A β-THALASSEmia VARIANT ASSOCIATED WITH UNUSUALLY HIGH HEMOGLOBIN A₂ IN AN IRANIAN FAMILY

To the Editor:

Despite the vast heterogeneity of mutations, the levels of increased hemoglobin (Hb) A₂ seen in individuals of different racial groups heterozygous for the different β-thalassemia mutations are remarkably uniform and rarely more than 6%. However, unusually high levels of Hb A₂ have been observed in some β-thalassemia heterozygotes; in one group, it is associated with a partial or complete deletion of the β globin gene. We describe here an Iranian family with a β₀-thalassemia mutation caused by a 290-bp deletion that removes the 5' region of the β gene including the messenger RNA (mRNA) cap site and entire exon I. As seen in previous cases, individuals heterozygous for this deletion have a typical β-thalassemia trait phenotype but unusually high levels of Hb A₂.

The propositus is a 53-year-old woman of Iranian origin and clinically asymptomatic. Hematologic investigations showed: Hb 10.3 g/dL; mean corpuscular volume (MCV) 64 fl; mean corpuscular Hb (MCH) 20.7 pg; Hb electrophoresis D, F, and a raised Hb A₂; Hb A₂ 7.1%; Hb F 8.8%. Her son is also heterozygous for
β-thalassemia with Hb 12.1 g/dL, MCV 66 fl, MCH 21.2 pg, Hb A₂ 7.8%, and Hb F 6.5%. The unusually high Hb A₂ level of the propositus, who is a compound heterozygote for Hb D and β-thalassemia, and her son is reminiscent of the hematologic phenotype of individuals heterozygous for β-thalassemia mutations due to deletions of the 5' region of the β gene.

The β globin complex in the propositus was analysed with a series of restriction enzymes and Southern blot hybridization. Digests of restriction enzymes (Bgl II, Pst I, Dra I, a.;d.Ava II) with sites that flank the 5' end of the β gene and hybridization with a β globin gene probe (Psp I) show an abnormal band that is ~300 bp smaller than the expected normal (data not shown). The deletion removes a site for Nco I in the initiation codon. The presence of the deletion was confirmed by enzymatic amplification of this region of the β globin gene by the polymerase chain reaction (PCR) using primers that flank the deletion (Fig 1). In normal individuals, a product of 1454 bp is obtained; in the propositus, an additional band of ~1,200 bp is seen. The endpoints of the deletion were defined by direct sequencing of the abnormal PCR product using the following approach. The PCR products were separated in 1.0% low-melting point agarose (Bethesda Research Laboratories, Bethesda, MD). After staining with ethidium bromide, the smaller fragment was localized and cut out under ultraviolet light. The agarose containing the fragment was melted and an equal volume of water added. Ten microliters of this product was subjected to another 30 cycles of amplification under reaction conditions similar to the initial PCR, with the exception of using a different set of primers: one 5'-biotinylated and internal to the previous (Bio-AP3), and the other as previously described (Fig 1). Single-stranded DNA (ss DNA) one 5'-biotinylated and the other complementary to the biotinylated–primed ssDNA, were prepared as described² and sequenced by the dideoxy chain termination method using Sequenase and the reagents as provided in the kit (US Biochemicals Corp, Cleveland, OH). Five microliters of the ssDNA and 1 pmol of the appropriate primer (internal or similar to that used in the PCR) were used per sequencing reaction.

The breakpoints of the deletion were determined by comparing the nucleotide sequence of the amplified DNA with that published of normal β gene in the region of the deletion. The deletion is 290 bp in length; three breakpoints are possible due to the presence of two guanine nucleotides common to both ends. The 5' breakpoint is 123 to 125 bp upstream of the cap site and the 3' end is at nucleotide 23 through to 25 of IVS-1 of the β globin gene (Fig 2). The deletion endpoints as determined by sequencing the biotinylated single strand were consistent with those determined from sequencing the complementary strand. Restriction analysis of

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**Fig 1.** Amplification of the 5’ end of the β globin gene by the PCR in the propositus (P) and a normal individual. AP1 represents the forward primer, 5'-TCCAGGCAAAACAGTTAGATGC-3', and AP2 the reverse primer, 5'-CATTCGTCGGTTTCCCATTCTA-3' used in the first PCR. The second PCR used the biotinylated primer, Bio-AP3 (5'-CGATCTCAATATTGCTACCAAG-3'), and AP2. Lanes M1 and M2 represent the markers phiX Hae III and A HindIII, respectively, BI, blank; P, patient; N, normal individual.

**Fig 2.** Breakpoint DNA sequences of the β-thalassemia deletion. The potential deletion breakpoints are indicated by the arrows and are located 123 to 125 bp upstream of the cap site and at nucleotides 23 through to 25 of IVS-1 of the β globin gene, close to a GACAGGT heptamer direct repeat (boxed). The inverted repeats are underlined.
DNA amplified by the PCR showed that the son has an identical deletion.

We have characterized a β^0-thalassemia mutation due to a 290-bp deletion that removes the promoter region, the entire exon 1, and part of the IVS-I of the β gene. This deletion appears identical to another recently described in a Jordanian3 and also in a Turkish family4 and, in view of the close geographic proximity, it is likely that these deletions are of a single origin. In both the Turkish and the Iranian family in this report, individuals heterozygous for the mutation have unusually high levels of Hb A2 (7.1% to 8.1%) and variable increases of the Hb F, in keeping with previous observations. Six deletions ranging in size from 290 bp to 12.6 kb affecting only the β globin gene have been described.3,5 The 619-bp deletion that accounts for 20% of the p-thalassemia in Asian Indians removes the 3' end, leaving the 5' end of the β gene intact, whereas the others are rare, isolated events and remove the 5' end of the β gene leaving the δ gene intact. Individuals heterozygous for the 619-bp deletion have increases in Hb A2 levels that are indistinguishable from the other common forms of β-thalassemia. In contrast, heterozygotes for the other five deletions have exceptionally high levels of Hb A2. The region extending from position −125 to +78 (relative to the mRNA cap site of the β gene) is removed in each of the five deletions that are associated with the variant of high Hb A2 β-thalassemia. This region includes the CAC box, the CAAT box, and the TATA box of the β globin gene promoter.

The mechanisms underlying the unusually high Hb A2 in these heterozygous β-thalassemias are not completely clear. Family studies of individuals heterozygous for β-thalassemia and a δ variant have shown that the increased Hb A2 is derived from both δ genes, the one in-cis as well as the one in-trans to the β-thalassemia gene. Recently, study of an individual heterozygous for the 1.39-kb deletion and a δ chain variant1 showed that, in addition to an increased HbA2 derived from both δ genes, there is a disproportionately increase of A2 derived from the δ gene in-cis. This would support the proposal that removal of the 5' β globin gene promoter removes competition for the hypersensitive sites in the upstream locus control region (LCR) such that the LCR could then interact with either the δ or γ gene in-cis to enhance their expression.10

Examination of the nucleotide sequences surrounding the endpoints of this 290-bp deletion shows a heptamer repeat (GAAGAGGT) at the breakpoints of the 5' and 3' normal sequences (Fig 2) as well as the presence of an inverted repeat (CTGTC). The deletion has removed one of the heptamers that supports the proposed mechanism underlying the generation of such short deletions, a model of 'slipped mispairing'11 that leads to the loss of one repeat and all sequences between the direct repeats.

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REFERENCES

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A beta-thalassemia variant associated with unusually high hemoglobin A2 in an Iranian family [letter]

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