A NEW MUTATION AT IVS1 nt 2(T → A), IN β-THALASSEMIA FROM ALGERIA

To the Editor:

Over 80 different point mutations leading to decreased or absent synthesis of β-globin chain have been characterized in β-thalassemia.1 Direct detection of mutant alleles, by the development of alleleshpecific oligonucleotide hybridization techniques (ASO), is now possible and used for prenatal diagnosis. However, this method can be used only if the main molecular defects prevalent in the population are known at the nucleotide level. The actual spectrum of β-thalassemia mutations has been determined in several populations and has shown that β-thalassemia alleles are population specific.3 This has not yet been done completely in northern Africa, with the exception of Tunisia.2 For this reason, we undertook a systematic study of Algerian β-thalassemic patients by directly sequencing their β-globin genes between position 1-2 and +620 relative to the cap site, after polymerase chain reaction (PCR) amplification of genomic DNA.

Briefly, a 773-bp double-stranded DNA fragment was synthesized, starting from 400 ng of leukocyte DNA, with primers β1 (5'GGACAGGTACGCCTGGCTCATC3'), located 135 nucleotides upstream to the cap site, and β2 (5'CGATCCTGAGACTTCGGACAGATCCCACA3'), located in the second intron 638 nucleotides downstream to the cap site. This DNA was purified by agarose gel electrophoresis, and used as template for synthesis of single-stranded DNA by asymmetric PCR amplification.4 Sequence was obtained from primer β1 on one strand and from an internal primer β3 (5'ATCAGGAGTGGACAGATCC') for the other strand.

A new thalassemic mutation was observed in the DNA of a patient who was found to be a compound heterozygote with a T → A transversion at IVS1 nt 2 on one chromosome and a deletion of A in the thalassemic DNA. With primer β1, two bands, corresponding to A and T respectively, are seen at position 2 of IVS1 in the thalassemic DNA. With primer β3, two superposed sequence ladders are seen, one shifted of one nucleotide from the other. This is the result of the deletion of one nucleotide in codon 6 in one of the chromosomes of this patient. However, a single A appears clearly in the adenine track, confirming the T → A transversion on the other chromosome at position 2 of IVS1.

Interestingly, of 33 thalassemic chromosomes on which we have actually determined a mutation, seven carried a T → C transition at position 2 in IVS1. This mutation has already been found in an American black patient, who was a compound heterozygote with HbS-β thalassemia.5 Our observation is the first in the Mediterranean area, and this mutation may be common in the Algerian population. Two patients were homozygotes for this substitution and had no detectable HbA by standard electrophoresis procedures, thus we conclude that this mutation leads to β-thalassemia. The three other patients were compound heterozygotes for this transition, the other chromosomes carrying a previously described β-thalassemic mutation. Unfortunately, we were unable to obtain fresh blood for globin chain synthesis studies from the patient carrying the T → A transversion who was transfusion-dependent. However, the T → A transversion is expected to result in the β-thalassemia phenotype.

Curiously, the third possible change, ie, T → G, at position 2 in IVS1 has been recently reported in a β-thalassemic patient of Tunisian origin.2 This position belongs to the 5' splice junction consensus “GT-AG,” conserved in all functional eukaryotic genes.6 Our observation that a change at this position completely abolishes β-globin chain synthesis is fully consistent with the Chambon rules.

ACKNOWLEDGMENT

Supported by the Ministère de la Recherche et de la Technologie (Grant 89 C 0779), the Institut National de la Santé et de la Recherche Médicale (Réseau Nord-Sud), the Centre National de la Recherche Scientifique (UMR 106), and the University Claude Bernard, Lyon I.

RACHID BOUHASS
MEZIANE AGUERCIF
Centre Emir Abdelkader
CHU Oran
Oran, Algeria

GUY TRABUCHET
JACQUELINE GODET
Centre de Génétique Moléculaire et Cellulaire
Université Claude Bernard Lyon I
Villeurbanne, France

Fig 1. Autoradiogram of sequencing gels of PCR amplified β-globin genes. Sequence was performed from primer β3 (A) or from primer β1 (B) on DNA from the thalassemic patient (T) or from a normal control (N). The sense sequence is written at the side and an arrow indicates the nucleotide substitution.
REFERENCES


A new mutation at IVS1 nt 2(T→A), in beta-thalassemia from Algeria

R Bouhass, M Aguercif, G Trabuchet and J Godet