

Short Communication

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IVSII-74 T > G: As harmless as we thought?

IVSII-74 T > G: Sandığımız kadar zararsız mı?

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Abstract

Background: IVSII-74 T > G is one of the most frequently identified polymorphic sites on the β -globin gene. In our report, we present three cases with low mean corpuscular volume (MCV) value in three and high red blood cell (RBC) value in two of the cases. The objective of this study was to further analyse the reason for condition of three patients, who were referred for the investigation of persistent anemia.

Materials and Methods: Following the HPLC analyses of Hb migration pattern, direct nucleotide sequencing of α - and β -globin genes was performed for all cases.

Results: The common finding was the homozygosity for the intronic change, IVSII-74 T > G.

Conclusion: In the intersection point of the variations on our patients, we claim a homozygous change at position 74 on the intron II of the β -globin gene alone may be sufficient to cause a β -thalassemia carrier phenotype.

Keywords: IVSII-74 T > G; β -Globin gene; β -Globin gene polymorphism; β -Thalassemia; β -Thalassemia carrier phenotype.

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Öz

Amaç: IVSII-74 T > G, β -globin geninin en sık gözlenen polimorfizmlerinden biridir. Çalışmamızda, MCV değeri düşük üç ve RBC değeri düşük iki olgu sunulmaktadır. Bu çalışmanın amacı kronik aneminin araştırılması için sevk edilen üç hastanın bulgularının nedeni olabilecek genetik değişikliklerin ayrıntılı analiz edilebilmesidir.

Gereç ve Yöntem: Hb migrasyon paterninin HPLC ile analizlerini takiben, tüm olgularda alfa- ve beta-globin genlerinin doğrudan nükleotid dizilemesi yapıldı.

Bulgular: Ortak bulgu, homozigot intronik IVSII-74 T > G polimorfizmi varlığı idi.

Sonuç: Hastalarımızdaki varyasyonların kesişim noktasında bulunan beta-globin geninin intron II'sinde pozisyon 74'te homozigot bir değişikliğin, beta-talasemi taşıyıcı fenotipine neden olmak için yeterli olabileceğini düşünmekteyiz.

Anahtar kelimeler: IVSII-74 T > G; β -Globin geni; β -Globin gen polimorfizmi; β -Talasemi; β -Talasemi taşıyıcılığı

Introduction

Up to 900 genetic variants have been characterized in the β -globin gene, which may or may not cause thalassemia phenotype. Turkey is one of the hotspot regions for the variations on the globin genes, so most of these variants are carried by individuals with Turkish origin [1].

β -thalassemias are characterized by reduced or diminished production of the β -globin chains. A β -thalassemia carrier state is caused by the reduced synthesis of the β -globin chains of the hemoglobin tetramer, and carriers of β -thalassemia are clinically asymptomatic. The phenotype of β -thalassemia carriers is characterized by microcytosis, hypochromia, variations in size and shape of red blood cells (RBC) in the blood smear and increased levels of HbA₂ [2]. The Hb electrophoresis pattern of β -thalassemia

heterozygotes (also known as β -thalassemia carriers) is characterized by $\geq 3.5\%$ HbA₂, and variable amounts of HbF [3].

Materials and methods

Complete blood count

Complete blood count (CBC) of the cases were evaluated using the Sysmex XS-1000i analyzer (Sysmex Corp, Japan).

Hemoglobin chromatogram by HPLC

HPLC analyses were performed using an in-house method occupying an ultra-speed HPLC system with a 415 nm UV/VIS-Detector (Shimadzu Corp., Japan). The method for obtaining hemoglobin chromatogram was modified from Ou and Rognerud [4]. Before the establishment of system, the method was validated according to CLSI guidelines. The efficiency of the system was controlled before every analysis using a ready-to-use control solution containing hemoglobin variants

HbA_{1c}, HbF, HbA, HbE, HbA₂, HbD, HbS and HbC, and control hemolysates in two levels of concentration, each containing HbF, HbA, HbA₂ and HbS (RECIPE Chemicals + Instruments GmbH, Germany). The coefficient of variation (CV) values for the analytes HbA₂ and HbF are 8.2% and 7.6%, respectively.

Direct sequencing

In order to recruit detailed analyses of suspected mutations, the α - and β -globin genes were amplified by polymerase chain reaction (PCR) and the PCR products were analyzed by direct nucleotide sequencing using the Beckman Coulter CEQ 2000XL DNA sequencer (Beckman Coulter Inc, USA).

Results

Here, we present three cases, who were referred for the investigation of chronic anemia. The CBC screens revealed a low mean corpuscular volume (MCV) value in three and high RBC value in two of the cases. Case #3, a 9-months old baby-girl had a normal RBC count. The hypochromic

Table 1: Results of hematological and DNA analyses of the cases.

Parameters	Case #1	Case #2	Case #3
Age	7 years	40 years	9 months
Sex	F	M	F
β -globin genotype	IVSII-74 T>G (homozygous)	IVSII-16 G>C (heterozygous) IVSII-74 T>G (homozygous)	IVSII-16 G>C (homozygous) IVSII-74 T>G (homozygous) IVSII-81 (C>T) (homozygous)
Unknown peak (%)	14.1%	14.1%	14.3%
HbF (%)	0.9 \leftrightarrow (ARRI: 0-0.9%)	1.9 \uparrow (ARRI: 0-0.9%)	7.2 \leftrightarrow (ARRI: 0.6-11.6%)
HbA (%)	80.75 \downarrow (ARRI: 95-98%)	78.00 \downarrow (ARRI: 95-98%)	73.81 \downarrow (ARRI: 86-97%)
HbA ₂ (%)	2.65 \leftrightarrow (ARRI: 0-3.9%)	3.21 \leftrightarrow (ARRI: 0-3.9%)	2.09 \leftrightarrow (ARRI: 0-3.9%)
RBC (M/mm ³)	6.8 \uparrow (ARRI: 4.0-5.2 M/mm ³)	6.5 \uparrow (ARRI: 4.7-6.1 M/mm ³)	4.8 \leftrightarrow (ARRI: 3.7-5.3 M/mm ³)
MCV (fL)	56 \downarrow (ARRI: 77-95 fL)	64.2 \downarrow (ARRI: 80-92 fL)	58 \downarrow (ARRI: 70-86 fL)
MCH (pg)	17.5 \leftrightarrow (ARRI: 25-29 pg)	20.7 \leftrightarrow (ARRI: 27-31 pg)	23.4 \leftrightarrow (ARRI: 23-40 pg)
Hb (g/dL)	11.9 \leftrightarrow (ARRI: 11.5-15.5 g/dL)	13.4 \downarrow (ARRI: 14.8-17.8 g/dL)	11.2 \leftrightarrow (ARRI: 10.5-13.5 g/dL)
RDW (%)	15.8 \uparrow (RI: 11.5-14.5%)	17.5 \uparrow (RI: 11.5-14.5%)	21.0 \uparrow (RI: 11.5-14.5%)
Peripheral smear	- Microcytosis - Hypochromia	- Microcytosis - Hypochromia - Target cells	- Microcytosis - Hypochromia
Iron (ug/dL)	62 \leftrightarrow (ARRI: 50-120 ug/dL)	192 \uparrow (ARRI: 50-170 ug/dL)	75 \leftrightarrow (ARRI: 40-100 ug/dL)
Ferritin (ng/mL)	18 \leftrightarrow (ARRI: 12-73 ng/mL)	156 \leftrightarrow (ARRI: 30-490 ng/mL)	81 \uparrow (ARRI: 12-60 ng/mL)

RBC, Red blood cell count; MCV, mean corpuscular volume; Hb, hemoglobin; RDW, red cell distribution width; ARRI, Age-related reference interval.

\uparrow : Above the reference interval.

\downarrow : Below the reference interval.

\leftrightarrow : Within the reference interval.

micro-erythrocytes were observed in the peripheral smear. We also observed target cells in the peripheral smear of case #2, a 40 year-old male (Table 1).

The cases were forwarded for further analysis with HPLC in order to see Hb migration patterns and percentages of Hb types on the chromatogram. In the HPLC analyses of the cases, we observed an approximately 14%

unidentified peak on the HbE window for all of the cases (Figure 1).

Following the full sequencing of the α - and β -globin genes, the only common finding we observed in all three cases was the homozygous presence of IVSII-74 T>G (also named as c.315+74T>G), an intronic change that was known as a polymorphism that has no effect on the phenotype [5,

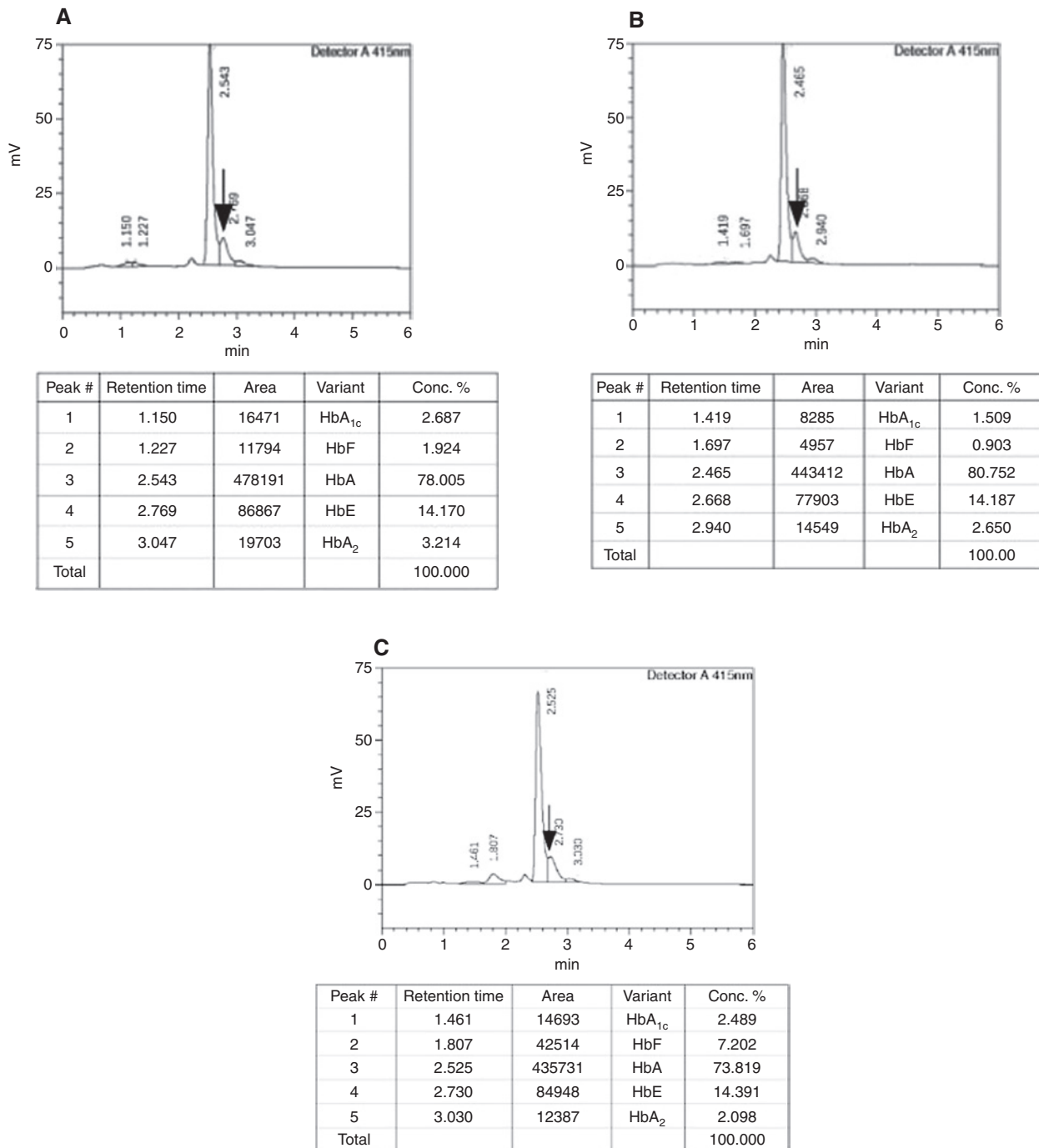


Figure 1: HPLC migration patterns for the hemoglobin types of the cases #1, #2 and #3, respectively. Unidentified abnormal patterns on the HbE windows were depicted with an arrow on the migration chromatograms.

6]. Other intronic changes, IVSII-16 G>C in heterozygous form was present in case#2, and IVSII-16 G>C and IVSII-81 (C>T) (both homozygous) in the case#3 (Figure 2).

The cases were recruited to our laboratory for detailed analysis in order to examine the underlying causes for the phenotypes characterized with microcytosis, hypochromia, and variations in size and shape of RBC.

The major possibilities for these phenotypes likely could be the cause of iron deficiency, α -thalassemia, β -thalassemia, δ -thalassemia, γ - δ - β -thalassemia, δ - β -thalassemia, or mild β -thalassemia [7].

Considerations argued against these conditions are as follows:

The probability of iron deficiency has been excluded with the measurement of iron and ferritin levels.

Direct sequence analyses of the β - and α -globin genes revealed no mutational sites for the β -globin genes; and no mutational sites or polymorphisms for the α -globin genes of the all cases. The variations found on the β -globin genes were depicted on the Table 1.

In the δ -thalassemia heterozygote condition, HbA₂ values are found to be below 1.5–2.5%, which is nearly half of the normal range without clinical manifestation. Moreover, HbA₂ is completely diminished in the homozygous version of δ -thalassemia, and serum iron levels are mostly normal [8].

Since HbA₂ consists of two α - and two δ -globin chains, defects on the δ -globin gene would imperiously modify the expression of the HbA₂ fraction [9]. Yet, the probands have normal HbA₂ levels. Thus, we also excluded the probability of variations on the δ -globin gene.

The γ - δ - β - and δ - β -thalassemia carrier conditions arise as a result of a deletion in both of the β - and δ -globin genes and the phenotypes of heterozygous and homozygous cases are characterized with normal HbA₂ values and low levels of MCV and MCH [10]. Additionally, HbF levels are markedly increased in δ - β -thalassemia cases as a result of increased γ -globin chain production [11]. Since the complete

sequencing of the β -globin genes did not reveal deletions, and owing to the normal and slightly increased levels of HbF in our cases, these possibilities were ruled out.

Also, due to the observed peaks on the HbE window, we expected to find possible mutations that lead to HbE or Hb Lepore.

In Hb Lepore, abnormal globin chain is the product of a hybrid gene that results from unequal crossing over between the δ - and β -globin genes during meiosis. As a result, this fused globin chain has the N-terminal amino acid sequence of a δ chain and the C-terminal amino acid sequence of a β chain, subsequently leading to a 74 kb deletion between the δ - and β -globin genes [7].

In the context of the study, we performed full sequence of the α - and β -globin genes. Since deleted gene regions were expected to be found on the β -globin gene as a result of Hb Lepore condition, we did not find any deletion on this gene. Therefore, we excluded this possibility.

In our report, we claim that, the subjects carrying a homozygous version of a so-called polymorphism, IVSII-74 T>G have a β -thalassemia trait phenotype with both hematological and peripheral smear findings.

IVSII-74 T>G was reported in several studies in a frequent fashion among the healthy controls and accepted as a polymorphism. IVSII-74 T>G is one of the most frequently identified polymorphic sites with a frequency of 22–53.8% [12, 13].

To date, this variation has been described as a common polymorphism with no pathogenic effect and occurred along with several polymorphic sites [10, 14, 15]. In several reports, heterozygous or homozygous forms of IVSII-16 G>C and IVSII-81 C>T were also found in most of the healthy controls and reported and described as neutral sequence changes [14].

In a report from India, the occurrence of IVSII-16 G>C and IVSII-74 T>G were reported in the thalassaemic patients and were considered as mutations causing the thalassaemic state [12].

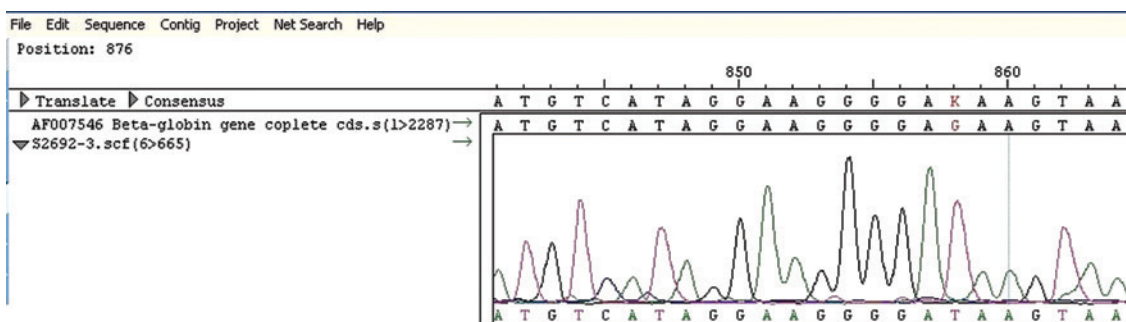


Figure 2: Direct sequencing analysis of the β -globin gene indicating the IVSII-74 T>G (homozygous) polymorphism.

In the previous reports, IVSII-74 T>G polymorphism has been reported with several other mutations in β -thalassemia cases. In the studies describing this variant as a polymorphism, it was mentioned that this variation was observed along different variations either in heterozygous or homozygous form [16, 17]. Thus, the effect of this polymorphism was not investigated further since the researchers identified other changes in the β -globin gene and claimed that the phenotypes became evident as a result of these other alterations. Thus, IVSII-74 T>G was accepted a harmless polymorphism to date, and the variation causing the thalassaemic state was thought to be a result of other genomic variants [16, 18, 19].

In the computer predicted secondary structure analysis of IVSII, positions 74 and 81 are localized at the end of the hairpin-loop. In this analysis, it has been revealed that, a G to T transition at position 74 and/or a C to T change at position 81 of IVSII change the free energy and changes the stable form of the hairpin-loop. They claim that the nucleotide change at position 81 alone or together with 74 of IVSII might be responsible for a β -thalassemia phenotype [20]. The idea that, IVS might be providing stability to the secondary structure of transcribed mRNA precursors is consistent with the studies, claiming the IVS in the globin genes are highly conserved [21].

In our report, we suggest that this change might have an effect in the protein level, resulting in a change on the electrical charge of the protein, leading to a different peak on the chromatogram.

In conclusion, as found in the intersection point of the variations, we suggest that, a homozygous change at position 74 revealed similar findings with β -thalassemia carrier phenotype in our cases.

Unfortunately, since the probands were lost to follow-up and it is not possible to provide additional data or samples, no further analysis could be performed. With this report, we advise to the scientists working in this area to consider the possibility of an IVSII-74 T>G mutation when assessing patients with a β -thalassemia carrier phenotype.

It will be of great interest to see how the change at position 74 on the intron II of the β -globin gene cause the β -thalassemia carrier phenotype with the help of the microarray studies recruited to exhibit the expression pattern of β -globin chain, globin chain synthesis and qRT-PCR of the β -globin gene of the probands' reticulocytes, which might provide additional data in order to unveil the further understanding of intronic changes on the β -globin gene and their possible effects on the phenotype.

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