The Molecular Spectrum of Beta Thalassemia Mutations in Southeastern, Turkey

Türkiye'nin Güneydoğu Bölgesindeki Beta Talasemi Mutasyonlarının Moleküler Spektrumunun Belirlenmesi

Derya Karaer[1], Bahtiyar Sahinoglu[2], Esra Pekpak Sahinoglu[3], Abdullah I. Gurler[2], Emre Kırat[4], Kadri Karaer[1]

[1]Pamukkale University Faculty of Medicine, Department of Medical Genetic, Denizli, Turkey
[2]Dr. Ersin Arslan Training and Research Hospital, Genetic Diseases Diagnosis Center, Gaziantep, Turkey
[3]Gaziantep University Faculty of Medicine, Department of Pediatric Hematology, Gaziantep, Turkey
[4]Bursa City Hospital, Department of Medical Genetic, Bursa, Turkey

ABSTRACT

Objective: Beta-thalassemia (β-thalassemia), which is very common in southern Turkey, is an autosomal recessive genetic disease caused by more than 400 mutations in the Beta-globin (HBB) gene. We aimed to investigate the beta thalassemia mutation profile in this region and contribute to treatment strategies with the study we conducted from patients who applied to our Genetic Diagnosis Center from Gaziantep and its surrounding southeast Anatolian provinces.
Method: In the study, HBB gene mutations were investigated by DNA sequence analysis in 313 unrelated patients who applied to our center. 41% of the patients included in the study were from Syrian migrant families.
Results: A total of 32 different beta globin mutations were detected. The most common mutations are: IVS-I-110 G>A (HBB: c.93-21G>A) 20.65%, Codon 39 C>T (HBB: c.118C>T) 8.63%, IVS I-6 T>C (HBB: c.92+6T>C) 7.10%, IVS I-1 G>A (HBB: c.92+1G>A), 6.88%, IVS II-1 G>A (HBB: c.315+1G>A) 6.24%, Codon 6 (GAG>GTG) (HbS) (HBB: c.20A>T) 4.52%, CAP +20 C>T (HBB: c.-31C>T) 4.52%, Codon 8 (-AA) (HBB: c.25_26del) 4.30%, -30 (T>A) (HBB: c.-80T>A) 4.09%, IVS II-745 C>G (HBB: c.316-106C>G) 3.87%. We also detected a new variation (HBB: c.92+56G>A) that was not reported before, and six different beta globin gene mutations (HBB: c.90C>T, HBB: c.47G>A, HBB: c. 93- 22_95del, HBB:c.30dup, HBB: c.180G>A , HBB: c.316-30A>C) not previously reported in Turkey. Four of these mutations were detected in Syrian patients (c.90C>T, c.47G>A, c.93-22_95del, c.30dup).
Conclusion: Our study reveals that the mutations that cause beta thalassemia and hemoglobinopathies in the Southeast Anatolia region, which includes Gaziantep and its surrounding provinces, are quite diverse and show some differences compared to other regions.

Keywords: Thalassemia, Beta-Globin gene, Mutations, Turkey.

Received: 09.02.2022 **Accepted:** 11.01.2022

ÖZET

Amaç: Türkiye'nin güneyinde oldukça sık görülen Beta-talasemi (β-talasemi), Beta-globin (HBB) genindeki dört yüzden fazla mutasyonun neden olduğu otozomal resesif geçişli genetik bir hastalıktır. Gaziantep ve çevresindeki güneydoğu Anadolu illerinden Genetik Tanı Merkezimize başvuran hastalardan yaptığımız çalışma ile bu bölgedeki beta talasemi mutasyon profilini araştırmayı ve tedavi stratejilerine katkıda bulunmayı amaçladık.
Yöntem: Çalışmada, merkezimize başvuran ve aralarında akrabalık olmayan, 313 hastada DNA dizi analizi ile HBB gen mutasyonları araştırıldı. Çalışmaya dahil edilen hastaların %41'i Suriyeli göçmen ailelerdendi.
Bulgular: Çalışma sonucunda, toplam 32 farklı beta globin mutasyonu tespit edildi. Bulunan en yaygın mutasyonlar şunlardı: IVS-I-110 G>A (HBB: c.93-21G>A) %20.65, Codon 39 C>T (HBB: c.118C>T) %8.63 , IVS I-6 T>C (HBB: c.92+6T>C) %7.10, IVS I-1 G>A (HBB: c.92+1G>A) %6,88, IVS II-1 G>A (HBB: c. 315+1G>A) %6,24, Codon 6 (GAG>GTG) (HbS) (HBB: c.20A>T) %4,52, CAP +20 C>T (HBB: c.-31C>T) %4,52, Codon 8 (-AA) (HBB: c.25_26del) %4,30, -30 (T>A) (HBB: c.-80T>A) %4,09, IVS II-745 C>G (HBB: c.316-106C>G) %3.87. Ayrıca daha önce bildirilmeyen yeni bir varyasyon (HBB: c.92+56G>A) ve Türkiye'de daha önce bildirilmemiş altı farklı beta globin gen mutasyonu tespit edildi (HBB:c.90C>T, HBB: c.47G>A, HBB: c.93-22_95del, HBB:c.30dup, HBB: c.180G>A , HBB: c.316-30A>C). Bu mutasyonlardan dördü (c.90C>T, c.47G>A, c.93-22_95del, c.30dup) Suriyeli hastalarda saptandı.
Sonuçlar: Çalışmamız, Gaziantep' in yoğunlukta olduğu güneydoğu Anadolu bölgesinde beta-talasemi ve hemoglobinopatilere neden olan mutasyonların oldukça çeşitli olduğunu ve diğer bölgelere göre bazı farklılıklar gösterdiğini ortaya koymaktadır.

Anahtar Sözcükler: Talasemi, Beta-Globin, Mutasyon, Türkiye

Geliş Tarihi: 02.09.2022 **Kabul Tarihi:** 01.11.2022

ORCID IDs: D.K.0000-0002-1874-0109,B. S.0000-0002-2208-540X,E.P.Ş.0000-0003-2143-1435,A.I.G.0000-0002-9034-715X,E.K.0000-0002-1063-0714,K.K.0000-0003-1415-9103

Address for Correspondence / Yazışma Adresi: Kadri Karaer, MD, Pamukkale University Faculty of Medicine, Department of Medical Genetics, Denizli, Turkey E-mail:kadrikaraer@yahoo.com

doi:http://dx.doi.org/10.12996/gmj.2023.12

INTRODUCTION

The beta-globin gene (*HBB*) (MIM#141900; GenBank genomic reference sequence NG_000007.3), which encodes the beta chain of human adult hemoglobin, a tetrameric protein, is a gene consisting of 3 exons and 2 introns located in the 15.4 regions (11p15.4) of the short arm of chromosome 11 (1, 2). Mutations in the beta-globin gene cause β-thalassemia, sickle cell anemia, and abnormal hemoglobin disorders (2). β-thalassemia is the most common autosomal recessive monogenic disorder (3). Beta-thalassemia patients suffer from hemolytic anemia and pathological complications such as splenomegaly, skeletal abnormalities and growth retardation, resulting from the reduced synthesis of beta-globin chains on hemoglobin (4, 5, 6). Today, it is known that there are more than 400 β-thalassemia mutations worldwide (7). Most of the mutations are single nucleotide changes, insertions or deletions that lead to frameshift. β-thalassemia is rarely caused by large gene deletions (4).

The frequency of beta-thalassemia in the Turkish population is 2.1%. However, the frequency shows regional variations, and in some areas, it is as high as 13%. Regional differences in the variety and frequency of mutations stem from the migration pattern and ethnic background. Molecular studies on the *HBB* gene in Turkey indicated the presence of more than 40 different mutations associated with β-thalassemia. In Turkey, the most common beta-thalassemia mutations are IVS I-110 (G>A), IVS I-6 (T>C), IVS II-1 (G>A), IVS II-745 (C>G), Codon 8 (-AA), and IVS I-1 (G>A) (8, 9). These mutations constitute approximately 72% of all beta-thalassemia mutations seen in Turkey (10). Gaziantep, located in southeast Turkey, is an endemic region for hemoglobinopathies and beta-thalassemia. The frequency of consanguineous marriages and the high birth rate in the region cause children with beta thalassemia to be born more often than expected. Early prenatal diagnosis of beta thalassemia, determination of heterozygous carriers and determination of beta globin gene mutations are necessary for treatment projects. This study revealed the molecular range of mutations in the beta-globin chain in Southeastern Anatolia, which includes Gaziantep and its surrounding provinces.

PATIENTS and METHODS

In this study, 313 unrelated beta-thalassemia or hemoglobinopathy patients and carriers who applied to Dr Ersin Arslan Training and Research Hospital for β-thalassemia mutation study between 2016-2021 were evaluated. Of the 313 patients included in the study, 41% (127 patients) consisted of Syrian patients.

Ethics committee approval

Approval for the research was obtained from the Pamukkale University , Non-Invasive Clinical Research Ethics Committee (dated 27/10/2022 and numbered E-60116787-020-277286).

Genomic DNA extraction

For mutation analysis, genomic DNA isolation was performed from the peripheral venous blood sample collected in ethylenediaminetetraacetic acid (EDTA) tubes by using the “Maxwell RSC” DNA isolation kit (Promega/ USA) from the blood with an automated system (Maxwell RSC Promega/USA). Spectrophotometric measurements were made for the obtained DNAs (Nano-drop/USA). 10-50 ng/µl DNA was used for the study.

DNA sequencing of HBB gene and Molecular analysis

The exon, intron regions, promoter and 5' untranslated region (5'UTR) and 3' untranslated region (3'UTR) of the HBB gene were amplified by PCR in accordance with the protocol of the kit used (**GML AG, Wollerau / Switzerland**). Obtained amplicons were checked by considering band patterns on agarose gel. Nucleotide sequences of suitable amplicons were determined using the Sanger Sequence method (ABI 3130 Genetic Analyzer (Applied Biosystems) Grand Island, NY 14072, USA). The obtained results were analyzed using the SeqScape v2.6 software program. The resulting variants were evaluated using databases such as 1,000 Genomes, dbSNP, ClinVar, Human Gene Mutation Database (HGMD).

RESULTS

In this study, a total of 32 different pathological variants, including 25 beta-thalassemia and 7 Hb variants, were detected in 313 beta-thalassemia patients and carriers (Table 1). These patients were detected with heterozygous mutations in 171 (55%), compound heterozygous mutations in 53 (17%) and homozygous mutations in 89 (28%) patients. As a result of the study, the frequency of the top 10 most common beta-globin mutations in the Gaziantep population is as follows: IVS I-110 G>A (HBB: c.93-21G>A) 20.65%, Codon 39 (HBB: c.118C>T) 8.63%, IVS I-6 T>C (HBB: c.92+6T>C) 7.10%, IVS I-1 G>A (HBB: c.92+1G>A) 6.88%, IVS II-1 G>A (HBB: c.315+1G>A) 6.24%, Codon 6 (GAG>GTG) (Hb S) (HBB: c.20A>T) 4.52%, CAP +20 C>T (HBB: c.-31C>T) 4.52%, Codon 8 (-AA) (HBB: c.25_26del) 4.30%, -30 (T>A) (HBB: c.-80T>A) 4.09%, IVS II-745 C>G (HBB: c.316-106C>G) 3.87%. These 10 mutations, one of which is Hb S, account for approximately 70% of the allelic frequency of all mutations. Apart from Hb S, we detected six more abnormal Hb variants: Hb D-Punjab (HBB: c.364G>C) 1.94%, Hb Lucknow (HBB: c.26A>G) 1.51%, HbC (HBB: c.19G>A) 0.43%, Hb City of Hope (HBB: c.208G>A) 0.22%, Hb E-Saskatoon (HBB: c.67G>A) 0.22%, Hb Ernz. (HBB: c.371C>A) 0.22%. The mutations listed here and all other mutations found, along with their allele frequencies, are summarized in Table 1. In this study, six different beta globin gene mutations that were not previously reported in Turkey were identified. These mutations were HBB:c.90C>T, HBB: c.47G>A, HBB: c.93-22_95del, HBB:c.30dup, HBB: c.180G>A, HBB: c.316-30A>C . Four of these mutations (c.90C>T, c.47G>A, c.93-22_95del, c.30dup) were detected in Syrian patients.

We performed mutation studies on 313 patients (626 chromosomes); 186 patients (372 chromosomes) were Turkish, and 127 patients (254 chromosomes) were Syrian. The mutations and allele frequencies found in Turkish and Syrian patients are shown and compared in Table1.

Table1. Summary of the 32 identified mutations and their frequencies and types in HBB gene in Gaziantep Province, Turkey and Comparison of mutations and allele frequencies detected in Turkish and Syrian cases

HGVS Name	Variant Name	HbName	Type	Allele Frequency	Frequency (%)	Turks	Syrian
HBB: c.93-21G>A	IVS I-110 (G>A)		β+	96	20.65	66	30
HBB: c.118C>T	Codon 39 (CAG>TAG)		β0	40	8.63	17	23
HBB: c.92+6T>C	IVS I-6 (T>C)		β+	33	7.10	18	15
HBB: c.92+1G>A	IVS I-1 (G>A)		β0	32	6.88	15	17
HBB: c.315+1G>A	IVS II-1 (G>A)		β0	29	6.24	12	17
HBB: c.-31C>T	CAP +20 C>T		Neutral	21	4.52	7	14
HBB: c.25_26del	Codon 8 (-AA)		β0	20	4.30	12	18
HBB: c.-80T>A	-30 (T>A)		β+	19	4,09	13	6
HBB: c.316-106C>G	IVS II-745 (C>G)		β+	18	3.87	6	12
HBB: c.92+5G>C	IVS I-5 (G>C)		β0	18	3.87	7	11
HBB: c.17_18del	Codon 5 (-CT)		β0	18	3.87	4	14
HBB: c.135del	Codon 44 (-C)		β0	15	3,23	9	6
HBB: c.90C>T	Codon 29 (C>T)		β+	14	3.01	0	14
HBB: c.52A>T	Codon 17 (AAG>TAG)		β0	12	2.58	8	4
HBB: c.93-1G>C	IVS I-130 (G>C)		β0	6	1.29	2	4
HBB: c.251del	Codon 82/83 (-G)		β0	6	1.29	6	0
HBB: c.47G>A	Codon 15 (TGG>TAG)		β0	6	1,29	0	6
HBB: c.27dup	CD 8/9 (+G)		β0	5	1.08	3	2
HBB: c.316-3C>A	IVS II-848 (C>A)		β+	4	0.86	2	2
HBB: c.180G>A	Codon 59 (AAG>AAA)		Neutral	2	0.43	2	0
HBB: c.112del	Codon 36/37 (-T)		β0	2	0.43	1	1
HBB: c.93-22_95del	IVS I (3' end) (-25 bp)		β0	2	0.43	0	2
HBB: c.92+56G>A*				1	0.22	1	0
HBB: c.316-30A>C	IVS II-821 (A>C)			1	0.22	1	0
HBB:c.30dup				1	0.22	0	1
HBB: c.20A>T	Codon 6 (GAG>GTG)	HbS	Sickling	21	4.52	8	13
HBB: c.364G>C	Codon 121 (GAA>CAA)	Hb D-Punjab		9	1.94	8	1
HBB: c.26A>G	Codon 8 (AAG>AGG)	Hb Lucknow		7	1.51	6	1
HBB: c.19G>A	Codon 6 (GAG>AAG)	HbC		2	0.43	1	1
HBB: c.208G>A	Codon 69 (GGT>AGT)	Hb City of Hope	Silent Hb	1	0.22	1	0
HBB: c.67G>A	Codon 22 (GAA>AAA)	Hb E-Saskatoon		1	0.22	1	0
HBB: c.371C>A	Codon 123(ACC>AAC)	Hb Ernz		1	0.22	1	0

* New variation identified for the first time

DISCUSSION

Although it is a preventable disease with the detection of carriers, genetic counseling, and prenatal diagnosis, at least 365.000 thalassemia patients are born every year. Beta-thalassemia is a severe disease that shortens life expectancy and negatively affects the quality of life if the treatment is not carried out correctly. The disease is difficult to treat, and the cost is very high. Therefore, it is imperative to take protective measures (11, 12).

Hemoglobinopathies are among the most common inherited blood diseases in our country. For endemic reasons, thalassemia and other hemoglobinopathies are essential public health problems, especially in the south and west of Turkey. The high rate of consanguineous marriage (an average of 20.9%) and fertility is one of the crucial factors in increasing the number of affected individuals in our country (13, 14). There are approximately 1.300.000 thalassemia carriers and approximately 5,500 patients with defined thalassemia and other hemoglobinopathies in Turkey. Although the incidence of hemoglobinopathies in our country is 2.1%, it varies between 0.7% and 13% according to regions (8). Gaziantep is a province located in the southeast of Turkey, near the Mediterranean provinces of Adana and Hatay. It is a city where thalassemia is a critical health problem, receiving immigration from surrounding provinces and Syria. In addition to all these, the high birth rate and the frequency of consanguineous marriages in Gaziantep increase the probability of having a child with thalassemia higher than expected. Especially in regions where thalassemia is common, identification and screening of thalassemia mutations, public education, and genetic counseling are very important to minimize thalassemia (15, 16). For this reason, we looked into the mutations of the beta-globin gene in Gaziantep province in our study. The study included 313 unrelated patients with beta-thalassemia or hemoglobinopathy and their carriers.

Since Gaziantep is a province with a high density of Syrian immigrants, 41% of its patients were Syrians. Thirty-two different beta-globin mutations were identified in this study; 25 beta-thalassemia mutations and 7 hemoglobin variant mutations (Table1). While 12 of these mutations were more common, 15 were less frequent. The frequency of the HbS mutation we found is similar to the frequency in Turkey. (17). In addition, 6 of the mutations found were mutations not seen before in Turkey. Four of these six mutations were detected in Syrian patients.

We identified a new variations not previously reported; HBB: c.92+56G>A. The this intronic variant (c.92+56G>A) occurred in intron 1. This variants were not present in gnomAD (v.2.1.1) (18) and were absent from our in-house databases containing more than 1000 exomes. According to the criteria, this variant was of uncertain significance (VUS) because it only met the criteria PP4 (Patient's phenotype or family history is peculiar for a disease with a single genetic etiology) and PM2 (absent from population databases).

The most common mutation in our study was IVS 1-110 (G>A), accounting for 20.65% of all mutant alleles. IVS I-110 (G>A) has been reported as the most common mutation in our country in many previous studies. This mutation is reported at rates varying between 28.60-52.70% in various studies covering our different regions. İnce et al. reported that the most common β- thalassemia mutation in the Southeast region was IVS I-110 (27.8%). They reported that this was followed by IVS I-6 (11.1%) and Codon 8 (-AA) (11.1%), IVS II-1 (8.3%) and IVS II-745 (5.5%) mutations (19). Although IVS I-110 was the first mutation in our study, the allele frequency was lower than in other regions of Turkey. This may be due to the lower frequency of IVS I-110 mutations in Syrian patients (15.70%) (20). This seems to have affected the allele frequency of the IVS I-110 (G>A) mutation in Gaziantep. Other beta-thalassemia and abnormal hemoglobin mutations detected in our study were compared with studies conducted in other regions of Turkey and studies conducted in Syria (Table 2).

It is thought that the IVSI-110 mutation originated in Turkey and spread to other countries with the migration flow (21). Additionally, it has been suggested that the Codon 29 (C>T) mutation originated in Syria (22). The difference in the frequency of this mutations between the two patient groups, Turkish and Syrian, was remarkable in the study. For example, while the allele frequency of IVSI-110 mutation was 66% in Turkish patients, it was 30% in Syrian patients. While the Codon 29 (C>T) mutation was seen at a high rate (14%) in Syrian patients, it was not seen in Turks. In addition, the allele frequency of the -30 (T>A) mutation in Turkish patients was found to be approximately twice that of Syrian patients. The mutations and allele frequencies detected in Turkish and Syrian patients are given in Table1 comparatively.

Table 2. Distribution and frequency of β-thalassemia mutations and some abnormal hemoglobins according to regions in Turkey and Syrian.

Variation Name	This study (%)	Turkey (17) (%)	Central Anatolia (8) (%)	Aegean Region of Turkey (23) (%)	Thrace Region of Turkey (24) (%)	Adana (25) (%)	Ankara (26) (%)	Antalya (27) (%)	Hatay (28) (%)	Siirt (29) (%)	Syrian (20) (%)
IVS I-110 (G>A)	20.65	39.20	40.88	41.7	28.60	35.14	49	52.70	30.1	38.90	15.70
Codon 39 (CAG>TAG)	8.57	3.80	4.12	4.60	8.40	5.94	3.59	-	5.37	5.60	13.30
IVS I-6 (T>C)	7.10	9.50	10.33	6.60	5.10	7.67	3.56	14.4	17.2	1.85	4.80
IVS I-1 (G>A)	6.88	5.50	5.72	8.90	8.20	8.66	8.00	10.0	9.60	-	13.50
IVS II-1 (G>A)	6.24	5.40	8.08	7.20	1.00	6.43	5.90	8.20	2.15	11.10	9.10
CAP +20 C>T	4.52	-	-	-	-	-	-	-	2.15	-	0.30
Codon 8 (-AA)	4.30	6.10	3.39	7.70	4.10	9.15	7.60	2.40	6.44	-	5.70
-30 (T>A)	4,09	3.10	4.22	-	-	7.42	-	8.20	-	9.25	1.80
IVS II-745 (C>G)	3.87	4.60	6.20	8.60	7.10	2.22	1.44	2.70	3.22	1.85	1.50
IVS I-5 (G>C)	3.87	1.10	1.00	-	-	3.71	1.08	-	3.22	9.25	4.50
Codon 5 (-CT)	3.87	2.20	1.69	1.90	3.10	2.97	0.67	2.10	1.07	3.70	4.50
Codon 44 (-C)	3,23	1.30	-	1.30	4.10	4.95	3.23	2.40	2.15	7.40	1.50
Codon 29 (C>T)	3.01	-	-	-	-	-	-	-	-	-	-
Codon 17 (AAG>TAG)	2.58	-	-	-	-	-	-	-	-	-	-
IVS I-130 (G>C)	1.29	<0.10	-	-	-	-	-	0.60	-	-	0.90
Codon 82/83 (-G)	1.29	-	-	-	-	-	-	-	-	-	0.30
Codon 15 (TGG>TAG)	1,29	<0.10	-	-	1.00	-	-	-	-	3.70	-
IVS II-848 (C>A)	0.86	<0.10	3.00	-	-	-	0.27	0.60	1.10	-	0.90
Codon 8/9 (+G)	1.08	1.50	-	-	3.10	2.47	-	-	-	1.85	0.30
Codon 59 (AAG>AAA)	0.43	-	-	-	-	-	-	-	-	-	-
Codon 36/37 (-T)	0.43	<0.10	-	-	-	0.74	-	-	-	3.70	0.60
IVS I (3' end) (-25 bp)	0.43	-	-	-	-	-	-	-	-	-	-
c.92+56G>A	0.22	-	-	-	-	-	-	-	-	-	-
IVS II-821 (A>C)	0.22	-	-	-	-	-	-	-	-	-	-
c.30dup	0.22	-	-	-	-	-	-	-	-	-	-
HbS	4.52	4.60	-	-	-	6.40	-	-	-	1.00	-
Hb D-Punjab	1.94	<0.10	-	-	3.10	0.23	-	-	-	-	-
Hb Lucknow	1.51	-	-	-	-	-	-	-	-	-	-
HbC	0.43	-	-	-	-	-	-	-	-	-	-
Hb City of Hope	0.22	-	-	-	-	-	-	-	-	-	-
Hb E-Saskatoon	0.22	-	-	-	-	-	-	-	-	-	-
Hb Ernz	0.22	-	-	-	-	-	-	-	-	-	-

In conclusion, beta-thalassemia is an important public health problem for Turkey. The surest solution to this disease, which has no definitive cure, will be to prevent the disease from occurring. Detection and profiling of beta globin gene mutations is important for treatment projects. We determined the distributions and frequencies of beta-globin gene mutations in this retrospective study that included patients from Gaziantep and surrounding provinces. As a result, we detected a total of thirty-two different mutations, six of which were reported for the first time in Turkey and one new variation. With the publication of our study and similar studies, the beta globin gene mutation profile in regions where beta thalassemia is more common will be understood more clearly and treatment programs will be created.

Acknowledgments
We thank Bio.Ömer Şimşek for his contributions to the study. This study has not been presented in a scientific congress before and the abstract has not been published.

Conflict of interest
No conflict of interest was declared by the authors.

REFERENCES

1.Thein SL. Genetic modifiers of beta-thalassemia. Haematologica. 2005;90:649–660.
2. Birgens H, Ljung R. The Thalassemia Syndromes. Scand J Clin Lab Invest. 2007;67:11-26.
3. Wetherall DJ, Clegg JB. The thallassemia syndromes. 4th ed. Oxford, UK: Blackwell Scientific Publications; 2008.
4.Cao A, Galanello R. β-Thalassemia. Genet Med. 2010;12(2):61–76.
5.Gulbay G, Yesilada E, Aydogdu I, Ozgen U, Otlu G. Beta thalassemia mutations in Malatya. Journal of İnonu University Medical Faculty. 2009;16(4):209-212
6.Kazazian HH, Boehm C. Molecular basis and prenatal diagnosis of β-thalassemia. Blood 1988;72:1107-1116.
7.Thalassemia Research Network. Interactive database for hemoglobin variants (ITHANET). Ithagene database. Available from: http://ithanet.eu/db/ithagenes.
8.Altay Ç. The frequency and distribution pattern of β-thalassemia mutations in Turkey. Türk J Haematol. 2002;19:309-315.
9. C¸Çürük MA, Yalın E, Aksoy K. Prevention of hemoglobinopathies in Turkey. Thalassemia Rep. 2013;3(1):1–4.
10. Tadmouri GO, Başak AN. β- Thalassemia in Turkey: A review of the clinical, epidemiological, molecular and evolutionary aspects. Hemoglobin 2001; 25(2):227-34.

11. Honig GR. Hemoglobin disorders. In: Behrman RE, Kliegman RM, Jenson BE, eds. Nelson Textbook of Pediatrics, ed. 16. Philadelphia: WB Saunders; 2000:1478–1488.

12. Ho WL, Lin KH, Wang JD, Hwang JS, Chung CW, Lin DT, Jou ST, Lu MY, Chern PS. Financial burden of national health insurance for treatingpatients with transfusion-dependent thalassemia in Taiwan. Bone Marrow Transplant. 2006;37(6):569–574.

13.TÜİK Family Structure Research, 2006.

14. Guler E, Garipardic M, Dalkiran T, Davutoglu M. Premarital screening test results for b-thalassemia and sickle cell anemia trait in east Mediterranean region of Turkey. Pediatr Hematol Oncol. 2010;27:608–613.

15. Weatherall DJ, Clegg JB. Inherited hemoglobin disorders: an increasing global health problem.Bull WHO. 2001;79:704–712.

16. Report of a WHO meeting: Primary health care approaches for prevention and control of congenital and genetic disorders, Cairo, Egypt;.6–8 Dec 1999.

17. Başak AN. The molecular pathology of β-thalassemia in Turkey: the Boğaziçi University experience. Hemoglobin 2007; 31: 233-241.

18.1000 Genomes Project Consortium. 2011

19. Ince HH, Ayyildiz O, Kalkanli S, Batun S, Muftuoglu E. Molecular basis of beta-thalassemia mutations in Diyarbakir in the southeastern region of Turkey. Hemoglobin 2003; 27 (4):275-278.

20. Jarjour RA., Murad H, Moasses F et al. Molecular update of β-thalassemia mutations in the Syrian population: Identification of rare β- thalassemia mutations, Hemoglobin. 2014; 38(4): 272-276.

21. Zahed L. The spectrum of b-thalassemia mutations in the Arab populations. J Biomed Biotechnol. 2001;1(3):129–132.

22. Murad H, Moasses F, Dabboul A, Mukhalalatay Y, Bakoor AO Achkar WA, Jarjour RA. Geographical distribution of b-globin gene mutations in Syria. Hematology. 2018; 23(9):697–704.

23. İrken G, Oren H, Undar B, Duman M, Gulen H, Ucar C, Sanlı N. Analysis of thalassemia syndromes and abnormal hemoglobins in patients from the Aegean region of Turkey. Turk J Pediatr 2002;44:21-4.

24. Yalcıntepe S. Beta globin mutations in beta thalassemia minor patients in Genetics Diagnosis Center of Trakya University Faculty of Medicine. Ege Journal of Medicine 2019;58 (4):330-335

25. Guvenc B, Canataroglu A, Unsal C, Yıldız SM, Turhan FT, Bozdogan ST, Dincer S, Erkman H. β-Thalassemia mutations and hemoglobinopathies in Adana, Turkey: results from a single center study Arch Med Sci 2012;8(3):411–414.

26. Beksac MS, Gumruk F, Gurgey A, Cakar N, Mumusoglu S, Ozyuncu O, Altay C. Prenatal diagnosis of hemoglobinopathies in Hacettepe University. Pediatr Hematol Oncol. 2011;28(1):51–55.

27. Kurtoglu A, Karakus V, Erkal O, Kurtoglu E. β-Thalassemia gene mutations in Antalya, Turkey: results from a single centre study. Hemoglobin. 2016;40(6):392-395,

28. Aldemir O, Izmirli M, Kaya H. The Spectrum of β- thalassemia mutations in Hatay, Turkey: Reporting three new mutations. Hemoglobin. 2014;38(5);325-328.

29.Yılmaz S. The Spectrum of β-thalassemia mutations in Siirt province, southeastern Turkey, Hemoglobin. 2019;43(3):174-181.