BRIEF REPORT

Absence of Hemoglobin A in a Double Heterozygote for F-Thalassemia and Hemoglobin S

By G. STAMATOYANNOPULOS, C. SOFRONIADOU AND A. AKRIVAKIS

In an ongoing population study in Karditsa (Thessaly, Greece) including screening for hemoglobins by starch gel electrophoresis, a 45 year old male was found to have an electrophoretic pattern characterized by normal levels of hemoglobin A2, large amounts of hemoglobins S and F and absence of hemoglobin A. In addition, he had erythrocyte morphologic abnormalities and increased osmotic resistance. His wife was normal. They had three children; one had sickle-cell trait, while the other two displayed increased fetal hemoglobin on electrophoresis together with increased osmotic resistance and abnormal red cell morphology. On the basis of the screening data, a tentative diagnosis of double heterozygosity for Hb S and F-thalassemia was made for the father. Because study of subjects doubly heterozygous for β-chain variants and F-thalassemia provides important information relative to β-chain synthesis in F-thalassemia, a more detailed study of the propositus and his family was undertaken.

METHODS

Routine hematologic techniques were performed including measurements of hemoglobin, volume of packed red cells, counts of red blood cells and reticulocytes and serum iron determination. The following methods for hemoglobin analysis were also applied. Starch gel electrophoresis in Tris-EDTA-Borate buffer pH 8.6 and in phosphate buffer pH 7.0. Agar gel electrophoresis in citrate buffer pH 6.2. Measurement of hemoglobin A2 by starch block electrophoresis in Tris-EDTA-Borate buffer pH 8.6, and by DEAE-Cellulose chromatography; Hb A2 in the father was measured by starch block electrophoresis in Tris-HCl buffer pH 9.2. Fetal hemoglobin was quantitated by the method of Betke et al. In order to detect Hb A in the hemolysate of the father, the two-dimensional paper agar electrophoresis and DEAE-Sephadex chromatography were used. In addition, the fraction of hemoglobin migrating more rapidly than Hb S on starch block electrophoresis was eluted, concentrated and submitted to agar gel electrophoresis stained subsequently with benzidine. Erythrocytes were studied for inclusions of hemoglobin H by the test of Couttus et al, and for fetal hemoglobin by the elution technique of Kleihauer et al.
RESULTS

Detailed history and clinical examination of the father did not reveal hemolytic episodes; the only clinical finding was slight splenomegaly. He did not have anemia (Hb = 12.0 gr%, Ht = 38%, RBC = 4,690,000); the reticulocyte count was 2.5%. Fetal hemoglobin was 23.6%, Hb A2 2.1%, the remaining hemoglobin fraction was hemoglobin S. Hemoglobin A could not be detected in his hemolysate by the various techniques outlined above (0.25 percent Hb A can be detected by two-dimensional paper agar electrophoresis).

The propositus' wife (40 years old) was found to be normal. Their first child, an eight year old boy, had: Hb = 10.9 gr%, Ht = 35%, RBC = 5,420,000, serum iron 158 y%, erythrocyte morphology clearly indicating thalassemia trait, hemoglobin A2 2.3%, and hemoglobin F, 12.1%. The second child, a five year old boy, had: Hb = 11.4 gr%, Ht = 34%, RBC = 4,600,000, serum iron 113 y%, ± anisocytosis and ± hypochromia of red cells and on starch gel electrophoresis hemoglobins A and S in an approximate 60:40 ratio. The third child, a three year old girl, had: Hb = 10.9 gr%, Ht = 35%, RBC = 5,740,000, serum iron 139 y%, erythrocyte morphology suggesting thalassemia trait, hemoglobin A2 2.5%, and fetal hemoglobin 10.3%.

Hemoglobin Bart's or traces of Hb H were not found in the hemolysate of any family member after starch gel electrophoresis in phosphate buffer at pH 7.0. Erythrocyte inclusions of hemoglobin H were also not found, even after several smears were screened from each individual. The distribution of fetal hemoglobin in red cells was unequal in both the father and the two children with elevated Hb F. In the latter, the hemoglobin was eluted from almost 90% of the erythrocytes.

DISCUSSION

The detailed hematologic studies indicate that the first and third children showed the F-thalassemia trait; they had characteristic morphological abnormalities of the red cells and low MCH in the absence of iron deficiency, as well as low Hb A2 and elevated fetal hemoglobin which was unequally distributed in the red cells. The findings argue against other conditions which resemble F-thalassemia and must be distinguished from it particularly when individual cases are studied. Hereditary persistence of fetal hemoglobin combined with a-thalassemia can be misdiagnosed as F-thalassemia since it presents the typical hematological characteristics of thalassemia trait (due to a-thalassemia) together with increased Hb F and low Hb A2 (due to persistent Hb F anomaly). However, such mixed heterozygotes also present traces of Hb Bart's and Hb H, rare erythrocytes with inclusions of Hb H, and fetal hemoglobin homogeneously distributed in the red cells. In the absence of such findings in our cases, this possibility was excluded. Hereditary persistence of fetal hemoglobin complicated by iron deficiency may be misdiagnosed as F-thalassemia since it manifests with erythrocyte abnormalities and low MCH. In our cases, normal serum iron in the children and the unequal distribution of fetal hemoglobin in the red cells of the father and the children with elevated Hb F exclude this possibility.
Since the diagnosis of F-thalassemia in the first and third children was well documented, the second child was an Hb S carrier, and the mother was normal, it is most reasonable to conclude that the father (the propositus) was a double heterozygote for F-thalassemia and Hb S. It is conceivable that the father was a double heterozygote for Hb S and an A2 thalassemia gene which completely suppressed β-chain synthesis. His normal levels of Hb A2 argues against this possibility. Furthermore, in such a case, it is necessary to postulate that the first and third children in this family are the illegitimate offspring of males with F-thalassemia trait. However, only 1.3% of the population of the village (3 out of 239 parents tested) showed the F-thalassemia trait. In addition, extensive genotyping of the family (ABO, Rh, Kell, Duffy, Kidd, Lewis, P, + MNS, Lutheran, Gc, Transferins, phosphoglucomutase, acid phosphatase, 6PGDhydrogenase, Adenylatekinase) provided no evidence in favor of illegitimacy.

Although F-thalassemia has been phenotypically differentiated from the other β-thalassemias since 1961, only recent study of some critical cases has permitted some insight into this condition. Two homozygotes have been detected, both presenting absence of hemoglobins A and A2. A mixed heterozygote for F-thalassemia and Hb B2 in repulsion also displayed absence of Hb A2. A large number of simple heterozygotes and double heterozygotes for both A2 and F-thalassemia have provided additional information on the production of hemoglobins A, F, and A2. The overall picture seems to indicate that in this mutation, complete β and δ chain suppression takes place in cis position, accompanied by genetically determined elevation of fetal hemoglobin, impaired red cell hemoglobinization and thalassemic hematologic picture. So far, no satisfactory hypothesis putting all these findings together under one common pathogenetic mechanism has been proposed.

The absence of hemoglobin A in the F-thalassemia/Hb S combination of the present family provides further evidence supporting the complete β-chain suppression in F-thalassemia. F-thalassemia is a relatively rare mutation; and thus far, only three other examples of combinations with β-chain variants have been reported. In one case studied by Silvestroni and Bianco, hemoglobin A was absent. Russo et al. and Russo and Mollica have reported two additional cases of F-thalassemia combined with Hb S. In the first, hemoglobin A was not detected by paper electrophoresis. In the second case, a male originating from Calabria (Italy), hemoglobins A2, S, F, A were found in the approximate proportion of Hb S 50%, Hb F 21%, Hb A 29% (means of two determinations). The large amount of hemoglobin A synthesized in this case, contrasted to the absence of Hb A in two F-thalassemia homozygotes and three combinations of F-thalassemia with Hb S, may indicate heterogeneity in the biochemical expression of F-thalassemia gene. However, the alternative possibility of a combination of hereditary persistence of fetal hemoglobin with Hb S has not been definitely ruled out in the case of Russo and Mollica. First, evidence was not presented that the two presumed F-thalassemia heterozygotes in the family (mother, niece of the patient) were not, in fact, examples of Hereditary Persistence of fetal hemoglobin either
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combined with α-thalassemia or complicated by iron deficiency; Hereditary Persistence of fetal hemoglobin is observed in Calabria, with a frequency equal to that of F-thalassemia. Second, the presence of hemoglobin A in the patient does not a priori argue against double heterozygosity for Hereditary Persistence of fetal hemoglobin and hemoglobin S, since it has already been suggested that in one type of Hereditary Persistence of fetal hemoglobin, the mutation is expressed by simultaneous synthesis of all β, γ, and δ chains in cis position. Double heterozygotes for this type of Hereditary Persistence of fetal hemoglobin and hemoglobin S are expected to synthesize large amounts of both hemoglobins A and F in addition to hemoglobin S. It seems, therefore, reasonable that the concept of complete suppression of β-chains in cis position be reserved for F-thalassemia, until other fully documented F-thalassemia homozygotes or combinations with β-chain variants are reported to present hemoglobin A.

SUMMARY

An adult with minor hematologic abnormalities and hemoglobin electrophoretic pattern characterized by large amounts of hemoglobins S and F together with absent hemoglobin A, was shown to be doubly heterozygous for F-thalassemia and hemoglobin S. Absence of Hb A in this double heterozygote provides further evidence that in F-thalassemia the suppression of the β-chains in cis position is complete.

SUMMARIO IN INTERLINGUA

Esseva trovate que un adulto con minor anormalitates hematologic e un configuration electrophoretic de hemoglobina characterisate per grande quantitates de hemoglobina S e de hemoglobina F in le absentia de hemoglobina A esseva duplemente heterozygotic pro F-thalassemia e hemoglobina S. Le absentia de hemoglobina A in iste duple heterozygoto provide evidentia additional que in F-thalassemia le suppression del catenas β in position cis es complete.

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