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ORIGINAL ARTICLE

Novel Beta (β)-Thalassemia Mutation in Turkish Children

Mustafa Ulasli · Serdar Oztuzcu · Sevil Kirkbes · Ali Bay · Yusuf Ziya Igci · Recep Bayraktar · Mehri Igci · Sercan Ergun · Ecir Ali Cakmak · Elif Aytekin · Ahmet Arslan

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Abstract Beta (β)-thalassemia is the most frequently observed hereditary blood disorder in the world. It is characterized by deficiency of hemoglobin β-globin gene and is also a profoundly heterogeneous both at the molecular and clinical level. In the case of β-thalassemia, there is reduced (β^+ type) or absent (β^o type) synthesis of the beta chains of hemoglobin. β-Thalassemia clinically occurs in three main forms: major, intermedia and minor according to requirement of transfusion. The objective of this study was to evaluate β-thalassemia mutations in 89 patients ranging from 2 months to 16 years of age, who enrolled to Medical School Research and Training Hospital, Gaziantep University. The direct DNA sequence analysis was performed for mutation scanning of β-globin gene. 89 children with β-Thalassemia including all types were analyzed, 16 different β-thalassemia mutations were detected. We have also identified a novel mutation (HBB.c.-80delT, rs397509430) in the promoter region (−30 TATA box) of β-globin gene, and clinical data of patient having novel mutation was given. The β-Thalassemia mutations were determined as β-Thalassemia major type in 42 patients (47.19 %), β-Thalassemia intermedia in 4 (4.49 %), β-Thalassemia minor in 43, (48.31 %) patients. The most frequent mutation was IVS I-110 G>A, followed by IVS I-1 G>A, IVS I-6 T>C, IVS II-1 G>A, respectively.

Keywords Beta-thalassemia (β-Thal) · Beta globin gene · β-Thal mutations · Molecular diagnosis

Introduction

Beta-thalassemia (β-Thal), is the most frequently observed hereditary blood disorder in the world and is characterized by deficiency of hemoglobin β-globin gene [1]. In the case of β-Thal, there is reduced (β^+ type) or absent (β^o type) synthesis of the beta chains of hemoglobin [1, 2].

β-Thal clinically occurs in three main forms: major, intermedia and minor according to requirement of transfusion [3]. β-Thal major is characterized by completely inhibited synthesis of beta chains [4] so β-Thal major must be treated (generally transfusion therapy) otherwise 85 % of patients die in early childhood due to severe anemia [5]. β-Thal major phenotype has homozygotes or compound heterozygotes for β^o or β^+ genes [6]. In contrast to major type, β-Thal minor patients are carrier, only one allel has a mutation and other allel is normal, and have slight phenotype (except to dominantly inherited β-Thal [7]). β-Thal intermedia clinically differs from major and minor ones with respect to necessity of transfusion. The degree of anemia for β-Thal major is more aggravated than the grade of anemia for β-Thal intermedia [5, 8]. The genotype of β-Thal intermedia is mostly homozygotes or compound heterozygotes [6].

β-Globin gene is found in β-globin locus which is localized on the short region of chromosome 11(11p15.4) and composed of five genes (β-globin and beta-like genes span 70 kb) [7]. β-Globin gene spans 1,600 bp and encodes 146 amino acid residues, and consists of three exons and

M. Ulasli (✉) · S. Oztuzcu · S. Kirkbes · Y. Z. Igci · R. Bayraktar · M. Igci · S. Ergun · E. A. Cakmak · A. Arslan
Department of Medical Biology, Faculty of Medicine, University of Gaziantep, 27310 Şehitkamil, Gaziantep, Turkey
e-mail: mulasli@gmail.com

A. Bay · E. Aytekin
Division of Pediatric Hematology, Department of Pediatrics, University of Gaziantep, 27310 Şehitkamil, Gaziantep, Turkey

two introns (intervening sequences, IVS). Exon 1 encodes for amino acid residues from 1 to 29 and also encodes the first two bases of codon 30, exon 2 encodes the last base of codon 30 and amino acids from 31 to 104 and exon 3 codes for amino acids from 105 to 146 [7]. In addition, 5′flanking, promoter, splicing and 3′untranslated (3′-UTR) regions also are important for β-globin protein activity [7, 9].

In this retrospective study, 89 β-thalassemic patients from 2 months to 16 years of age enrolled to Medical School Research and Training Hospital, University of Gaziantep, Turkey were included. Genomic sequence of β-globin gene was scanned for mutations working direct DNA sequence analysis. This assay enables more comprehensive detection of rare and novel mutations [3]. We reported the number and the frequencies of known mutations, and type of β-Thal mutations and then carried out mutation analysis of β-Thal in Gaziantep of Turkey. In addition, we defined a novel mutation located in promoter region of β-globin gene and informed about the β-Thal phenotype.

Materials and Methods

Patient Selection

This study included 89 patients who were enrolled Department of Pediatric Hematology between 2009 and 2013 in Medical School Research and Training Hospital, University of Gaziantep, which serves patients from South-east part of Turkey and in this part of Turkey. β-Thal is a significant public health problem in South-east part of Turkey. 89 β-Thal patients (ranging from 2 months to 16 years of age) were included into the study group. The study was approved by the Institutional Review Board of Faculty of Medicine in University of Gaziantep.

Mutation Analysis

89 β-Thal patients were included in this study. Genomic DNA was isolated from peripheral blood samples using the QIAamp DNA Blood Mini Kit (Qiagen Sample and Assay Technologies, Hilden, Germany). β-Globin gene was amplified by using polymerase chain reaction (PCR) Seq finder sequencing system HBB Kit (Wollerau, Switzerland). PCR was performed in 25 µl reaction volumes containing 12.5 µl master mix, 2 µl of primer mix (forward and reverse), 2 µl distilled water, 4 µl GC enhancer, 2.5 µl of genomic DNA and 0.2 µl Taq polymerase. PCR reactions were carried out in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA) and cycling conditions were for 95 °C, 10 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 59 °C for 90 s, and elongation at 72 °C for 60 s and a final elongation at 72 °C for 7 min. The samples were purified by ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced with the 3130×l Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Genomic and amino acid sequences for β-globin gene was obtained from Ensemble: ENSG00000244734, ENSP00000333994.3

Results

DNA samples from 89 children with β-Thal including β-Thal major, β-Thal intermedia and also β-Thal minor were analyzed, 16 different β-Thal mutations were detected, and one of them is a novel mutation. Molecular analysis of β-globin gene mutations in CODON 5 (−CT), CODON 8 (−AA), CODONS 9/10 (+T), CODON 17 A>T, CODONS 36-37 (−T), CODON 39 C>T, CODON 44 (−C), CODON 82/83 (−G), IVS I-1 G>A, IVS I-6 T>C, IVS I-110 G>A, IVS I-130 G>C, IVS II-1 G>A, -30 T>A, -30 (−T),IVS II-848 C>A were carried out.

β-Thal is classified into major, minor, intermedia. Table 1 shows the frequencies of β-Thal types. In 89 β-Thal patients, β-Thal major type was accounted for 47.19 % (42/89). 43 β-Thal cases were β-Thal minor type which their mutations have a frequency of approximately 48.31 % (43/89). The most common mutation was IVS I-110 G>A for both β-Thal major and β-Thal minor forms. β-Thal intermedia form was found only in 4 patients.

The most frequent mutation was IVS I-110 G>A, identified in 30.66 %, followed by IVS I-1 G>A with 12.41 %, IVS I-6 T>C with 9.49 %, IVS II-1 G>A with 8.76 %, respectively. The other frequencies of mutations are also shown in Table 2. In addition, Table 2 lists the molecular features of mutations, rs numbers and HGVS codes. The mutations were found in exons, intron regions and also promoter regions. The ratio of β^{o}/β^{+} detected, Thalassemia mutations was 1.18, except novel mutation. Moreover, a novel mutation (HBB:c.-80delT) was and tagged by rs397509430. The novel mutation gave rise to β-Thal major phenotype for this patient. The hemoglobin levels for the patient were HbA_1: 45.3 %, HbA_2: 3.7 % and HbF: 51 %, respectively and also first hemoglobin (Hb) concentration was 7.4 g/dl. He has received blood transfusions in 3 weeks regularly. The patient does not have a laparoscopic splenectomy. The patient was admitted to the bone marrow transplantation. The parents are heterozygous for this mutation and their HbA_2 levels were approximately 4 %.

Discussion

β-Thal, is the most frequently observed hereditary blood disorder in the world, is characterized by deficiency of

Table 1 Distribution of β-Thalassemia mutations in the β-gobin gene in 89 Turkish children

Mutation	Variant common name	Number of patients
β-Thal major (n = 42, 47.19 %)	IVS I-110 G>A homozygous	10
	IVS I-1 G>A homozygous	6
	IVS I-6 T>C homozygous	5
	CODON 17 A>T homozygous	3
	IVS II-1 G>A homozygous	3
	CODON 39 C>T homozygous	2
	CODON 8 (−AA) homozygous	1
	CODONS 9/10 (+T) Homozygous	1
	(−)30 (−T) homozygous	1
	CODON 82-83 (−G) homozygous	1
	IVS II-848 C>A homozygous	1
	IVS I-130 G>C Homozygous	2
	CODON 17 A>T/IVS I-1 G>A compound heterozygous	1
	CODON 44 −(C)/IVS II-1 G>A compound heterozygous	1
	CODON 36-37(−T)/CODON 44 (−C) compound heterozygous	1
	IVS II-1 G>A/CODON 44 (−C) compound heterozygous	1
	IVS II-1 G>A/CODON 39 C>T compound heterozygous	1
	IVS I-110 G>A/CODON 82-83 (−G) compound heterozygous	1
β-Thal intermedia (n = 4, 4.49 %)	(−)30 T>A homozygous	2
	CODON 8 (−AA) homozygous	1
	(−)30 T>A/IVS I-6 T>C compound heterozygous	1
β-Thal minor (n = 43, 48.31 %)	IVS I-110 G>A heterozygous	20
	CODON 8 (−AA) heterozygous	5
	CODON 39 C>T heterozygous	4
	CODON 17 A>T heterozygous	3
	IVS II-1 G>A heterozygous	2
	IVS I-6 T>C heterozygous	2
	IVS I-1 G > A heterozygous	3
	CODON 5 (−CT) heterozygous	1
	CODON 44 (−C) heterozygous	1
	IVS I-110 G>A/CODON 8 (−AA) compound heterozygous	1
	IVS I-1 G>A/IVS II-1 G>A compound heterozygous	1
Total	89	

hemoglobin β-globin gene and also is a profoundly heterogeneous, both at the molecular and clinical level [1, 10]. Approximately 300 different β-globin gene mutations for β-Thal have been reported and 40 of them are common in Turkey [11]. The first β-thalassemia study for Turkey was published in 1985 [1, 12]. When considering the distribution of these mutations, it is seen that heterogeneity increases from the western part of the country to the east [13]. The distributions of most common mutations in Southeastern part of Turkey are IVS I-110, IVS I-6, CODON 15, IVS I-1 and others which have frequencies approximately 25, 15, 10, 5, 60 %, respectively [13].

In our study, we have identified 16 different β-Thal mutations in 89 patients; the mutations were noted in 37.5 % of other studies reported for β-Thal mutations from Turkey [11, 13, 14]. In addition, we detected a novel β-globin gene mutation located in the promoter region of the gene and found β-Thal major phenotype.

Three most frequent β-globin gene mutations were reported in 62.16 % of our study, and the most common mutation was detected as IVS I-110 G>A, similar to previous reports [1, 11, 15]. The other most common mutations of IVS I-1 G>A (13.51 %) and, IVS I-6 T>C (10.81 %) were similar to previous report. The other studies in Turkey indicated that second most common mutations was CODON 8 (−AA) and also it is most common mutation in Azerbaijan [1, 11], but other previous study detected as 6.1 % [13] similar to our data (7.30 %). The −30 T>A and CODON 39 (C>T) mutations were frequently seen in Balkan countries [1], observed as 3.65 and 6.57 % in our cases.

The four most common mutations counted up 61.31 %, and the first 7 common mutations was 82.48 % of overall mutations. The most common mutation frequencies of patients with β-Thal major and β-Thal intermedia were detected in IVS I-110 G>A homozygous, and IVS I-1 G>A homozygous by Fettah et al. as similar to our data. However surprisingly CODON 8(−AA) was not frequently detected in β-Thal major but the frequency of CODON 8(−AA) heterozygous in β-Thal minor was detected as second common mutation and also this mutation was observed in β-Thal intermedia [11].

These mutations were mostly β^o type mutations except IVS I-6 T>C and IVS I-110 G>A, (−)30 T>A, IVS II-848 C>A but the ratio of β^o: β^+ thalassemia mutations was 1:18. In addition, it was previously described that the ratio of β^o: β^+ mutations 1:1. Furthermore, IVS I-110 G>A forms the majority of β^+ type and this mutation causes severely lesions so homozygous or compound heterozygous combinations for the mutations reasons β-Thal major form [13]. In this study the majority of β^+ type patients also contained IVS I-110 G>A lesion.

Homozygous and compound heterozygous individuals have usually β-Thal major and intermedia phenotype [6]. However, in our cases, two compound heterozygous patients were found as β-Thal minor (see Table 1). In addition, the same mutation can be seen in both β-Thal major and intermedia [11] just as we found in our study. Because of mentioned conditions above, β-Thal phenotype can be influenced by not only the β-globin gene mutations

Table 2 Distribution and frequencies of mutated alleles of β-gobingene

SNP No	HGVS code	Variant common name	Type	Number of alleles	Frequency (%)
rs35004220	HBB:c.93-21 G>A	IVS I-110 G>A	β^+	42	30.66
rs33971440	HBB:c.92 + 1 G>A	IVS I-1 G>A	β^o	17	12.41
rs35724775	HBB.c.92 + 6 T>C	IVS I-6 T>C	β^+	13	9.49
rs33945777	HBB:c.315 + 1 G>A	IVS II-1 G>A	β^o	12	8.76
				Σ	61.31
rs33986703	HBB:c.52 A>T	CODON 17 A>T	β^o	10	7.30
rs35497102	HBB:c.25_26delAA	CODON 8 (−AA)	β^o	10	7.30
rs11549407	HBB.c.118 C>T	CODON 39 C>T	β^o	9	6.57
				Σ	82.48
rs33980857	HBB.c.−80T>A	(−)30 T>A	β^+	5	3.65
rs80356820	HBB:c.135delC	CODON 44 (−C)	β^o	4	2.92
rs63751478	HBB.c.250delG	CODON 82/83 (−G)	β^o	3	2.19
rs33943001	HBB:c.93−1 G>C	IVS I−130 G>C	β^o	4	2.92
rs34548294	HBB:c.30_31insT	CODONS 9/10 (+T)	β^o	2	1.46
rs33913413	HBB.c.316−3C>A	IVS II−848 C>A	β^+	2	1.46
rs397509430[a]	HBB:c −80delT	(−)30 (−T)	unknown	2	1.46
rs34889882	HBB:c.17_18delCT	Codon 5 (−CT)	β^o	1	0.73
rs63750532	HBB:c.112delT	CODONS 36-37 (−T)	β^o	1	0.73

[a] Novel mutation

but also other factors, e.g., α/β-globin chain imbalance, changes in other genes involved in bilirubin metabolism and iron absorption [13].

We described a novel mutation (−30 (−T)) for β-globin gene located in the TATA box (positions −28 to −31) which is cis acting element for promoter region and bound transcription factors [7] so the mutation may prevent to the binding to transcription factors in the TATA box in order to reduction or inhibition of transcription. This mutation gives rise to β-Thal major in homozygous form so transcription of β-globin gene is severely affected. The mutation types (β^o or β^+) are not known for this mutation and it will be investigated.

Conclusion

As a conclusion, in this retrospective study, we determined the distributions of β–Thal mutations in south-east part of Turkey which were all studied in Gaziantep University. 16 different mutations were found, and a novel mutation was reported additionally. Correlation between this novel mutation and clinical manifestations were established so that detection of novel mutation was significant for literature.

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