The effect of HBB:c.*+96T>C (3'UTR +1570 T>C) on the mild β-thalassemia intermedia phenotype

HBB:c.+96T>C (3'UTR +1570 T>C) mutasyonunun ılımlı tip beta-talasemi intermedia fenotipi üzerine etkisi*

Türker Bilgen[1], Duran Canatan[2], Yunus Arıkan[1], Akif Yeşilipek[3], İbrahim Keser[1]

[1]*Department of Medical Biology and Genetics, Faculty of Medicine, Akdeniz University, Antalya, Turkey*
[2]*Mediterranean Blood Disease Foundation, Hemoglobinopathy Diagnosis Center, Antalya, Turkey*
[3]*Department of Pediatric Hematology, Faculty of Medicine, Akdeniz University, Antalya, Turkey*

Abstract

Hemoglobin beta (HBB):c.*+96T>C substitution is very rare among β-globin gene mutations and its clinical significance remains to be clarified. The present study aimed to investigate the role of HBB:c.*+96T>C in the β-thalassemia intermedia phenotype in a Turkish family. The proband and parents were screened for β-globin gene mutations via direct sequencing. Hematological and physical examination results were recorded, and correlated according to genotype. The proband was compound heterozygous for Cod 8 (-AA) and HBB:c.*+96T>C, whereas his mother and father were heterozygous for Cod 8 (-AA) and HBB:c.*+96T>C, respectively. The father had almost normal hematological findings, whereas the mother had the typical β-thalassemia trait phenotype. The proband was diagnosed as mild β-thalassemia intermedia based on hepatosplenomegaly and hematological findings. To the best of our knowledge this is the first report of HBB:c.*+96T>C mutation in a Turkish family. HBB:c.* 96T>C substitution is a very rare, but clinically relevant β-globin gene mutation. Additionally, we think that if 1 spouse is a carrier for β-globin gene mutation the other should be screened for silent mutations, such as HBB:c.*+96T>C mutation of the β-globin gene, even if she/he does not have any clinical or hematological signs of the β-thalassemia trait phenotype. *(Turk J Hematol 2011; 28: 219-22)*
Key words: Mild β-thalassemia intermedia, β-globin gene, HBB:c.*+96T>C

Received: October 23, 2010 *Accepted: January 12, 2011*

Özet

Hemoglobin beta (HBB):c.*+96T>C, β-globin geninde çok nadir görülen ve klinik önemi henüz tam olarak aydınlatılamamış bir değişimdir. Bu çalışmada; bir Türk ailede HBB:c.*+96T>C değişiminin beta-talasemi intermedia fenotipi üzerine etkisini araştırmayı amaçladık. Beta-talasemi intermedia ön tanılı çocuk ile anne ve babasının β-globin gen mutasyonları DNA dizi analizi yöntemiyle tarandı. Aile bireylerinin hematolojik ve klinik bulguları elde edilerek, genotipleri ile birlikte değerlendirildi. Direkt

Address for Correspondence: Prof. İbrahim Keser, Department of Medical Biology and Genetics, Faculty of Medicine, Akdeniz University, 07059 Antalya, Turkey Phone: +90 242 249 69 73 E-mail: keser@akdeniz.edu.tr

doi:10.5152/tjh.2011.57

DNA dizi analizi yöntemiyle mutasyon taranması sonucunda, olgunun Cod 8 (-AA) ve HBB:c.*+96T>C mutasyonları açısından birleşik heterozigot olduğu belirlendi. Annenin Cod 8 (-AA) mutasyonu, babanın ise HBB:c.*+96T>C mutasyonu yönünden taşıyıcı oldukları görüldü. Anne klasik beta-talasemi taşıyıcı bulgularına sahip iken, babanın klinik ve hematolojik açıdan normale yakın olduğu belirlendi. Diğer yandan olgu, hematoloji bulgularına ek olarak, hepato-splenomegalinin varlığı ile ılımlı tip beta-talasemi intermedia tanısı aldı. Bu çalışmada, HBB:c.*+96T>C değişimi ilk kez bir Türk ailesinde bildirilmektedir. Cod 8 (-AA) ve HBB:c.*+96T>C değişimleri açısından birlikte heterozigot olan olguda, sadece Cod 8 (-AA) mutasyonu ile açıklanamayacak klinik bulguların gözlenmesi nedeniyle, HBB:c.*+96T>C' nın taşıyıcılarda sessiz, ancak başka bir mutasyonla birlikte olduğunda hastalık şiddetini artıran, klinik açıdan önemli bir β-globin geni mutasyonu olduğu sonucuna varıldı. Ayrıca, çiftlerden birinin taşıyıcılığının genetik testlerle kesinleştiği durumlarda, diğerinin klinik ve hematolojik olarak taşıyıcı bulgularına sahip olmasa bile, HBB:c.*+96T>C gibi hematolojik açıdan sessiz mutasyonlar için taranması gerektiğini önermekteyiz. *(Turk J Hematol 2011; 28: 219-22)*
Anahtar kelimeler: Ilımlı tip beta-talasemi intermedia, Beta-globin geni, HBB:c.*+96T>C

Geliş tarihi: 23 Ekim 2010 *Kabul tarihi: 12 Ocak 2011*

Introduction

β-thalassemia is a hereditary blood disorder characterized by anomalies in hemoglobin (Hb) beta-chain synthesis and is the most frequently observed genetic condition in the world [1]. Diagnosis of β-thalassemia is based on hematologic and molecular genetic test results [2]. To date, more than 200 mutations have been reported; the disease exhibits a high level of molecular and clinical heterogeneity [3,4]. Among β-globin gene mutations, HBB:c.25_26delAA (Cod 8 [-AA]), which results in frame shift and stop codon at the 21st amino acid, is a rare mutation with an incidence of 2.1% among all β-globin gene mutations in Antalya Province [5]. HBB:c.*+96T>C or 3'UTR+1570 T>C is a very rare mutation located 12 nucleotides upstream of the polyadenylation signal in 3' UTR of the β-globin gene. There are a limited number of reports on the prevalence and clinical role of HBB:c.*+96T>C [6]. Additionally, there is a lack of consensus concerning the clinical importance of HBB:c.*+96T>C. To the best of our knowledge the present study is the first to report HBB:c.*+96T>C substitution in Turkey. The present study aimed to investigate the effect of the co-existence Cod 8 (-AA) and HBB:c.*+96T>C substitution on mild-type β-thalassemia intermedia in a Turkish family.

Materials and Methods

The proband, with ultrasound findings of hepatomegaly and splenomegaly, and unusual blood parameters, and his parents were referred from the hematology department to our laboratory for screening of β-globin gene mutations. The family's hematological findings were recorded. Informed consent was obtained from all the family members. Following genomic DNA extraction from whole blood using the salting-out method [7], genomic DNA was measured using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The proband and his parents were screened via direct sequencing of the region including 5' UTR (-101 position) of the β-globin to 3' UTR (Poly-A signal) in the β-globin gene. Sequencing was performed using a BigDye Terminator v3.1 Cycle Sequencing kit and an ABI prism 3130 genetic analyzer (Applied-Biosystems, Foster City, CA).

Results

Clinical examination showed that the proband had hepatomegaly with a longitudinal measure of 167 mm (normal: <155 mm) and splenomegaly with a longitudinal measure of 172 mm (normal: <130 mm), based on abdominal ultrasound findings. Hematological findings for the proband and parents are given in the Table. The proband was compound heterozygous for HBB:c.*+96T>C and Cod 8 (-AA) mutations according to DNA sequencing. His mother was a carrier of the Cod 8 (-AA) mutation, and his father was a carrier of the HBB:c.*+96T>C mutation. β-globin DNA sequencing showed HBB:c.*+96T>C and Cod 8 (-AA) mutations in the proband (Figure).

Following clinical and hematological examinations, the proband was diagnosed as mild type β-thalassemia intermedia. Although the patient's MCH level was low (18.60 pg/cell), his Hb level (11.5

g/dL) was compensated by an elevated RBC count (6.16 million mm^3). The proband's mother had hematological findings that correlated with the β-thalassemia trait due to Cod 8 (-AA) mutation, whereas the father (who only had the HBB:c.*+96T>C mutation) did not have hematological findings associated with the β-thalassemia trait.

Discussion

β-thalassemia due to structurally abnormal hemoglobin or an insufficient quantity of Hb is the most common genetic disease with a wide spectrum of phenotypic heterogeneity caused by genetic variation. The modifier factors that play in the severity of β-thalassemia are not well known. Cod 8 (-AA) is a frame shift mutation that creates a stop codon at the 21st codon, leading to β0-thalassemia. The modified C-terminal amino acid sequence in the β-globin gene with Cod 8 (-AA) mutation is as follows: (8)Val-Cys-Arg-Tyr-Cys-Pro-Val-Gly-Gln-Gly-Glu-Arg-(20)Gly-COOH (http://globin.bx.psu.edu/cgi-bin/hbvar/query_vars3). On the other hand, Cai et al. reported that HBB:c.*+96T>C was the only genomic alteration responsible for the β-thalassemia trait phenotype with elevated HbA_2 levels in 5 members of an Irish family. They also reported that the β-thalassemia trait phenotype in the family was caused by the known mutation IVS-II-654 C>T [8,9]. Additionally, Divoky et al. reported that HBB:c.*+96T>C was not associated with β-thalassemia phenotypes in Czech patients and that HBB:c.*+96T>C is linked to IVS-II-654 C>T mutation [6]. We examined our sequence results for such a mutation, but IVS-II-654 C>T mutation was not present in the presented family. Giambona et al. reported HBB:c.*+96T>C mutation in an Italian patient with a slightly elevated HbA_2 level [10]. Moreover, Boussiou et al. observed the HBB:c.*+96T>C mutation in 7 Greek patients without any clinical symptoms [11].

The presented proband had compound heterozygosity for Cod 8 (-AA) mutation and HBB:c.*+96T>C, and had clinical and hematological findings associated with mild β-thalassemia intermedia, whereas his mother had some findings indicative of anemia, such as paleness and fatigue, in addition to hematological parameters consistent with the trait phenotype due to Cod 8 (-AA) mutation (she occasionally takes folic acid and zinc tablets). Compared to the mother's blood parameters, the proband's Hb (11.5 g/dL), MCV (59.9 fL), and MCH (18.6 pg/cell) levels were lower, and RBC (6.16×10^{12} mm^3), HbA_2 (5.65%), and HbF (2.20%) levels were higher (Table 1). On the other hand, the proband's father was clinically normal, despite being a carrier of the HBB:c.*+96T>C mutation.

The father's laboratory tests did not show any significant hematological findings that could be due to HBB:c.*+96T>C mutation other than a slightly reduced RBC count (4.0×10^{12} mm^3) and Hb level (12.9 g/dL). This implies that HBB:c.*+96T>C carriers might be hematologically asymptomatic. Although by itself HBB:c.*+96T>C mutation does not cause the β-thalassemia trait phenotype in carriers, it probably has an additive affect when it co-exists with other β-globin gene mutations such as Cod 8 (-AA).

It was suggested that T>C substitution at position +1570 in the β-globin gene (12 bp upstream of the AATAAA polyadenylation signal) may contribute to the β-thalassemia phenotype by destabilizing β-globin mRNA [12]. Destabilization of mRNA processing due to HBB:c.*+96T>C mutation together with Cod 8 (-AA) mutation may have resulted in

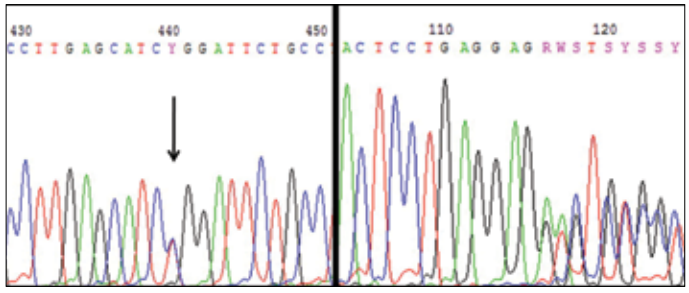

Figure 1. T>C transition at the c.*+96 position (left) and the frame shift mutation Cod 8 (-AA) (right) detected via DNA sequencing in the proband

Table 1. Hematological and molecular findings in the family members

	Age/ gender	β-globin gene mutation(s)	RBC (mm^3)	Hb (g/dL)	MCV (fL)	MCH (pg/cell)	MCHC (g/dL)	HbA_2 (%)	HbF (%)
Proband	20/M	Cod 8 (-AA)/ HBB:c.*+96T>C	6.16	11.5	59.9	18.60	31.1	5.65	2.20
Father	59/M	HBB:c.*+96T>C	4.00	12.9	92.60	32.30	34.9	2.20	0.20
Mother	49/F	Cod 8 (-AA)	6.14	12.5	61.9	20.30	32.8	4.40	0.30

the mild β-thalassemia intermedia phenotype in the presented proband, as patients that are heterozygous for the Cod 8 (-AA) mutation do not have the mild β-thalassemia intermedia phenotype with hepatosplenomegaly. As such, we think that the combination of β-globin gene mutations is important for the expression of the mild β-thalassemia intermedia phenotype.

To the best of our knowledge the present study is the first to report the combination of HBB:c.*+96T>C and Cod 8 (-AA). In addition, based on the very low frequency of HBB:c.*+96T>C substitution in the thousands of patients that have been sequenced in our laboratory, as well as in other laboratories, it can be considered a clinically important mutation that causes mild β-thalassemia. More detailed molecular studies on other factors, such as mRNA instability and other globin genes, should be performed to further clarify the role of HBB:c.*+96T>C mutation in the β-thalassemia trait phenotype.

In conclusion, we think that if 1 spouse is a carrier of a β-globin gene mutation, the other should be screened for silent mutations of the β-globin gene, even if she/he does not have any clinical or hematological signs of the β-thalassemia trait phenotype.

Acknowledgements: This study was supported by the Akdeniz University Scientific Research Projects Management Unit.

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.

References

1. Trent RJ. Diagnosis of the haemoglobinopathies. Clin Biochem Rev 2006;27:27-38.
2. Galanello R, Melis MA, Ruggeri R, Addis M, Scalas MT, Maccioni L, Furbetta M, Angius A, Tuveri T, Cao A. Beta 0 thalassemia trait in sardinia. Hemoglobin 1979;3:33-46.
3. Giardine B, van Baal S, Kaimakis P, Riemer C, Miller W, Samara M, Kollia P, Anagnou NP, Chui DH, Wajcman H, Hardison RC, Patrinos GP. Hbvar database of human hemoglobin variants and thalassemia mutations: 2007 update. Hum Mutat 2007;28:206. [CrossRef]
4. Wong C, Antonarakis SE, Goff SC, Orkin SH, Boehm CD, Kazazian HH, Jr. On the origin and spread of beta-thalassemia: Recurrent observation of four mutations in different ethnic groups. Proc Natl Acad Sci U S A 1986;83:6529-32.
5. Keser I, Sanlioglu AD, Manguoglu E, Guzeloglu Kayisli O, Nal N, Sargin F, Yesilipek A, Simsek M, Mendilcioglu I, Canatan D, Luleci G. Molecular analysis of beta-thalassemia and sickle cell anemia in Antalya. Acta Haematol 2004;111:205-10.
6. Divoky V, Baysal E, Oner R, Cürük MA, Walker EL 3rd, Indrak K, Huisman TH. The t-->c mutation at position +96 of the untranslated region 3' to the terminating codon of the beta-globin gene is a rare polymorphism that does not cause a beta-thalassemia as previously ascribed. Hum Genet 1994;93:77-8.
7. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
8. Cai SP, Eng B, Francombe WH, Olivieri NF, Kendall AG, Waye JS, Chui DH. Two novel beta-thalassemia mutations in the 5' and 3' noncoding regions of the beta-globin gene. Blood 1992;79:1342-6.
9. Eng B, Waye JS, Chui DH. The t----c substitution at nucleotide + 1570 of the beta-globin gene is a polymorphism. Blood 1992;80:1365.
10. Giambona A, Passarello C, Vinciguerra M, Li Muli R, Teresi P, Anza M, Ruggeri G, Renda D, Maggio A. Significance of borderline hemoglobin a2 values in an italian population with a high prevalence of beta-thalassemia. Haematologica 2008;93:1380-4.
11. Boussiou M, Karababa P, Sinopoulou K, Tsaftaridis P, Plata E, Loutradi-Anagnostou A. The molecular heterogeneity of beta-thalassemia in greece. Blood Cells Mol Dis 2008;40:317-9.
12. Cooper DN. Human gene mutations affecting rna processing and translation. Ann Med 1993;25:11-7.