A NEW SINGLE NUCLEOTIDE CHANGE AT THE INITIATION CODON (ATG → AGG) IDENTIFIED IN AMPLIFIED GENOMIC DNA OF A CHINESE β-THALASSEMIC PATIENT

To the Editor:

β-Thalassemia is heterogeneous and the clinical severity is dependent upon many factors, among which are the nature of the mutation in the β-globin gene1 and the co-inheritance of α-thalassemia.2 Here we report a new mutation identified in the initiation codon (ATG → AGG) with the use of the polymerase chain reaction followed by direct sequencing of the amplified DNA.3

At 18 months, the patient was found to have splenic enlargement, a hemoglobin level of 7.1 gm/dL, and a high fetal hemoglobin of 83.7%. Triton-urea gel confirmed the total absence of the β-globin chain (Fig 1). She only reappeared for treatment at age 3. It was unusual for a homozygous β-thalassemic to have survived with no blood transfusion for 3 years.

Dot blot hybridization of the amplified DNA with probes corresponding to the mutations common for the Chinese4 showed that the patient and her mother carried the deletion-TCTT at the codons 41/42. However, it was intriguing that DNA from her father produced no signal.

When the amplified fragment was directly sequenced (3: but with sequenase from USB), a new single nucleotide mutation was identified at the initiation codon in the patient (Fig 2) and her father. This is the first report of a mutation in the initiation codon of the β-globin gene, although mutations of this kind have been reported for the α-thalassemias.5 It was shown that a single base substitution (ATG → AGG) in the initiation codon of the α-2 globin gene in a Sardinian patient decreases the amount of steady-state messenger RNA produced.6 In the case here, the absence of the β-globin chain in triton-urea gel strongly suggests that the ATG → AGG at the initiation codon has seriously affected β-globin chain synthesis and that the resultant type would most likely be β0.

This mutation abolishes the Ncol site. A 746-base pair fragment was observed in the amplified DNA from the patient and her father.

Fig 2. Direct sequencing of the amplified DNA from the patient. Lanes from left-to-right are A, C, G, T. The ATG → AGG at the initiation codon was identified.

Fig 3. Identification of the ATG → AGG mutation with Ncol restriction digestion. Left-to-right: 1, marker; 2, normal; 3, patient; 4, patient’s father; 5, patient’s mother.

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but not from the mother (Fig 3). Hence, restriction digestion of the amplified DNA provides a rapid method for detection and obviates the use of radioactive probes. This has important implications for many third world centers.

Restriction fragment length polymorphisms showed that the patient has inherited the \( \alpha^{+}/\alpha^{-} \) genotype as well as the deletion at the codons 41/42 of the \( \beta \)-globin gene from her mother. The new mutation at the initiation codon was from her father. The combined \( \alpha^{0} \) thalassemic genotype could have accounted for the relatively mild clinical presentation in this patient. This emphasizes the need for detail characterization of the genotypes.

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REFERENCES


