First report from Turkey of a rare frameshift mutation [codons 9/10 (+T)] in the beta-globin gene

Türkiye'de beta globin geninde nadir olarak gözlenen ilk çerçeve kayması mutasyonu [codons 9/10 (+T)] raporu

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To the Editor,

Beta-thalassemia (β-thal) is one of the most common autosomal recessive single gene disorders worldwide [1]. At least 200 different mutations in the β-globin gene that result in the β-thal minor and major phenotypes have been described [2,3]. The incidence of β-thal is high in Mediterranean regions, Iran, India, The Arabian Peninsula, Southeast Asia, and Turkey [4]. β-thal is characterized by point mutations, small deletions, or insertions that result in a decrease in or lack of expression of the β-globin chain, and each ethnic group or population has its own set of common mutations. In Turkey β-thal is common and 12 mutations accounted for 83.3% of 1500 unrelated cases with homozygous β-thal [5]; the remaining mutations were rare or newly identified. To date, at least 39 nucleotide insertions in 3 exons of the β-globin gene that result in a modified C-terminal sequence of the β-globin protein have been reported (http://globin.bx.psu.edu/cgi-bin/hbvar/query_vars3). Codon 9/10 (+T) insertion mutation was first described in a Greek family by Wave et al. in 1994 [6], followed by the report of an Iranian patient of Kurdish origin by Rahimi et al [7]. Herein we present a 30-year-old Arab male with the β-thal trait living in Hatay, Turkey that had β-globin gene codon 9/10 (+T) frameshift mutation, which was noted during premarital genetic screening. To the best of our knowledge this is the first case reported from Turkey and only the third case worldwide.

In our laboratory where molecular testing for the premarital screening of thalassemia mutations are routinely performed, we encountered a case of a 30 year-old male meeting the diagnostic criteria of β-thal trait. After written informed consent, the patient accepted to undergo mutation analysis and laboratory tests.

Genetic analysis showed a frameshift mutation-an insertion of T between codons 9 and 10 in the first exon of the β-globin gene. Hematological data

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were obtained via an automated cell counter and routine methodology. Red cell lysates were analyzed using high-performance liquid chromatography (HPLC). Hemoglobin species assay via HPLC showed the following: Hb A: 86.8%; Hb A2: 4.7%; Hb F: 1.0%. Complete blood count findings were as follows: Hb: 12.3 g/dL; Hct: 39.0%; MCV: 62.2 fL (low), MCH: 19.6 pg (low); MCHC: 31.5 g/dL (low)-red blood cell count, white blood cell count, and platelet count were as follows; 5.2 million/mm^3, 6200/mm^3, 255000/mm^3, respectively. Iron status, serum ferritin, and other hematological indices were normal. DNA was isolated from whole blood samples in EDTA using a commercially available DNA extraction kit (RTA Lab, Ltd., Sti, Turkey). Regions of the β-globin gene were sequenced using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA., USA), according to the manufacturer's instructions, and the sequence reaction was analyzed using an ABIPRISM 310 genetic analyzer (Applied Biosystems, Foster City, California, USA). The mutation was confirmed via sequencing of the anti-sense DNA strand, which was performed twice (Figure 1).

Genetic defects that result in β-thal are primarily point mutations, rather than gene deletions or insertions. The mutations result in defects in transcription, RNA splicing and modification, translation via frameshifts, and nonsense codons that produce highly unstable β-globin, which cannot be utilized. Codon 9/10 (+T) is a rare mutation in the β-globin gene and was first described in a Greek family with β-thal, and then in an Iranian of Kurdish origin. The present case had the β-thal trait, is the first case reported from Turkey, and is only the third case worldwide. The insertion of a thymine nucleotide between codons 9 and 10 in exon 1 in the β-globin gene causes a reading frameshift from this point, leading to an in-frame stop codon at codon 22. The insertion of a T nucleotide in exon 1 alters the protein structure due to a reading frameshift and the formation of a stop codon in position 22. The resulting truncated protein is completely inactivated because of the premature termination of translation at codon 22, instead of at codon 147. A similar frameshift mutation in the β-globin gene [codon 9/10 (+G)] was observed in a patient of Turkish origin by Aulehla-Scholz et al. [8]. Codon 9/10 (+G) and codon 9/10 (+T) mutations are similar, but differ in terms of nucleotide insertion. The presented case had thymine insertion between the codon 9 and codon 10 regions, whereas the case reported by Aulehla-Scholz et al. had guanine insertion between codon 9 and codon 10.

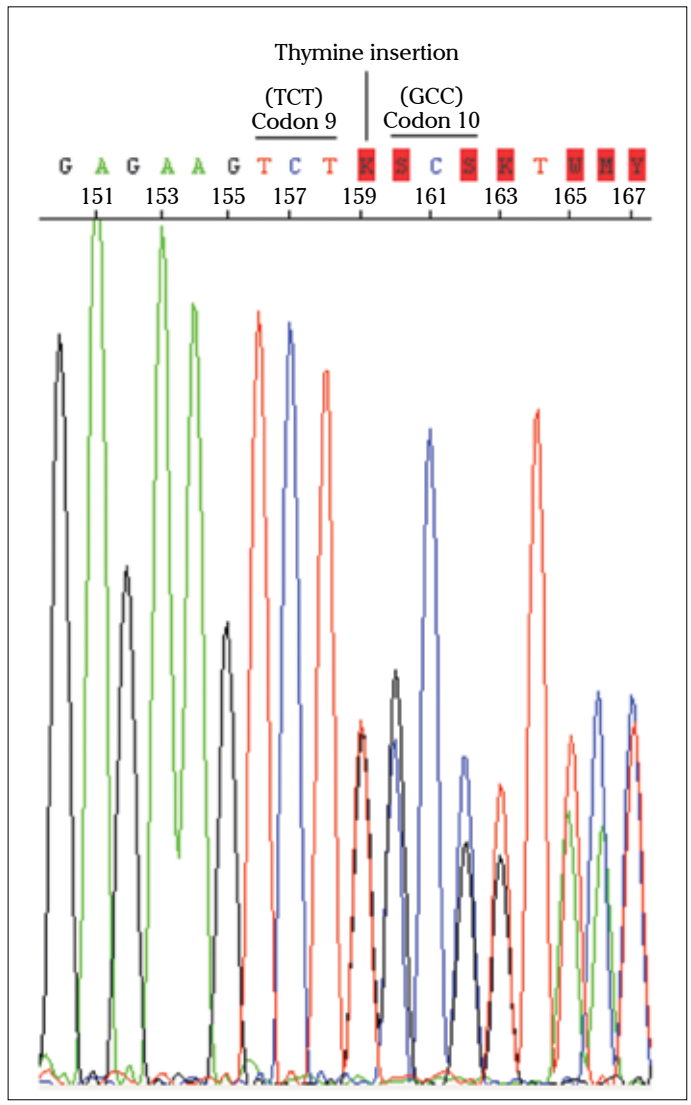

Figure 1. Sequence Electropherogram Showing a Frameshift Mutation Due to a Thymine Insertion Between Codons 9 and 10 in Exon 1 of Beta Globin Gene

β-thal mutations are very heterogeneous and at least 40 different mutations are common in Turkey. This molecular heterogeneity might be due to the historical migration of individuals from non-Mediterranean regions to Turkey. β-thal mutations are usually population specific and each ethnic group has distinct common mutations. Research on different populations indicated that 4-6 mutations account for 90%-95% of β-thal chromosomes in any given ethnic group. The prevalence of a limited number of mutations in such populations has

facilitated molecular testing using primer-specific amplification or reverse dot blot hybridization, with a set of sequence-specific primers or probes for the frequently occurring β-thal mutations. In Turkey, β-thal mutations are very heterogeneous and the above-mentioned techniques are not sufficient for detecting rare or unknown mutations; therefore, direct DNA sequence analysis of the β-globin gene could prove to be extremely useful for prenatal diagnosis and carrier identification. Detection of rare β-thal mutations may also be useful for establishing a national mutation database and in genetic counseling.

Conflict of interest statement

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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