Association of Hemoglobin H Disease With Hb J-Iran (β77 His → Asp): Impact on Subunit Assembly

By Samuel Rahbar and H. Franklin Bunn

A young Iranian female was found to be heterozygous for hemoglobin (Hb) J-Iran (β77 His → Asp) in combination with Hb H disease. The proportion of Hb J in the patient's hemolysate was surprisingly high: 65% Hb J, 30% Hb A. Thus, the interaction of a negatively charged β subunit variant of Hb with α-thalassemia leads to a marked increase in the relative amount of the variant Hb within red cells. This observation provides further support for an electrostatic model of Hb subunit assembly.

METHODS

Routine hematologic data were obtained by standard techniques. Erythrocytes were examined for inclusion bodies after treatment with brilliant cresyl blue. Hb stability was assessed by incubations of hemolysates in isopropanol. Hb electrophoresis was performed on cellulose acetate (Tris-EDTA-borate buffer, pH 8.6) and on acid citrate agar (Fig 1). Previously, the association of another negatively charged β subunit variant with α-thalassemia was reported in Turkey and in a Russian-Armenian family. The proportion of each variant in heterozygotes was always found to be nearly equal to that of Hb A. In this report we describe a 14-year-old female who had Hb J-Iran in combination with Hb H disease. Surprisingly, electrophoresis on both cellulose acetate and on citrate agar revealed twice as much Hb J as Hb A. This observation provides additional insights into how the surface charge on mutant Hb affects subunit assembly.

RESULTS

A 14-year-old Persian girl was referred for investigation of mild jaundice and anemia. On physical examination her spleen was found to be moderately enlarged. Two other siblings were also found to have mild anemia and splenomegaly. Erythrocytes from the proband and from two of her siblings displayed characteristic inclusion bodies of Hb H disease. The proportion of red cells containing inclusion bodies was much less in the proband than that found in her two siblings. Nevertheless the isopropanol stability test was positive in all three individuals. Hb electrophoresis on cellulose acetate at alkaline pH confirmed the presence of Hb H in the proband as well as in these two siblings. In addition to Hb H, the proband showed a fast-moving Hb band in the position of Hb J. The distribution of Hbs in the proband's hemolysate was 65% Hb J, 30% Hb A, 3% Hb H, and 2% Hb A2. The α-to-β synthetic ratio in the proband was 0.2, consistent with three-gene deletion α-thalassemia. Three other siblings were also heterozygous for Hb J but lacked Hb H. The variant in their red cells composed about 50% of the total Hb. The marked difference in Hb distribution between the proband and her siblings was confirmed by citrate agar electrophoresis at pH 6.5 as well as by electrophoresis and chromatography of globin chains. Structural analysis of the variant demonstrated that it was Hb J-Iran (β77 His → Asp), a variant frequently found among Iranians.

DISCUSSION

This is the first description of the association of Hb H disease with a negatively charged β subunit variant. This severe form of α-thalassemia imposes a marked increase in the proportion of the variant Hb in the circulating red cells (Fig 1). Previously, the association of another negatively charged variant, Hb J-Baltimore (β16 Gly → Asp), with probable homozygous α-thalassemia-2 (α-/α-) was noted to cause a moderate increase of the variant within the red cells. In contrast, several reports have described the association of α-thalassemia (including Hb H disease) with positively charged β subunit variants such as Hb S, Hb C, Hb E, and Hb D. In all cases the proportion of these variants

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Fig 1. Effect of α-thalassemia on the proportion of negatively charged (0) and positively charged (1) β globin variants in heterozygotes. In all cases Hb A constitutes the great majority of the remaining Hb. In three-gene deletion α-thalassemia, a small amount of Hb H (0) is present. Modified with permission.1

In heterozygotes correlates inversely with the number of α genes deleted (Fig 1).

The substitution of an aspartic acid residue for a histidine in Hb J-Iran gives this variant a gain of 1.5 negative charge units. The higher negative charge on the surface of the flu-Iran subunit would be expected to enhance its electrostatic attraction for the normal positively charged α subunit, thereby allowing the formation of more Hb J-Iran than Hb A in the heterozygote’s red cells. In the presence of α-thalassemia, the competition between αA and αJ subunits for the deficient α subunits is greatly enhanced, and even more Hb J-Iran is formed. The same mechanism applies to the observations noted earlier on Hb J-Baltimore. In fact, in vitro competition experiments using purified βA and βJ-Baltimore subunits have verified that when α subunits are deficient, the formation of Hb J exceeds that of Hb A by a factor of 1.5.16 In contrast, the lower levels of the positively charged variants imposed by α-thalassemia can be explained by decreased electrostatic attraction between the α subunit and the variant β subunit.

In conclusion, the interaction of α-thalassemia with the negatively charged β globin variant Hb J-Iran provides informative and independent support for the importance of electrostatic attraction in the assembly of Hb subunits.1

REFERENCES
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