normal-α-globin chain synthesis ratio was 1.5 (90 minutes incubation). Hb electrophoresis on cellulose acetate and gelsolin chain synthesis analysis on CM52 columns failed to detect any abnormal hemoglobin. Heat and isopropanol stability tests gave normal results. Both parents (Fig 1A, I-1 and I-2) and her sibling (II-2) showed normal red blood cell indices, normal HbF and HbA2, levels, and normal α/normal-α-globin chain synthesis ratio. Direct sequencing of the amplified DNA from the proband detected a TG insertion at codon 94 in the second exon in one of the β-globin genes, while the other was normal (Fig 1B). This mutation produces an out-of-frame reading and a read through of the β-globin mRNA, until a new in-phase termination codon is encountered, resulting in an extended β-chain, 157 amino acids long, with a different carboxy terminus from codon 94. To confirm the presence of this mutation, we carried out a dot blot analysis with an oligonucleotide probe specific for this mutation with the following sequence: 5'-GCACTGTTGACAAAGCTG 3'. A positive signal was seen in the DNA of the proband but not in the parents (Fig 1C).

The abnormal β-chain described in this study was named Hb Agnana from the town where the proband was born. It is most likely a de novo mutation because both parents were phenotypically and genotypically normal. The molecular diagnosis in our proband was obtained by DNA sequencing of the β-globin gene, because conventional chromatographic and electrophoretic techniques failed to detect any abnormal Hb. It is possible that more sensitive chromatographic techniques or shorter incubation times in the globin chain synthesis analysis could have detected the abnormal variant. Hb Agnana should be added to the growing list of elongated β-globin chain structural variants.
Hb variants (eg, Hb Geneva, Hb Manhattan) that result from mutations in exon 3 of the β-globin gene. We were not able to analyze the composition of the red blood cell inclusions, but, in analogy to Hb Geneva, they are most likely composed of α-chains in excess. Accumulation of α-chains that precipitate and damage the red cell membrane is most likely the result of the exhaustion of the proteolytic system, because of the continuous enzymatic breakdown of the abnormal β-chains. From this and previous studies, we can conclude that the presence of abnormal, very unstable Hb should be suspected in any sporadic patient showing the clinical phenotype of thalassemia intermedia and having both parents hematologically normal, or in families in which this phenotype shows a Mendelian dominant transmission pattern. Molecular diagnosis may be rapidly accomplished by direct sequencing of amplified β-globin gene sequences.

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A spontaneous mutation produced a novel elongated beta-globin chain structural variant (Hb Agnana) with a thalassemia-like phenotype [letter]

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