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C→T mutation at -158 $^G\gamma$ HPFH associated with 4 bp deletion (–225–222) in the promoter region of the $^A\gamma$ gene in homozygous β^0 39 nonsense thalassemia

Sir,

Two Caucasian brothers from Central Spain were found to have homozygous β^0 thalassemia with mild anemia and mild physical stigmata of thalassemia. Molecular studies revealed that both subjects were homozygotes for the nonsense mutation of codon 39 (C→T), and heterozygotes for the C→T mutation at position –158 to the $^G\gamma$ gene [*Xmn* I-γ (+)] and for the 4 bp deletion (–225–222) in the promoter of the $^A\gamma$ gene.

β-thalassemias are a heterogenous group of genetic alterations characterized by a deficient synthesis (β+) or an absence (β^0) of β globin chains. The clinical expression of this disease can range from asymptomatic cases in most heterozygote forms of β-thalassemia (thalassemia minor) to severe forms of the disease (thalassemia major) in which the patients, usually homozygotes or double heterozygotes, are transfusion dependent. However, between these two extreme clinical forms there are a wide range of clinical phenotypes.[1]

We have studied two Caucasian brothers, 26 (II_1) and 31 (II_2) years old, from Central Spain. Physical examination revealed normal body structure with a splenomegaly of 5 cm in II_1 and 6 cm in II_2, and mild signs of thalassemic facies and conjunctival jaundice in both. Their father (I_1) and mother (I_2) were not related but both had thalassemia minor. The subjects had a more severe phenotypic expression than their parents with mild anemia (Table 1).

Both subjects were homozygotes for the nonsense mutation of codon 39 (C→T) and their parents were heterozygotes for this mutation (Figure 1). This mutation produces a lack of expression of the β gene (β^0) and has been reported to be responsible for thalassemia major.[2] The existence of α-thalassemia, which would have produced a less pronounced phenotypic expression of the disease,[3] was ruled out by Southern blot analysis with *Bam* HI, *Bgl* II, *Hph* I, *Nco* I and *Eco* RI restriction enzymes and α and ζ probes.

In the last decade some forms of non HPHF-deletion, which can "improve" the expression of the disease, have been described. These forms are due to point mutations of one base upstream of the $^G\gamma$ or $^A\gamma$ gene. Most of these mutations are associated with levels of HbF from 5 to 25% in heterozygotes and levels of HbF greater than 5% when associated with heterozygote β-thalassemia.[4] In the two cases reported here the parents are carriers of heterozygous β-thalassemia and the levels of HbF are lower than 3% in both (Table 1). On the other hand, the substitution C→T at position –158 of the $^G\gamma$ gene [*Xmn* I-γ (+)] is associated with increases in HbF in situations of severe anemia and stress erythropoiesis (homozygote SS, homozygote or double heterozygote β-thalassemia) which would result in a decrease in the clinical severity of these situations.[5-7] However, these *Xmn* I-γ (+) are not associated with a significative increase in HbF in normal individuals or heterozygote β-thalassemias.[7] The molecular studies revealed that the mother and the two brothers had the C→T mutation at position –158 to the $^G\gamma$ gene [*Xmn* I-γ (+)] in the heterozygote form (Figure 1). This finding could explain the clinical picture of the disease, with a mild anemia of 10.5 to 11.5 g/dL of HbF and a $^G\gamma/^A\gamma$ ratio of 2:1, higher than the expected 2:3, in the brothers, and a HbF level less than 3% in the mother who has heterozygote β-thalassemia. Other forms of non HPHF-deletion are associated with levels of HbF greater than 5% when associated with heterozygote β-thalassemia.[4] In this context, the presence of another form of non HPHF-deletion associated in this family is not probable.

At the level of the promoter of the gene $^A\gamma$ both the brothers and the parents had a 4 bp deletion (–225–222) (Figure 1). This deletion of 4 base pairs is

Table 1. Hematologic values and biochemical studies.

Measurement	I_1 (father)	I_2 (mother)	II_1	II_2
RBC × 10^{12}	6.9	6.3	4.5	4.6
PCV (L/L)	41.6	38.1	33.7	31.4
Hb (g/dL)	13.6	12.5	11.3	10.6
MCV (fL)	59.2	61	75	67.6
MCH (pg)	19.6	20	25.5	22.8
MCHC (g/L)	33.1	32.8	33.6	33.8
RDW (%)	15.5	14.9	26.1	26
Reticulocytes (‰)	6.2	5.9	7.4	8.3
IMR: (MFR+HFR)×100/LFR	3.5	6.9	13.4	10.7
Hb A_2 (%)	5	5.2	2.5	3.1
Hb F (%)	2.9	2.3	97.5	96.9
$^G\gamma/^A\gamma$	-	-	2/1	2/1
LDH (U/L)	-	-	226	186
Total bilirubin (mg/dL)	-	-	4.8	3.40
Serum iron (g/dL)	-	-	171	108
TIBC (g/dL)	-	-	193	184
Ferritin (ng/mL)	-	-	368.2	454.8

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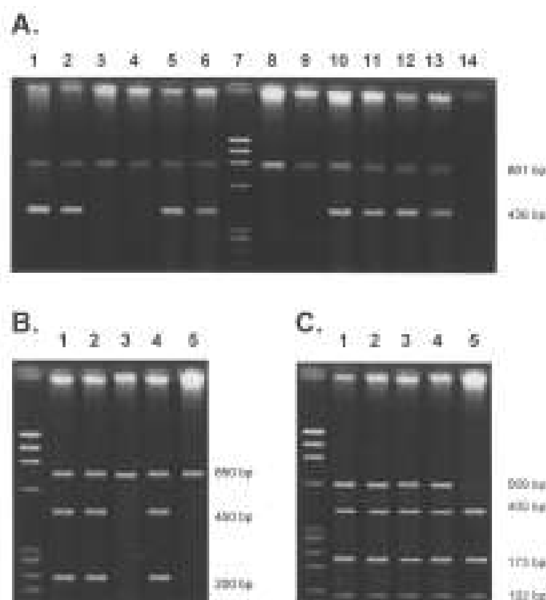

Figure 1.
A. Ethidium bromide-stained gel illustrating the products obtained after amplification using primers specific for detection by PCR-ARMS of the point mutation CD39 (C→T) (436 bp). The first six lanes correspond to the normal assay. Lane 1 and 2 are heterozygote controls, Lanes 3 and 4 represent the propositus II_1 and II_2 respectively. Lanes 5 represent the father (I_1) lane 6 the mother (I_2), respectively. Lane 7 is the DNA Marker X174 *Hae* III. Lines 8 to 13 correspond with the mutate assay; lane 8 and 9 heterozygote controls, lanes 10 and 11 represent the propositus II_1 and II_2, respectively. Lane 12 represent the father (I_1) and line 13 the mother (I_2). Lane 14 is the H_2O control. The internal control corresponds to the 861 bp fragment.

B. Ethidium bromide-stained gel illustrating the products obtained after amplification using specific primers and digestion with *Xmn* I for the polymorphic variation of C→T in the 158 bp 5' of the Cap site of the $^G\gamma$ gene. Lane 1 represents propositus II_1 [heterozygote for –158 (C→T)] with 650, 450 and 200 bp fragments. Lane 2 represents propositus II_2 [heterozygote for –158 (C→T)] with 650, 450 and 200 bp fragments. Lane 3 represents the father (I_1) (*Xmn* I– (–) haplotypes) with a 650 bp fragment, lane 4 the mother (I_2) [heterozygote for –158 (C→T)] with 650, 450 and 200 bp fragments. Lane 5 represents a negative control with a 650 bp fragment. DNA Marker X174 *Hae* III.

C. Agarose gel electrophoresis of Fnu 4HI-digested A promoter DNA amplified by PCR. Lanes 1 and 2 represent the propositus II_1 and II_2 respectively with 569, 400, 173 and 102 bp fragments (heterozygotes for 4 bp deletion). Lanes 3 and 4 represent the father (I_1) and mother (I_2) respectively with 569, 400, 173 and 102 bp fragments (heterozygotes for 4 bp deletion). Lane 5 is a normal control DNA with 400, 173 and 102 bp fragments. DNA Marker X174 *Hae* III.

closely associated in *cis* with haplotype II in cases of β-thalassemia with the mutation of codon 39, and has been reported to cause decreased expression of $^A\gamma$ when it is associated in *trans* with haplotype I and IX β^0 39.[8] It has not been well determined whether this decrease in $^A\gamma$ expression can affect expression of the gene $^G\gamma$ in *cis* or in *trans*.[9,10] This way, the presence of the deletion of 4 base pairs from 225 to 222 in the promoter region of the $^A\gamma$ gene, in our two cases (II_1 and II_2), would favor the expression of $^G\gamma$[10] which would already be augmented by the presence of *Xmn* I-γ (+).

Although the existence of other related genetic factors which produce an increase of HbF in the *Xmn* I-γ (+) cannot be ruled out, the C→T substitution at position –158 to the $^G\gamma$ gene is in a region which contains sequences which are important in regulation of γ gene expression[5] and probably, in addition to being a genetic marker, is responsible for most of the $^G\gamma$ synthesis in these *Xmn* I-γ haplotypes. It is, therefore, important to study this factor in patients with β-thalassemia because of the prognostic implications of this disease.

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