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SHORT REPORT

Identification of a Novel Frameshift Mutation [Codon 3 (+T)] in a Turkish Patient with β-Thalassemia Intermedia

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β-Thalassemia, one of the most widespread genetic diseases in the world, is an autosomal recessive disease generally caused by point mutations in the β-globin gene that is located as a cluster on the short arm of chromosome 11 (1-3). Modern molecular techniques have provided a wealth of information about the nature of mutations and their distributions within the world population. More than 200 different mutations affecting diverse levels of β-globin gene expression have so far been identified (2,4,5). These mutations such as frameshifts of the insertion/deletion type and/or nucleotide substitutions have been reported to interfere with the transcription of the β-globin gene, splicing procedures and translation of β-globin mRNA (1-5). All these mutations result in either an absence or reduction of synthesis of β-globin chains (3,6). Insertion or deletion of one or more nucleotides in the coding region of the β-globin gene disrupts the normal reading frame and causes the frameshift. As a result of frameshift the β-globin chain is synthesized as either elongated or truncated (7, 8).

We report a novel frameshift mutation in a 4-year-old Turkish male with β-thalassemia intermedia. DNA sequencing revealed a frameshift mutation with insertion of thymidine at codon 3 (+T) and a G->A transition at position 110 of intron 1. The co-inheritance of these mutations (IVSI-110 and Cod 3(+T)) has not been reported previously. For this reason, such cases may be considered important in terms of understanding the heterogeneity of molecular defects causing β-thalassemia.

A 4-year-old boy attended the Thalassemia Center in Antalya State Hospital because of soft skin, anemia, weakness, and lack of appetite. After clinical examination, he was clinically diagnosed as having β-thalassemia intermedia. He had received blood transfusions of 1 unit annually. Hematologic data of the patient and his parents are given in the Table. His blood counts were Hb; 9 g/dl, MCV; 50.6 fl, MCH; 17.1 pg, and Reticulocytes; 2.63%. HbA1, HbA2, and HbF levels were 64%, 3.7% and 32.4%, respectively.

DNA Analysis

DNA was extracted from whole blood using the salting-out method (9). Polymerase chain reaction (PCR) was performed using specific primers of the β-globin gene and amplified DNA was analyzed to investigate known common mutations in the Mediterranean region using a β-globin strip assay kit (Vienna Lab) based on reverse dot blot hybridization (RDBH), and a PCR-based amplification refractory system (ARMS) as described previously (10).

Table. Hematological parameters of patient and his parents.

	Patient	Mother	Father
Weight (kg)	11	60	80
Height (cm)	78	160	170
Liver (cm)	Normal	Normal	Normal
Spleen (cm)	Normal	Normal	Normal
Skin and mucosa	Normal	Normal	Normal
RBCx10^{12}/l	5.24	4.83	6.61
Hb (g/dl)	9	12.4	14.2
MCV (fl)	50.6	76.8	65.1
MCH (pg)	17.1	25.7	21.5
Reticulocytes (%)	2.63	-	-
HbA1 (%)	64	96	90,9
HbA2 (%)	3.7	3.7	6
HbF (%)	32.4	0.3	2.3
Ferritin	167.3	-	-
WBC x 10^9/l	12.3	5.1	5.6
Platelets x 10^9/l	417	256	195

To explore the other mutations in the β-globin gene, DNA including approximately 700 bp was amplified using amplification primers (Primer F: 5'- GCCAAGGACAGGT CGGCTGTCAT C-3' and Primer R: 5'-CCCTTCCTATGACATGA ACTTAACCAT-3') designed for the initial site of the β-globin gene. PCR product was separated in a 1.5% agarose gel. The amplified fragment was isolated from agarose gel using purification kit (MBI Fermantas, K0513). About 500 ng of purified fragment was sequenced using the dideoxy termination procedure of Sanger et al. (11). Before the samples were loaded on sequencing gel, the gel was pre-run at 40 W. Then 4 µl of each sample was loaded on denaturing 8% polyacrylamide gel. After the gel had dried, it was exposed to X-ray film for 12 h at room temperature and then developed.

The G->A substitution at position 110 of intron 1 was screened using RDBH and confirmed using ARMS. DNA sequencing of the patient's β-globin gene demonstrated the insertion of thymine (+T) before the beginning of codon 3 in exon 1 of the β-globin gene in the Figure. The proband's mother and father were heterozygote for IVS.1.110 and codon 3 (+T), respectively. This mutation disrupts the normal reading frame and causes the shift in the protein reading frame.

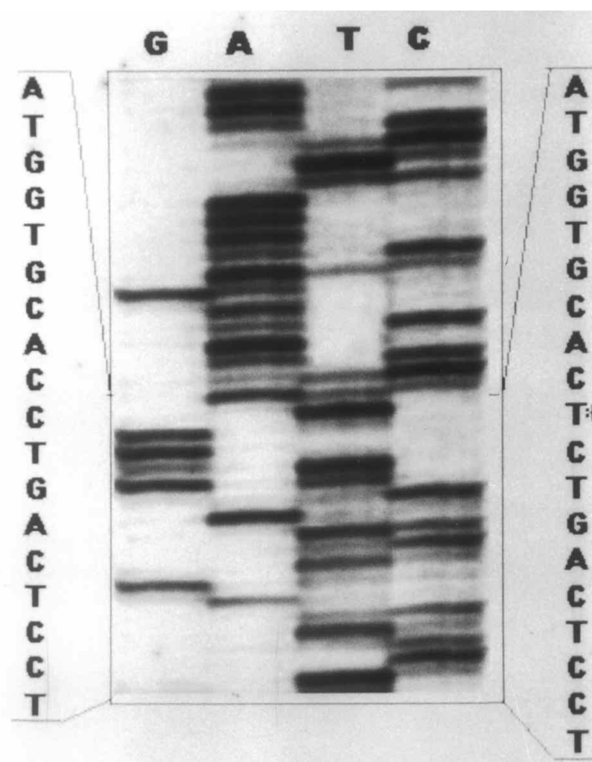

Figure. Direct DNA sequence analysis of the mutated strand of a β-globin gene amplified fragment. The normal sequence is shown on the left, and the abnormal allele (+T) on the right. The inserted T at codon 3 is indicated by *.

The remarkable phenotypic diversity of β-thalassemia in Turkey as well as worldwide reflects the heterogeneity of mutations at the β-globin locus (12). More than 200 different mutations have been documented to date (1,13), and more than 40 of these mutations have been described by several Turkish laboratories for β-thalassemia (12). We know that as these mutations affect various levels of β-globin gene expression, they differ greatly in their phenotypic effects. Despite this marked molecular heterogeneity, the prevalent molecular defects are limited in each risk population (12,13). Usually, 5 to 10 mutations account for the great majority of β-globin disease-causing alleles in the at risk population. Mutation analyses are available for common mutations and are easily detected by PCR-based techniques. IVSI-110 was the most common β-thalassemia defect with a frequency of 38.6%, followed in decreasing order by IVSI-6 (9.4%), –30 (8.4%), IVSII-1 (6.9%), IVSII-745 (5.9%), Cod44 (3%), and IVSI-1(3%), while frameshift mutations were observed less frequentlyin our region (14). Compound heterozygosity for mild/silent b+ and

severe mutations produce a variable phenotype ranging from β-thalassemia intermedia to β-thalassemia major (1-3,6).

The frameshift mutation that occurred through insertion of T at codon 3 can cause a truncated protein. Therefore, when the +T is inserted as the 7th nucleotide, Exon I ends at codon 6 with a stop codon (UGA) in mRNA. The mild phenotype in our case may be due to the very short truncated protein. Molecular analysis of β-thalassemia has shown that many rare and several novel β-globin mutations responsible for the disease are present in the Turkish population, in addition to common mutations.

The consanguinity rate in Antalya was reported to be 37% (15). Due to this relatively high rate, the high incidence of β-thalassemia in the population and the presence of rare and novel mutations in the rich genetic pool in our region are not suprising. For this reason, our group started to register all patients and their families with hemoglobinopathies in 1999. We believe that this recording system is useful in controlling the disease and in detecting many rare and novel mutations that are likely to be important in our understanding of the heterogeneity of molecular defects causing β-thalassemia in our region.

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