



## A Rare $\beta^0$ -Thalassemia Frameshift Mutation in a Turkish Individual: (+T) at Codon 9/10

LETTER  
TO THE EDITOR

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$\beta$ -thalassemia is an autosomal recessive disorder caused by mutations that lead to deficiency ( $\beta^+$ ) or absence ( $\beta^0$ ) of  $\beta$ -globin chains (1). It is one of the most common inherited disorders of hemoglobin in Mediterranean countries, North Africa, and Southeast Asia (2). In Turkey, previous studies have reported the frequency of  $\beta$ -thalassemia at 2.0% (3). There are currently over 300 mutations affecting different levels of  $\beta$ -globin gene expressions by a variety of mechanisms that are known to result in  $\beta$ -thalassemia phenotype (4). About 40 mutations associated with  $\beta$ -thalassemia have been described in Turkey (5, 6). Here, we report a rare  $\beta$ -globin gene mutation in a Turkish individual.

A 23-year-old woman consulted our Genetic Diagnosis Center from Hemoglobinopathy Disorders Center in Adana for hypochromic-microcytic anemia and high HbA2 levels in a routine test before marriage. Complete blood count (CBC) and high-performance liquid chromatography (HPLC) results were compatible with  $\beta$ -thalassemia heterozygous shown in Table 1. HPLC analysis showed levels of HbA2 and HbF at 4.3% and 1.3%, respectively. Iron deficiency anemia was excluded.

Hemoglobin electrophoresis and CBC values of her husband were found to be normal. There was no consanguinity between her parents. Two sisters of our patient and their father had also anemia history, but we could not evaluate them.

$\beta$ -globin gene mutation analysis was first performed by the strip assay technique (ViennaLab cat. no. 4-120; ViennaLab Diagnostics, Vienna, Austria), which is based on the reverse-hybridization principle automatically. After the strip assay technique, direct sequencing of the Polymerase Chain Reaction (PCR) products was performed on an ABI 3130 Genetic Analyzer. The 3 exons of  $\beta$  globulin gene as well as 5'UTR, 3'UTR, promoter, and introns sequences replicated by PCR using primers given in Table 2. There was no mutation on strip assay, but Sanger sequencing detected Fsc 9/10 (+T) heterozygous mutation (Figure 1). This mutation is a rare mutation for  $\beta$ -thalassemia (7-10). A written informed consent for the publication was given by the patient.

Over the past two decades, a wide range of methods for DNA analysis have allowed us to identify such defects in globin genes that are associated with hemoglobin disorders. In this case, we report a rare mutation in the  $\beta$ -globin gene. This mutation is an insertion of one base (T) at codon 10 causing a frameshift mutation, resulting in a stop codon at codon 22 (Figure 2). mRNA reverse transcriptase-PCR should be used to evaluate the functional effects of this mutation for measuring level of  $\beta$ -globin. In this study, we could not use this method, but frameshift mutations of the  $\beta$ -globin gene frequently generate a new premature termination codon, which makes it impossible to synthesize normal functional protein and results in  $\beta^0$  thalassaemia (1).

Consequently, hypochromic-microcytic anemia with elevated HbA2 should be evalu-

**Table 1.** Haematological data of patient detected hypochromic-microcytic anemia and high HbA2 levels

HGB g/dL	RBC G/uL	MCV fL	MCH pg	HbA2	HbF
10.5	6.18	59.3	17.0	4.3	1.4

**Table 2.** Oligonucleotide sequences for PCR amplification and sequencing of the HBB gene

Regions	Forward	Reverse
$\beta$ globin 1. Region	5'-CCAACTCCTAAGCCAGTGCC-3'	5'-TGCAATCATTCGTCTGTTTCCC-3'
$\beta$ globin 2. Region	5'-TCCCTAATCTCTTTCTTTCAG-3'	5'-TTTCCCAAGGTTTGAAGTAGC-3'

PCR: polymerase chain reaction

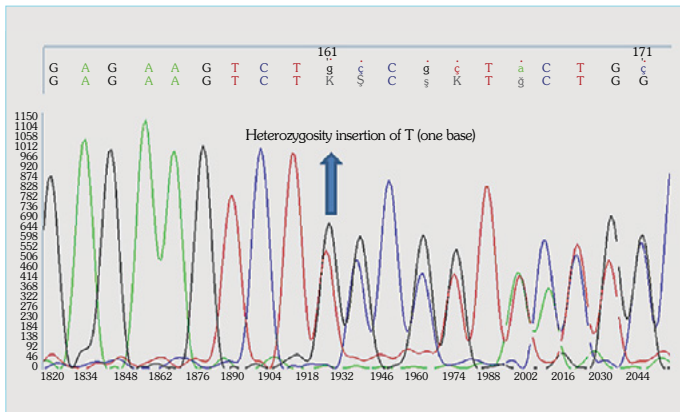
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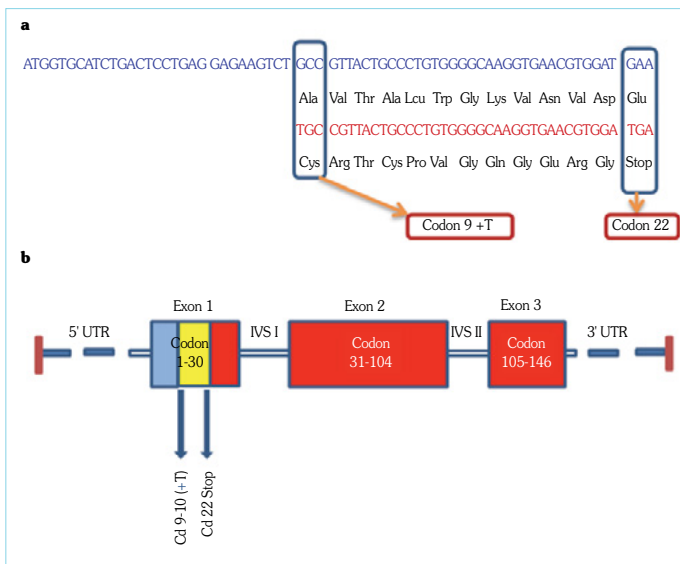
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**Figure 1.** Partial DNA sequence showing a heterozygosity insertion of T at codon 10



**Figure 2. (a, b).** (a) Insertion of a single T nucleotide within the exon 1 at codon 10 of the  $\beta$  globin gene. With insertion of T at codon 10 and frameshift mutation causing amino acid sequence changings from codons 10 to 21, and then 22. codon converts to stop codon. (b) Normal amino acid sequences, differed amino acid sequences, and not synthesized region were demonstrated with blue, yellow and red, respectively

ated carefully, and additional methods such as Sanger sequencing should be used if  $\beta$ -globin strip assay is normal. This situation may be important for genetic counseling especially when the individual's spouse has thalassemia trait.

In conclusion, it is very important to report new or rare nucleotide changes in hemoglobin diseases (11). Defining the changes in this group will not only help explain the clinical findings of the patient

but also understand the possible interactions with known molecular defects. Also, describing the genetic changes will be definitely helpful for the accurate genetic counseling for couples at risk.

**Informed Consent:** Informed consent was obtained from patients who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Authors' Contributions:** Conceived and designed the experiments or case: AA, ÖÖY. Performed the experiments or case: AA, SY. Analyzed the data: PS, SC. Wrote the paper: AA, SY, ÖÖY. All authors have read and approved the final manuscript.

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