DETECTION OF A NOVEL DOUBLE MUTATION IN A β-THALASSEMIA PATIENT BY RNA SINGLE-STRANDED CONFORMATION POLYMORPHISMS AND DIRECT SEQUENCING

To the Editor:

Applications of various molecular methods for the analysis of mutations in the β-globin gene has led to the identification of nearly 100 different β-thalassemia mutations in the world.\(^1\)

Recently, two methods have been described for mutation detection that rely on the analysis of single-stranded conformation polymorphisms (SSCP) of DNA\(^2\) or of RNA.\(^3\) We find that DNA SSCP and RNA SSCP analysis both appear to be equally capable of detecting mutations in the β-globin gene but RNA SSCP analysis appears to be more informative. We have been able to identify a β-thalassemia gene with two mutations: one at codon 30 (a G to C change), and another at intervening sequence I (IVS I) nt 5 (G to C change) in one individual by RNA SSCP analysis. To our knowledge this is the first time a double mutation has been found for the consensus splice donor site in the same patient.

DNA was isolated by the method of McCabe et al\(^4\) from blood obtained as dried spots on filter papers from finger pricks of 20 putative β-thalassemia heterozygotes from Calcutta, India. Preliminary screening of these samples by polymerase chain reaction (PCR) amplification using allele-specific oligonucleotide primers by the method of Varawalla et al\(^5\) showed that 12 of the 20 samples had the IVS 1 nt 5 G to C mutation. We then analyzed these 12 samples by DNA and RNA SSCP following previously published procedures.\(^2,3\) By DNA SSCP analysis we were also able to distinguish these samples from four normal samples (Fig 1). RNA SSCP analysis was also able to separate the mutants from the normal samples, but additionally one sample (patient no. 8) appeared to have a distinctly different pattern from the other mutants (Fig 2). Direct sequence analysis of the PCR amplified products showed that the DNA from sample no. 8 indeed had a double mutation in the β-globin gene (Fig 3). The sequences from a normal and one other heterozygote are shown in Fig 3. Because the double mutations were both heterozygous it was of interest to determine if one

![Fig 1. DNA SSCP analysis of a region of β-globin gene amplified by PCR. N1 to N4 represent four normal volunteers and the rest represent samples heterozygous for the IVS 1 nt 5 (G to C change). Samples with the mutation show shift in mobility of the lower band compared with the bands seen for the normal samples.](image1)

![Fig 2. RNA SSCP analysis of four normal samples (N1 to N4) and three samples having the mutation at IVS 1 nt 5 (G to C change). The samples no. 1, 5, and 8 all show a different electrophoretic pattern from the normal samples. Sample no. 8 has a distinctly different pattern from samples no. 1 and 5 as shown by arrows in the figure.](image2)

![Fig 3. Direct sequence analysis of sample N1 and samples no. 8 and 5 are shown. Samples no. 5 and 8 have the IVS 1 nt 5 (G to C change). Sample no. 8 has an additional mutation at codon 30 (G to C change). Both mutations are indicated by arrows.](image3)
allele had both the mutations or whether the mutations were present separately on each allele. We cloned the PCR product directly into a modified bluescript vector PCR-Script SK(+) obtained from Stratagene (La Jolla, CA) and screened 10 colonies obtained from ampicillin agar plates. Two of the positive colonies (restriction digest showed correct sized insert) upon sequencing showed that the two mutations existed on separate alleles, as shown in Fig 4. This suggested that this particular individual had separately inherited one mutation from each parent. The IVS1 nt 5 G to C change is fairly common amongst Indian patients from eastern India whereas the codon 30 G to C change has never been reported from this region. The same report identified 7 patients out of a total 780 patients who had the codon 30 mutation, but all 7 of these patients were from the western part of the country. However, this mutation is common in Tunisian \(\beta\)-thalassemia patients. Our work suggests that for routine mutation screening for detection of \(\beta\)-thalassemic mutations, DNA SSCP analysis is adequate but RNA SSCP analysis may be more informative in terms of being able to distinguish between types of mutations.

**ACKNOWLEDGMENT**

Supported by National Institutes of Health Grant No. CA08010 and in part by a Collaborative Core Unit grant from Howard University. We thank Drs G. Talukder and D.K. Bhattacharya for help with sample collection. Details of PCR conditions and primer sequences are available on request: address correspondence to J.R.B.

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**REFERENCES**

Detection of a novel double mutation in a beta-thalassemia patient by RNA single-stranded conformation polymorphisms and direct sequencing [letter]

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