A Rare Mutation in Two Thalassemia Patients from a Family: Case Report

Aynı Aileden İki Talasemi Hastasında Nadir Görülen bir Mutasyon: Olgu Sunumu

A Rare Mutation for Thalassemia / Talasemide Nadir Bir Mutasyon

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Özet

Beta thalassemia is one of the most prevalent autosomal recessive disorders in the world. The responsible gene HBB (hemoglobin beta) is localised on the short arm of 11th chromosome. More than 200 different mutations on beta globin gene have been identified. In this case report, we wanted to show the detection of a rare mutation (direct sequencing of amplified DNA detected a C→G substitution in the promotor site of exon 1 (CR920788, cDNA.41C>G, g.2239C>G) that causes beta thalassemia and the importance of DNA sequencing in thalassemia patients. Different nucleotide substitutions affecting the beta-globin gene in combination with severe beta-thalassaemia, can produce a mild form of thalassemia differing in severity from thalassaemia intermedia to late-presenting thalassaemia major.

Anahtar Kelimeler

Talasemi; Mutasyon; DNA

Abstract

Beta talasemi, dünyada en sık görülen otozomal resesif bozukluklardan birisidir. Sorumlu olan HBB (Hemoglobin Beta) geni 11. kromozomun kısa kolunda lokalizedir. Beta globin geni üzerinde yaklaşık 200'den fazla farklı mutasyon tanımlanmıştır. Bu olgu sunumunda, beta talasemiye neden olan nadir bir mutasyonu (amplifiye edilmiş DNA'nın direkt sekanslanmasıyla, exon 1 promotor bölgesinde C→G substitüsyonu, CR920788, cDNA. 41C>G, g.2239C>G) ve dolayısıyla talasemi hastalarında DNA sekanslaması yapılmasının ne kadar önemli olduğunu göstermek istedik. Beta globin genini etkileyen farklı nükleotid substitusyonlarıciddiyeti ortadan ağır seyirli olan talasemiye kadar farklı tablolara yol açabilir.

Keywords

Thalassemia; Mutation; DNA

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Introduction

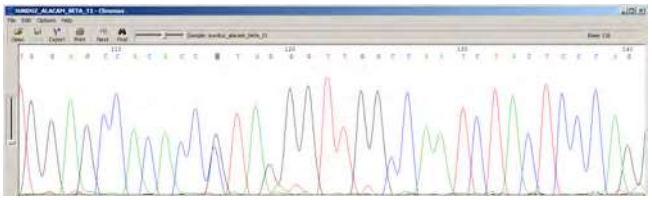
Beta thalassemia is one of the most prevalent autosomal recessive disorders in the world that affects nearly 150–200 million people from more than 60 countries around the world [1]. The general rate of carriers for beta thalassemia is 2% in Turkey, but in some provinces of Turkey, it is 10% [2]. The responsible gene HBB (hemoglobin beta) is localised on the short arm of 11th chromosome (11p15.5) and it consists of 3 exons, 2 introns and 5' and 3' regulatory regions [3]. So far more than 200 different mutations on beta globin gene that lead to beta thalassemia disease have been identified [4]. Although 35 mutations have been reported in Turkey, the prevalance and the distribution of these mutations differs according to different provinces in Turkey [5]. The most seen reasons of beta thalassemia are point mutations [6]. Point mutations prevent the beginning of RNA transcription, RNA processing and RNA stability, so normal RNA globin synthesis is prohibited. The other common mutations are small deletions, or addition of few nucleotides [7].

Some mutations are classified as common mutations, while others are known as rare mutations [1]. One of these rare mutations, called regulatory mutations, can affect the regulation of the genes. Changes in the 3'untranslated region may cause the mRNA to be unstable [8]. A change in this region may create or abolish a critical binding site for a micro-RNA or a protein that regulates translation. For example the regulator gene can produce another protein that controls the transcription of other genes or can affect the transcription regulation of another region. In both cases, regulatory mutations can disrupt this process, they can activate or inactivate the gene [9]. CR920788 (HGMD code) (cDNA.41C>G, g.2239C>G, (rs33994806) mutation is one of the rare mutations causing beta thalassemia disorder. In this case report, we wanted to show the importance of DNA sequencing in thalassemia patients. For example in reverse hybridisation, we can not detect these rare mutations, but in Turkey there are many thalassemia patients and the molecular screening must be essential in our country.

Case Report

An 34 years old-female patient from Middle East province in Turkey was referred to our Genetics Laboratory Center from Hematology clinics for the identification of hemoglobin disorders. Following complete blood count (CBC) (Table 1), hemoglobin electrophoresis was carried out using Minicap capillary electrophoresis method (Sebia, France). Hematological indices and electrophoresis results were compatible with beta thalassemia pattern. For the detection of mutation involved in manifestation of beta thalassemia disease, we used DNA sequencing of beta globin gene using Amersham DNA sequencing instrument (Applied Biosystems, Foster City, CA, USA) after amplification of the gene with specific primers. Result of the sequencing showed a mutation for the patient: CR920788 (cDNA.41C>G, g.2239C>G) in heterozygote state (Figure 1). Finally, the parents of the patient were screened and the mother was diagnosed as a carrier and the father was diagnosed to be normal. The mother's phenotype was not as severe as her daughter, but she used also some drugs for iron deficiency anemia. Treatment of the patient was started before full identification of mutations, and after finding the mutation, the treatment has been continued with higher precision. Informed consent of the patient and her parents was taken.

Figure 1: Sequencing of beta globin gene showing location of CR920788 (cDNA.41C>G, g.2239C>G) mutation of beta globin gene.



Discussion

Several studies have been carried out since 1980s to identify beta globin gene mutations and the rate of finding new mutations significantly increased after invention of PCR technique. There are always some rare mutations among beta thalassemia patients, and identification of all rare mutations in each region can help to improve screening protocols of the carriers and prevent affected child birth [1]. In two patients from a family, direct sequencing of amplified DNA detected a C→G substitution in the promotor site of exon 1 (CR920788, cDNA.41C>G, g.2239C>G) and this was a regulatory mutation. Both of our patients showed a severe phenotype although they were carriers. The location of the regulatory mutations are relative to the transcriptional initiation site, initiaton codon, polyadenylation site or termination codon. CR920788 (cDNA.41C>G, g.2239C>G mutation is also a rare regulatory mutation, which is located relative to the transcriptional initiation site. This mutation causes beta thalassemia major, so it causes a severe phenotype. Some of the mutations for thalassemias cause severe phenotype although the patients are carriers. Especially the regulatory mutations cause more severe phenotypes than the others (10).

The aim of this study was to report a beta thalassemia patient with CR920788 (cDNA.41C>G, g.2239C>G) mutation in Central Anatolia. This mutation is reported as a disease causing mutation by Huisman in 1992 [10]. Finding this case shows that there are also some rare mutations that can be missed during the screening of beta thalassemia carriers, and it is recommended for this mutation to take place in screening protocols for beta thalassemia carriers in different regions of Turkey. Sometimes, some molecular techniques (for example reverse hybridisation method) are not enough for some patients, because some rare mutations can cause the disease and to detect these muta-

Table 1. Hematological results of the patient and her parents

Gender	Age (y)	RBC (x106/⊠L)	H b(g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	HbA (%)	HbA2 (%)	HbF (%)
Case	34	5.06	11.9	37.6	74.3	23.5	31.6	89.14	5	5.86
Father	60	6.00	15.9	39.1	80.1	30.1	35.8	95.01	3.0	1.89
Mother	55	5.04	10.6	37.2	74.5	24.0	31.0	92.04	4.2	5.52

tions DNA sequence analysis is necessary. The results of this study indicate that different nucleotide substitutions affecting the proximal CACCC box of the beta-globin gene in combination with severe beta-thalassaemia, can produce a mild form of thalassemia differing in severity from thalassaemia intermedia to late-presenting thalassaemia major.

Competing interests

The authors declare that they have no competing interests.

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