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Thalassemic trait caused by IVS II-1 (G→A) mutation detected in a Spanish family

We have detected, for the first time in Spain, a β° thalassemia caused by the mutation IVS II-1 (G→A). This mutation, relatively frequent in Mediterranean countries, is very rare in the Iberian peninsula. This finding besides its epidemiological relevance, increases knowledge of the spectrum of Spanish mutations of β thalassemia.

Sir,

In three members of a Spanish family from the province of Valencia (east of Spain) we detected, for the first time in Spain, a β^0 thalassemia caused by G→A transition at nt 1 of IVS-II of the β globin gene. The propositus (I_1) was diagnosed in the course of routine hematologic screening at the age of 57 years and was symptom-free. Unfortunately, the parents could not be included in the study as they had died. Investigation of the other siblings of the family (two sisters) revealed the presence of thalassemia in one of the sisters (I_2) of the propositus who frequently complained of fatigue and lassitude, but the other sister (I_3) was disease-free. The hematologic study of the propositus' children (three daughters) revealed disease in one of them (II_2), but the other two were disease-free.

The hematologic parameters of the three thalassemic individuals (I_1, I_2 and II_2) showed slightly high RBC, low levels of Hb (range: 9.9-12.1 g/dL), MCV (range: 60.2-67.2 fL), and MCH (range:17.7-20 pg) and high levels of RDW (15.2-17%) (Table 1). The three thalassemic subjects had a high percentage of HbA_2 (range: 4.2-5) and a normal percentage of HbF (Table 1). The slightly lower level of HbA_2 in carrier II_2 with regard to the others might be caused by her sideropenia. Two of the three carriers (I_1 and I_2) had normal serum iron levels, but the other (I_2) showed low levels. Ferritin was high in carriers I_1 and I_2 and low in the other (II_2) (Table 1). Molecular studies, carried out by PCR-ARMS[1] and corroborated by sequencing (Figure 1), confirmed the presence of transition G→A at nt 1 of IVS II of the β-globin gene in one of the alleles (heterozygosity). This mutation affects the splicing process causing the premature interruption of translation and consequently a β^0 thalassemia.[2] The thalassemia caused by G→A transition at nt 1 of IVS II (IVS II-1) is relatively frequent in the countries bordering the Mediterranean basin, but is very rare in other countries around the world. Among the Mediterranean countries the IVS II-1 mutation is especially fre-

Table 1. Hematologic values and biochemical studies.

Measurement	*Normal range*	*I_1*	*I_2*	*II_2*	*II_1*	*I_3*	*II_3*
RBC(x10^6/μL)	4.7-6.1	5.3	6.1	5.3	5.2	4.5	4.3
Hb (g/dL)	12.0-18.0	9.9	12.1	9.4	13.8	13.5	13.5
PCV (%)	37.0-45.0	33.1	40.8	32.2	44	42.5	40.9
MCV (fL)	80.0-99.0	62.9	67.2	60.2	84.7	65.3	96.2
MCH (pg)	27.0-31.0	18.9	20	17.7	26.5	30.3	31.9
MCHC (g/L)	32.0-36.0	28.9	29.7	29.3	31.3	31.8	33.1
RDW (%)	11.0-17.0	15.2	15.6	17	13	13.1	12.3
Hb A_2 (%)	1.3-3	5	4.7	4.2	2.3	2.3	2.4
Hb F (%)	0.3-1.5	1.5	0.6	1	0.1	0.1	0.1
Ferritin (ng/mL)	15-125	181.3	129.8	5.66	52.7	46.9	61
Serum iron (g/dL)	40-150	68	59	38	115	56	76

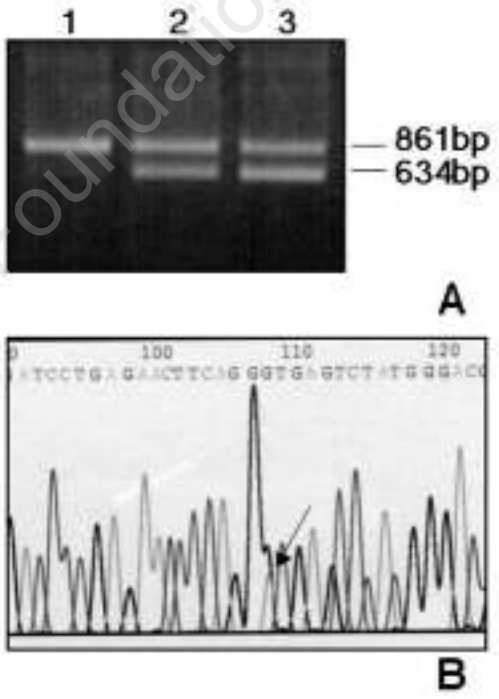

Figure 1. A. Detection of the β IVS II-1 (G→A) mutation by ARMS: the 861 bp fragment represents the DNA amplified by the control primers (forward 5'CAATGTATCATGCCTCTTTGCACC 3'; reverse 5' GAGTCAAGGCTGAGAGATGCAGGA 3') which serves to monitor the amplification procedure; the 634 bp fragment represents the allele specific PCR product for the β IVS II-1 (G→A)mutation obtained with the ARMS primers: common primer 5' ACCTCACCCTGTGGAGCCAC 3';primer for wild-type allele 5' AAGAAAACATCAAGGGTCCCATAGACTGAC 3'; primer for the mutated allele 5' AGAAAACATCAAGGGTCCCATAGACTGAT 3'. Lane 1, normal control; lane 2, subject heterozygous for the IVS II-1 mutation; lane 3, propositus (I_1). B. Sequence analysis of the amplified β globin gene, using the ABI377 DNA sequencer (Applied Biosystem). The arrow indicates the presence of a G→A substitution at nt 1 of IVS II causing the β°thalassemia. For PCR and sequencing reaction the following primers were used: forward 5' GGTGAACGTGGATGAAGTTG 3'; reverse 5' TCCTATGACATGAACTTAAC 3'.

quent in Hungary, Turkey and Lebanon[3] where it represents between 3 to 7% of the β-thalassemias. However it is very scarce in the island of Sardinia. This mutation was also reported in inland countries of Europe and the Middle East such as Czechoslovakia and Azerbaijan,[3] where it represents 14-16% of the molecular abnormalities detected in β-thalassemias. On the Iberian peninsula, to our knowledge, only a single case of IVS II-1 among 88 thalassemics has been reported in a study carried out in the north of Portugal.[4] There have been no reports until now of this molecular abnormality in Spain. This abnormality, according to our experience in molecular diagnosis of β-thalassemia, represents 1 out of 40 β thalassemics.[5]

Although this molecular abnormality is a β^0, its rarity lessens its clinical importance. However, awareness of its existence increases the spectrum of known mutations in Spain and, consequently, in the Iberian Peninsula.

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Azoospermia in a patient with sickle cell disease treated with hydroxyurea

This report describes a case of reversible azoospermia in a patient treated with hydroxyurea. This occurrence has not been previously documented in patients with sickle cell disease. We suggest that patients treated with this therapy should be informed of this potential side effect and that they should be given the necessary clinical follow-up investigations.

Sir,

Sickle cell disease (SCD) is a hemoglobinopathy characterized by an amino acid substitution in the beta chain of hemoglobin (hemoglobin S, HbS), which determines, under conditions of hypoxia, the polymerization and precipitation of hemoglobin within the erythrocytes and their subsequent deformation (sickling). The clinical picture of SCD is characterized by recurrent painful crises, hematologic crises (aplasia, splenic sequestration, hemolysis) and infections.The use of hydroxyurea (HU) causes an increase of HbF[1] which interferes with the process of polymerization of HbS; in fact at the dose of 20 mg/kg/day HU increases the value of HbF which causes an improvement in the general state of health of the patients and a reduction in the frequency and severity of painful crises (especially during the first months of therapy).[2] In our center we treat 120 patients of whom 79 are affected by thalassemia major, 24 patients by thalassemia intermedia, 15 patients by double heterozygosis for HbS and beta thalassemia (thalassodrepanocytosis) and 2 patients by SCD. One of the two SCD patients is a male who was born in 1971 and both his parents are HbS carriers with normal values of HbA_2. Our patient has required periodic blood transfusions because of recurrent severe painful crises (first crisis in 1972 at the age of 8 months); he was splenectomized in 1977. This patient was offered HU therapy at the above stated dosage. Before starting the therapy we performed: spirometry, chest X-rays, liver, biliary ducts, kidney and urinary tract ultrasound, echocardiogram, hemochrome with erythroblast count and reticulocyte assessment, Hb phoresis by HPLC, LDH, bilirubin, ALT and AST, creatinine, LH, FSH, prolactin, FT3, FT4, TSH, testosterone, and spermatic fluid analysis. All the clinical tests gave normal values except the liver ultrasound which showed a slightly enlarged liver and a non-homogeneous echostructure (the patient is $HCVAb^+$, $HCV\ RNA^+$).