Homozygosity for nondeletion δ-β⁰ thalassemia resulting in a silent clinical phenotype

Renzo Galanello, Susanna Barella, Stefania Satta, Liliana Maccioni, Carlo Pintor, and Antonio Cao

The clinical phenotype of homozygous β thalassemia varies in severity from the mild thalassemia intermedia to the severe thalassemia major. This variability depends largely on the molecular heterogeneity of β thalassemia defects. We report the first case of a homozygous state for nondeletion Sardinian δ-β⁰ thalassemia, which resulted in a symptomless clinical phenotype with a peculiar hemoglobin (Hb) pattern (99.8% Hb F and 0.2% Hb A₂). The molecular defect was characterized by the presence of 2 nucleotide substitutions: −196C>T in the promoter of the Aγ-globin gene and β 39C>T nonsense mutation. The absence of typical β thalassemia clinical findings was due to the high Hb F output, which compensated for the absence of β chains. The near absence of Hb A₂ may have resulted from either alterations in the globin gene transcriptional complex with preferential activation of γ-globin genes and suppression of δ-globin genes or preferential survival of red blood cells with the highest Hb F content and low Hb A₂ level. (Blood. 2002; 100:1913-1914)

Introduction

Thalassemia intermedia is a clinical definition for a heterogeneous group of conditions ranging in severity from the β thalassemia carrier state to the transfusion-dependent thalassemia major phenotype. Characteristic of these mild clinical forms is the absence of an absolute requirement for regular transfusions for survival. The remarkable variability in clinical severity of thalassemias intermedia results largely from the genetic heterogeneity of thalassemias. Progress in molecular biology has allowed definition of globin gene defects and partial elucidation of the relation between phenotype and genotype.¹ The defined molecular mechanisms for thalassemia intermedia have a common hallmark a reduction in the imbalance of synthesis of α and non-α globins. One of these mechanisms is an increase in production of γ-globin chains sufficient to reduce the α/γ imbalance, which results from the coinheritance of genetic determinants such as δ-β thalassemia or hereditary persistence fetal hemoglobin (HPFH).

δ-β Thalassemia is usually caused by large deletions of variable extent in the β-globin cluster.² One form of nondeletion δ-β⁰ thalassemia has been described; this disorder results from the presence in cis in the β-globin cluster of 2 different nucleotide substitutions, one in the promoter of the Aγ gene (−196C>T) and the other in the β-globin gene (the β 39C>T nonsense mutation).³ Carriage of nondeletion δ-β⁰ thalassemia is characterized by high levels of hemoglobin F (Hb F; range, 10%-20%) containing mainly Aγ chains and normal levels of Hb A₂.⁴ Nondeletion δ-β⁰ thalassemia is a rare form of δ-β thalassemia in the Sardinian population. Compound heterozygosity for δ-β⁰ thalassemia and the common β 39 nonsense mutation results in the clinical phenotype of thalassemia intermedia.⁵ Here, we report the first case of a homozygous state for nondeletion δ-β⁰ thalassemia, which produced a symptomless clinical phenotype with a peculiar Hb pattern.

Study design

An 18-month-old girl was admitted to the hospital because of high fever. Venous blood was drawn for hematologic and molecular analysis. Red blood cell (RBC) indices were determined by using a Coulter STKS device (Beckman Coulter, Milan, Italy). Qualitative and quantitative Hb analysis was done with high-pressure liquid chromatography (Variant; Bio-Rad, Milan, Italy)⁶ and globin-chain analysis with reversed-phase high-performance liquid chromatography (Gold System; Beckman Coulter). Analysis of globin-chain synthesis was carried out according to the Clegg method.⁷ Serum levels of erythropoietin (Epo) and transferrin receptor (TIR) were determined by enzyme immunoassays (ImmuliTe Epo; DPC, Los Angeles, CA, and Ramco Laboratories, Houston, TX, respectively). DNA was extracted from peripheral blood (PB) leukocytes, and cluster mutations in the β-globin gene were defined by sequencing DNA amplified by polymerase chain reaction.⁸

Results and discussion

Analysis of RBC indices and Hb level performed as part of a routine hematologic evaluation in our patient showed a normal Hb level (125 g/L), microcytosis (mean corpuscular volume [MCV], 61.6 fL), and an Hb pattern indicative of homozygous β⁰ thalassemia with only Hb F and a near absence of Hb A₂ (Hb F, 99.7%; and HbA₂, 0.3%; Figure 1). Hb F was composed of 72% Aγ and 28% Aγ chains (ratio of Gγ to Aγ, 0.38). Analysis of globin-chain synthesis in PB reticulocytes showed an α-to-γ ratio of 1.9, which is in the range of that of carriers of β thalassemia. A PB smear revealed only mild morphologic changes in RBCs (hypochromia and anisopoikilocytosis) and absence of nucleated RBCs. Clinical examinations at presentation and follow-up (6 years) showed no...
enlargement of the liver or spleen, growth within the normal range (10th percentile for both weight and height), and absence of thalassemia-associated bone changes.

DNA analysis of the β-globin gene cluster in the patient revealed homozygosity for the codon 39 nonsense (C>T) mutation and the −196C>T substitution in the promoter of the Aγ-globin gene. These mutations are typical of nondeletion Sardinian δ-βthalassemia. The patient’s mother was found to have the hematologic characteristics of carriers of this genetic determinant (MCV, 73 fl; Hb A2, 2.8%; and Hb F, 18.8%), and DNA analysis confirmed the presence of heterozygous state for the mutations observed in the patient. The patient’s father was not available for testing. Repeated clinical and hematologic evaluations of the patient during follow-up (up to 6 years) did not show any clinical abnormalities. In particular, Hb levels remained within the expected range for the patient’s age, and erythropoiesis was not quantitatively increased as indicated by normal reticulocyte numbers and normal serum levels of Epo and TfR (Figure 1). The total bilirubin level was not increased (8.5 μM/L).

Compound heterozygotes for nondeletion Sardinian δ-βthalassemia and the β0 39 nonsense mutation have the classic clinical phenotype of thalassemia intermedia characterized by moderate to severe anemia, hepatosplenomegaly, and mild jaundice because the increased production of Hb F associated with the δ-β thalassemia determinant partly compensates for the absence of β chains. The homozygous state for nondeletion δ-βthalassemia in our patient resulted in a marked increase in Hb F that mostly compensated for the absence of β chains. The increased number of RBCs contributed to achievement of normal Hb levels. The imbalance in the ratio of α to γ, similar to that in heterozygous β thalassemia, explains the reduction in MCV and mean corpuscular Hb. Heterozygotes for Sardinian nondeletion δ-β thalassemia have a high proportion of Aγ-globin chains (range, 80%-93%) resulting from overexpression of the Aγ gene associated with the absence of β-globin synthesis due to the presence of the β0 39 nonsense mutation. Aγ chains were also prevalent in our homozygous patient, who had 72% Aγ-globin chains. The higher Gγ content compared with that in the heterozygous state may have been due to mild stress erythropoiesis, which was also reflected in the increased RBC production.

Patients who are heterozygous for nondeletion δ-β thalassemia have reduced Hb A2 levels (0.6 pg/cell compared with 0.75 pg/cell in healthy subjects and 1.1 pg/cell in carriers of β thalassemia). Interestingly, our patient with nondeletion homozygous δ-βthalassemia had almost no Hb A2 (0.3%). We previously reported that the δ-globin gene sequence in a δ-βthalassemia chromosome from position −360 to the Cap site to 343 nucleotides 3’ to the termination codon was entirely normal. Therefore, the reduced expression of the δ-globin gene of the Sardinian δ-β thalassemia chromosome may result from the suppressive effect of the in cis Aγ −196C>T mutation. This suppressive in cis effect has already been reported for similar mutations, such as the −202 Gγ HPFH, and may be explained by assuming a reciprocal and coordinated regulation of globin gene expression through the interaction of the locus control region and the individual genes. However, the very low Hb A2 level could also be related to the preferential survival of RBCs with the highest Hb F content, since it is well known that Hb A2 levels have a strong negative correlation with Hb F levels.

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References

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