A 13-bp Deletion in the 3' Untranslated Region of the β -Globin Gene Causes β -Thalassemia Major in Compound Heterozygosity with IVSII-1 Mutation

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Key Words

Deletion • β -Globin • β -Thalassemia • Untranslated region

Abstract

Objective: To describe hematological and molecular features of a 13-bp deletion in the 3' untranslated region (3' UTR) of the β -globin gene in carrier individuals and a compound heterozygous patient. **Subjects and Methods:** Five members of an Iranian family of Persian ethnic origin were studied. Red blood cell indices and hemoglobin analysis were carried out according to standard methods. Genomic DNA was obtained from peripheral blood cells by salting-out procedures. β -Globin gene amplification and DNA sequencing were performed. **Results:** One patient had a 13-bp deletion in the 3' UTR of the β -globin gene that causes the β -thalassemia phenotype in combination with the IVSII-1 (G→A) mutation. The patient had inherited the IVSII-1 (G→A) mutation from his mother, while the second β -globin gene (inherited paternally) had a 13-bp deletion at nucleotide 90 downstream of the termination codon (CD +90 del 13 bp). The patient's father and paternal grandmother, who are carriers of this deletion, had no hematological abnormalities. **Conclusion:** This case showed a patient with a 13-bp dele-

tion in the 3' UTR of β -globin gene that could cause a slight decrease in the stability of the mRNA, but did not have a hematological effect in the heterozygotes. The 13-bp deletion could be clinically important only in situations where β -chain synthesis in trans is compromised.

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Introduction

β -Thalassemia is a genetic hereditary disorder characterized by the decrease or absence of β -globin chain production due to mutations in coding and non-coding sequences of the β -globin gene. The 3' untranslated regions (3' UTR) have critical roles in pre-mRNA processing through the polyadenylation signal. The sequences downstream and upstream of this signal have been shown to be responsible for proper processing [1, 2]. Therefore, some 3' UTR mutations are involved in the reduction of β -globin chain production [1, 3]. In this study, we describe hematological and molecular characteristics of a 13-bp deletion in the 3' UTR of an Iranian family detected during prenatal diagnosis.

Table 1. Hematological data and β -globin mutation of 3 generations of the family

	Paternal grandfather	Paternal grandmother	Father	Mother	Patient (post-transfusion)
Age, years	63	56	30	25	5
Sex	M	F	M	F	M
Hb, g/dl	15.4	14.8	16.4		7.7
RBC, $\times 10^{12}/l$	5.27	4.86	5.2		4.04
Hematocrit, %	43	43.3	47.7		24.8
Mean cell volume, fl	82	89	91.8	58.8	61
Mean cell Hb, pg	29	30	31.5		19
MCHC, %	36	30	34.4		31
HbA, %	95	95.6	97		94.3
Fetal Hb, %	1.4	1.6	0.6		2.2
HbA2, %	3.4	2.8	2.4	4.9	3.5
Ferritin, ng/ml	–	–	318	–	750
β -Genotype	N/N	term CD +90 del 13 bp/N	term CD +90 del 13 bp/N	IVSII-1 (G→A)/N	IVSII-1 (G→A)/term CD +90 del 13 bp

RBC = Red blood cells; MCHC = mean cell Hb concentration; N = normal.

Subjects and Methods

Five members of a family from Tehran, Iran, of Persian ethnic origin who were referred for prenatal diagnosis for β -thalassemia were studied. Red blood cell indices and hemoglobin (Hb) analysis were carried out according to standard methods. After obtaining written informed consent, molecular studies were conducted on genomic DNA isolated from peripheral blood cells by a salting-out procedure [4]. The entire β -globin gene was amplified and DNA was sequenced with the use of a primer set encompassing exons 1 and 2 (fragment A: Beta1F 5'-GGGCCAAGAGATATA-TCTTAG-3', Beta1R 5'-AATGACATGAACTTAACCATAG-3') and another set encompassing exon 3 and 3' UTR (fragment B: Beta2F 5'-GCACCATTTCTAAAGAATAACAG-3', Beta2R 5'-GT-TTGAAGTAGCTCTTCATTTC-3').

The sequencing reactions were performed as described elsewhere [5, 6]. The nucleotide numbering is based on GenBank accession number U01317.

Results

The 13-bp deletion in the 3' UTR of the β -globin gene was identified in 3 members of 3 generations. One patient had a 13-bp deletion in the 3' UTR of the β -globin gene that causes β -thalassemia intermedia in combination with IVSII-1 (G→A) mutation. In addition, 2 subjects who are β -thalassemia carriers with term CD +90 del 13 bp mutation had normal hematological indices (table 1). The hematological and molecular features of this mutation in carrier individuals and the compound heterozygous patient are summarized in table 1.

Our patient (third generation), who is a compound heterozygote for the 13-bp deletion and the IVSII-1 (G→A) mutation, had hematological indices and clinical manifestations consistent with thalassemia major. He has splenomegaly and is blood transfusion dependent (every 3 weeks).

Discussion

This small deletion (GCATCTGGATTCT) begins 90 bp downstream of the termination signal TAA and ends 4 bp upstream of the poly A sequence, spanning a region of 13 bp. The heterozygotes did not show any clinical symptoms or hematological changes. This result is completely different from a previous study that reported typical features of the β -thalassemia trait in carriers of this 13-bp deletion in the 3' UTR of the β -globin gene [3]. To the best of our knowledge, this mutation is the first report from an Iranian family and the second in the world [3].

The functional effect of this deletion, which has been previously studied [7], indicated a strong negative transcription effect. However, the carriers of the deletion in our study appear to have normal hematological parameters. Therefore, it may be concluded that β -globin chain synthesis has not been affected in these individuals. However, this mutation, when it occurs in trans with β^0 mutation compound heterozygosity, could lead to a severe thalassemia phenotype, as in our patient. It is important

to bear in mind that the hematologic profile of the patient is post-transfusion and does not reflect his true status. Furthermore, this mutation is a pitfall, a potential source of misdiagnosis in screening for β -thalassemia carriers and in prenatal diagnosis. Therefore, caution must be taken to ensure that any at-risk person is not overlooked in the context of thalassemia prevention programs or prenatal diagnosis settings. When parents or at-risk individuals seek genetic counseling antenatally or premaritally, the first rule should be screening of both members of the couple and not just one. In some national thalassemia prevention programs, the man is screened first and if his hematology profile appears to be normal, his partner is not tested to avoid stigmatization of the woman. Second-

ly, if one of the partners is definitely a carrier of β -thalassemia, the other partner has to be scrutinized at the molecular level for β -globin gene variation irrespective of an apparently normal hematological profile.

Conclusion

This case showed a patient with a 13-bp deletion in the 3' UTR of β -globin gene that could cause a slight decrease in the stability of the mRNA but did not have a hematological effect in the heterozygotes. The 13-bp deletion could be clinically important only in situations where β -chain synthesis in trans is compromised.

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