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Evaluation of Beta Globin Gene Mutations in Mersin, Turkey: A Single Center Experience

Mersin, Türkiye'de Beta Globin Gen Mutasyonlarının Değerlendirilmesi: Tek Merkezli Bir Deneyim

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Öz

Beta-talasemi, β-globin zincir sentezinin eksikliği veya yokluğundan kaynaklanan otozomal resesif kalıtım gösteren, dünya çapında en yaygın tek gen hastalığıdır. Çalışmamızın amacı Mersin ilinde beta talasemi tanısı alan hastaların mutasyon tiplerini ve sıklığını değerlendirmektir. 2017-2019 yılları arasında Mersin Üniversitesi Tıp Fakültesi hastanesinde hemoglobinopati taraması yapılan 292 hastanın klinik verileri retrospektif olarak incelendi. HBB geninde mutasyon bulunan 292 hastanın %55,5'inde (n=162) beta talasemi vardı. Beta talasemili hastalarda, 4'ü anormal Hb varyantı olmak üzere 32 farklı mutasyon ve bu hastaların 22'sinde 12 farklı bileşik heterozigot β-tal mutasyonu tespit edildi. En sık görülen alel %25.2 frekansla c.93-21G>A idi. En yaygın bileşik varyasyon HBB:c.*233G>C/HBB:c.92+6T>C/A, (%27,4) idi. Türkiye'de daha önce bildirilmemiş olan HBB:c.92+5G>A mutasyonunu tespit ettik. Beta-talasemi mutasyonlarının tipleri ve sıklıkları coğrafi bölgeler arasında farklılık göstermektedir. Bu çalışmada, beta-talasemi mutasyonlarının yaygınlığını moleküler düzeyde incelemiş ve DNA dizi analizi ile tanımlanamayan mutasyonların tespit oranını artırmıştır. Beta-talasemide yeni mutasyonların tanımlanması genetik danışmanlık, prenatal tanı, tarama programları ve literatür için yararlıdır.

Anahtar Kelimeler: Beta-talasemi, HBB geni, Mersin, Mutasyon, Türkiye

Abstract

Beta-thalassemia is the most common single gene disease worldwide with an autosomal recessive inheritance caused by the deficiency or absence of β-globin chain synthesis. The aim of our study is to evaluate the types and frequencies of mutations in patients diagnosed with beta thalassemia in the province of Mersin. Clinical data of 292 patients who underwent hemoglobinopathy screening at Mersin University Faculty of Medicine Hospital between 2017 and 2019 were retrospectively analysed. Of the 292 patients with mutations in the *HBB* gene, 55.5% (n=162) had beta thalassemia. In patients with beta thalassemia, 32 different mutations, including 4 abnormal Hb variants, and 12 different compound heterozygous β-tal mutations were detected in 22 of these patients. The most commonly seen allele was c.93-21G>A with a frequency of 25.2%. The most common compound variation was HBB:c.*233G>C/HBB:c.92+6T>C/A, (27.4%). We detected the mutation HBB:c.92+5G>A, which has not been previously reported in Turkey. The types and frequencies of beta-thalassemia mutations vary among geographic regions. This study examined the prevalence of beta-thalassemia mutations at the molecular level and enhanced the detection rate of unidentified mutations by DNA sequence analysis. Identification of new mutations in beta-thalassemia is useful for genetic counseling, prenatal diagnostic, screening programs, and literature.

Keywords: Beta-thalassemia, HBB gene, Mersin, Mutation, Turkey

Introduction

Beta-thalassemia is a blood disorder caused by the absence (β0) or deficiency (β+) of the β globin chains in the two β globin chains and two alpha globin chains (alpha2beta2) that make up the hemoglobin (Hb) tetramer. One of the most common autosomal recessive disorders in the world, β-thalassemia, is highly prevalence in Cyprus (14%), Sardinia (12%), and Southeast Asia (1). While β-thalassemia carriage is seen at a rate of 5.1% worldwide, this rate was shown to be between 0.7% and 13.1% in study including 16 provinces in the Mediterranean, Aegean and Marmara regions in our country (2). Demographic events such as high birth rate, migration and high rates of consanguineous marriage have led to increased prevalence of β-thalassemia in some regions of Turkey.

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The hemoglobin β-globin (HBB) gene situated in the short arm of chromosome 11 is approximately 1.6 Kb long, encodes 146 amino acids, contains 3 exons, 2 introns and 5' and 3' untranslated regions (UTRs), and is regulated by the 5' promoter region (3). A variety of molecular lesions, from point mutations to tiny deletions restricted to HBB to massive deletions of the complete β-globin cluster, can cause hemoglobin's β-globin chains to be down-regulated. Deletions cause α-thalassemia, while most mutations causing β-thalassemia are point mutations (4). Among the point mutations affecting the expression of the β-globin gene are mutations causing defective β-globin gene transcription (promoter and 5' UTR mutations), mutations affecting messenger RNA (mRNA) synthesis (splice junction and consensus sequence mutations, polyadenylation and other 3' UTR mutations), and mutations causing abnormal mRNA translation (start codon mutations, nonsense and frameshift mutations) (5).

β0-thalassemias, defined by the complete absence of β chain synthesis, result from deletion, nonsense, frameshift, start codon, and splicing

mutations, particularly at the splice junction. The polyadenylation signal and 5' or 3' UTR, mutations in the promoter region (CACCC or TATA box), or splicing abnormalities lead to a decrease in the synthesis of β chains, causing β+-thalassemias. There are three categories of β-thalassemia mutations: severe, mild, and silent. The classification is based on the degree of reduction in β chain synthesis1. So far, 486 β-thalassemia mutations have been reported in the IthaGenes database (6).

In the molecular analysis of β-thalassemia patients carried out in Turkey, it has been demonstrated that more than 40 different β-globin gene mutation types responsible for the disease represent 90% of the total mutations (7). This diversity reflects the heterogeneity of the Turkish population. Worldwide, in high-frequency carrier populations, there are a few common mutations specific to a particular region, as well as a varying number of rare mutations (8). It is helpful to identify the relationship between genotype and phenotype as well as to detect and categorize β-thalassemia mutations, which are very common in our nation. The objective of our study was to to determine the spectrum of β-globin gene mutations and frequency of patients diagnosed with β-thalassemia in Mersin province and to identify infrequent and rare β-thalassemia mutations. Knowing the spectrum of prevalent and rare mutations will help determine responsible mutations accurately and in a timely manner.

Material and Method

In this study, Mersin University Medical Genetics Department Molecular Genetics Laboratory 292 patients who were referred from other clinics with preliminary diagnoses of anemia and β-thalassemia and whose mutation was detected as a result of *HBB* gene sequence analysis were retrospectively evaluated. These patients underwent *HBB* gene sequence analysis as part of the Hemoglobinopathy evaluation. Complete blood count parameters and HbA2 level at the time of diagnosis and demographic characteristics of these cases were obtained from file scans and computer data systems. Ethics committee approval for this study was obtained from Mersin University Local Ethics Committee with decision no: 650 dated 2022.

Reference ranges for HbA2 in normal subjects are usually between 2.0% and 3.3%, whereas HbA2 levels in β-thalassemia carriers are usually above 3.5%. HbA2 values between 3.1% and 3.5% are considered borderline. Mean corpuscular volume (MCV) <80 fL and/or mean corpuscular hemoglobin (MCH) <27 pg and HbA2 level >3.5% are interpreted in favor of thalassemia carrier status. For HbF values, <1% was considered normal, 1-5% slightly high and >5% high (9).

Before the β-thalassemia tests were performed, a consent form was acquired from all patients and genetic counseling was provided. Current classifications of the detected mutations were checked utilizing the Franklin by Genoox (10) and ClinVar (11) databases. Mutations are classified according to the ACMG (American College of Medical Genetics) Guidelines as pathogenic, likely pathogenic, variants of uncertain significance (VUS), likely benign, and benign[12]. Age and gender data of the patients with β-thalassemia were obtained from the hospital and department archives (12).

Statistical analysis

Results were presented as mean ± standard deviation. Categorical variables were presented as numbers and percentages.

Results

We evaluated the findings of 292 people who were referred to our laboratory from other clinics with preliminary diagnoses of anemia and β-thalassemia and underwent MCI gene screening. Since the incidence of β-thalassemia is high in our region, β-thalassemia was screened in the patient group in which anemia was investigated. Of 292 patients with HBB gene mutation, 130 (44.5%) had sickle cell anaemia alone, 27 had compound sickle cell anemia (9.2%) and 135 had beta-thalassemia (46.3%). This group with sickle cell anemia and compound sickle cell anemia were excluded from the statistical analysis in research. The results of 135 patients with β-thalassemia mutation were evaluated. In 135 patients who were diagnosed with anemia and underwent HBB gene mutation analysis, we detected a total of 32 different HBB mutations, 28 of which were β-globin gene mutations, together with 4 abnormal Hb variants, when HbS compound heterozygotes were excluded. In this study, a different β-globin gene mutation (HBB:c.92+5G>A), which has not been reported before in Turkey, was identified.

Ninety-nine (73.3%) of these individuals had heterozygous mutations, 22 (16.3%) had compound heterozygous mutations, and 14 (10.4%) had homozygous mutations. Of the 135 patients with β-globin gene mutation, 89 (66%) were under 18 years of age, and 46 (34%) were 18 years of age or older. The youngest patient with β-thalassemia was 1 year old, the oldest was 71 years old, and the average age of all patients was determined to be 16.1±12.4 years.

In this study, 90 cases with HbA2 elevation (>3.5%) were detected, and 86 of 90 cases had MCV <80 and/or MCH <27. Three of the four cases with high HbA2 had MCV>80 and MCH>27. However, in 1 case, MCV was high and MCH was normal. In 80 cases, HbF was >2%. Borderline HbA2 level was found in 6 cases. Of the 90 cases with elevated HbA2

Table 1. Distribution and frequency of beta globin gene mutations and some abnormal hemoglobins in Mersin, Turkey

HGVS Name	Variation Name	HbName	rs ID	Clinical Significance	Homozygous/ Heterozygous (n/n)	Frequency n (%)	Type	Mutation Type	Consequence
HBB:c.93-21G>A	IVS-I-110 G>A		rs35004220	Pathogenic	6/42	48 (25.2)	β+	SNV	Intronic
HBB:c.*233G>C	TTS +99 G>C 3′ UTR +101 (G>C)		rs12788013	Benign	2/42	44 (23.1)	benign polymorphism	SNV	3'UTR
HBB:c.92+6T>C	IVS-I-6 (T>C)		rs35724775	Pathogenic	3/8	11 (5.6)	β+	SNV	Intronic
HBB:c.92+1G>A	IVS I-1 G>A		rs33971440	Pathogenic	-/9	9 (4.7)	β^0	SNV	Splice-D/A
HBB:c.135del	Codon 44 (-C)		rs80356820	Pathogenic	-/9	9 (4.7)	β^0	Deletion	Frameshift
HBB:c.25-26delAA	Codon8 (-AA)		rs35497102	Pathogenic	1/7	8 (4.2)	β^0	Deletion	Frameshift
HBB:c.364G>C	Codon121 GAA>CAA	variant Hb D-LA	rs33946267	Likely Pathogenic	-/8	8 (4.2)	variant Hb D-LA	SNV	Missense
HBB:c.315+1G>A	IVS-II-1		rs33945777	Pathogenic	-/5	5 (2.7)	β^0	SNV	Splice-D/A
HBB:c.-80T>A	-30 (T>A)		rs33980857	Pathogenic	2/3	5 (2.7)	β+	SNV	5'UTR
HBB:c.68_74del	Codon 22-24 (-7 bp)		rs281864898	Pathogenic	-/4	4 (2.4)	β^0	Deletion	Frameshift
HBB:c.316-106C>G	IVS II-745 C>G		rs34690599	Pathogenic	2/2	4 (2.4)	β+	SNV	Intronic
HBB:c.118C>T	Codon 39 (C>T)		rs11549407	Pathogenic	-/4	4 (2.4)	β^0	SNV	Stop gain
HBB:c.364G>A	Codon121GAA >AAA	variant Hb O-Arab	rs33946267	Pathogenic	-/3	3 (1.6)	variant Hb O-Arab	SNV	Missense
HBB:c.17-18del	Codon 5(-CT)		rs34889882	Pathogenic	-/3	3 (1.6)	β^0	SNV	Missense
HBB:c.92+5G>C	IVS1-5G>C		rs33915217	Pathogenic	1/1	2 (1)	β^0	SNV	Intronic
HBB:c.92+5G>A*	IVS I-5 G>A		rs33915217	Likely Pathogenic	-/1	1 (0.5)	β+	SNV	Intronic
HBB:c.251delG	Codon 82/83 (-G)		rs193922555	Pathogenic	1/1	2 (1)	β^0	Deletion	Frameshift
HBB:c.82G>T	Codon 27 (G>T)		rs35424040	Pathogenic	-/2	2 (1)	β+	SNV	Missense
HBB:c.-31C>T	IVS II-745 C>G		rs63750628	Likely Benign	-/2	2 (1)	β+	SNV	5'UTR
HBB:c.112delT	Codon 36/37 (-T)		rs63750532	Pathogenic	-/2	2 (1)	β^0	Deletion	Frameshift
HBB:c.79G>A	Codon 26 (G>A)	Hb E	rs33950507	Pathogenic	-/2	2 (1)	Hb E	SNV	Missense
HBB:c.135del	Codon 44 (–C)		rs80356820	Pathogenic	-/2	2 (1)	β^0	Deletion	Frameshift
HBB:c.180G>A	Codon 59 (AAG>AAA)		rs34621955	Likely Benign	1/-	1 (0.5)	Neutral	SNV	Synonymous
HBB:c.*110T>C	Poly A (T>C) AATAAA>AACAAA		rs33978907	Pathogenic	-/1	1 (0.5)	β+	SNV	3'UTR
HBB:c.-78A>C	-28 (A>C)		rs33931746	Pathogenic	-/1	1 (0.5)	β+	SNV	5'UTR
HBB:c.-151C>T	-101 C>T		rs63751208	Pathogenic	-/1	1 (0.5)	β+	SNV	5'UTR
HBB:c.27dupG	Codon 8/9 (+G)		rs35699606	Pathogenic	-/1	1 (0.5)	β^0	Duplication	Stop gain
HBB:c.151A>T	Codon 50 ACT>TCT	Hb Zürich-Langstrasse	rs63750336	VUS	-/1	1 (0.5)	Hb Zürich-Langstrasse	SNV	Missense
HBB:c.47G>A	Codon 15 TGG>TAG		rs63750783	Pathogenic	-/1	1 (0.5)	β^0	SNV	Stop gain
HBB:c.315+260A>G	IVS II-260 A>G		rs111415391	Likely Benign	-/1	1 (0.5)	β?	SNV	Intronic
HBB:c.315+74T/G	IVS II-74 T>G		rs7480526	Benign	-/1	1 (0.5)	benign polymorphism	SNV	Intronic
**HBB:c.96T>C	CAP + 1570 T>C		rs34029390	Benign	-/1	1 (0.5)	β?	SNV	3'UTR
HBB:c.112delT	Codon 36/37 (-T)		rs63750532	Pathogenic	-/2	2 (1)	β^0	Deletion	Frameshift

HGVS: Human Genome Variation Society, ** New variation identified for the first time

(>3.5%), 67 were under 18 years of age, while 23 cases were 18 and over.

The patients were divided into two main groups according to their age: the pediatric group 15 years or younger (n=79; 58.5%) and the adult group older than 15 years (n=56; 41.5%).

The most prevalent allele frequency was found as 25.2% for HBB:c.93-21G>A, 23.1% for HBB:c.*233G>C, 5.6% for HBB:c.92+6T>C, 4.7% for HBB:c.92+1G>A and 4.7% for HBB:c.135del. These five mutations accounted for 63.3% of all mutations in the patients. The distribution and allele

prevalence of all noted mutations are provided in Table 1.

When the HbS compound was excluded, compound heterozygous mutation were detected in 12 different genotypes in 22 patients. The most prevalent of these mutations were compound heterozygous genotypes HBB:c.*233G>C/HBB:c.92+6T>C/A, which were found in six cases (27.4%). Others that are common are HBB:c.25-26delAA / HBB:c.*233G>C (18.2%); HBB:c.*233G>C/HBB:c.316-106C>G/HBB:c.-31C>T (9.2%); HBB:c.135delC/HBB:c.*233G>C (9,2%) and HBB:c.93-21G>A/HBB:c.*233G>C (4.5%), respectively. The genotypes of patients exhibiting compound heterozygous mutations are presented in Table 2.

Table 2. The distribution of compound heterozygous β-thalassemia mutations, in Mersin, Turkey

Variations	Mutation Type	n	%
HBB:c.92+6T>C/A(rs35724775), HBB:c.*233G>C (rs12788013)	β+, benign polymorphism	6	27.4
HBB:c.25-26delAA (rs35497102), HBB:c.*233G>C (rs12788013)	β0, benign polymorphism	4	18,2
HBB:c.*233G>C (rs12788013), HBB:c.316-106C>G(rs34690599), HBB:c.-31C>T (rs63750628)	benign polymorphism, β+, β+	2	9.2
HBB:c.135delC (rs80356820), HBB:c.*233G>C (rs12788013)	β0, benign polymorphism	2	9.2
HBB:c.93-21G>A (rs35004220), HBB:c.*233G>C (rs12788013)	β+, benign polymorphism	1	4.5
HBB:c.93-21G>A(rs35004220), HBB:c.135delC (rs80356820)	β+, β0	1	4.5
HBB:c.-80T>A (rs33980857), HBB:c.93-21G>A(rs35004220), HBB:c.*233G>C (rs12788013)	β+, β+, benign polymorphism	1	4.5
HBB:c.92+5G>A(rs33915217), HBB:c.151A>T(rs63750336)	β+, β0	1	4.5
HBB:c.92+6T>C/A(rs35724775), HBB:c.*233G>C (rs12788013), HBB:c.20A>T (rs334)	β+, benign polymorphism, βS	1	4.5
HBB:c.-80T>A (rs33980857), HBB:c.*233G>C (rs12788013)	β+, benign polymorphism	1	4.5
HBB:c.92+1G>A(rs33971440), HBB:c.93-21G>A (rs35004220)	β0, β+	1	4.5
HBB:c.93-21G>A (rs35004220), HBB:c.*110T>C (rs33978907)	β+, β+	1	4.5
TOTAL		22	100

HBB: c.*233G>C 42 of 44 cases with variation were heterozygous, and 23 of them carried only this variation. Of the 23 patients who were only HBB: c.*233G>C heterozygous, MCV<80 fL in 18 patients and MCV>80fL in 4 patients. Data could not be reached in 1 patient. While MCH was <27 pg in 16 patients with only HBB: c.*233G>C heterozygote, it was MCH>27 pg in 4 patients. In 3 patients, data could not be reached. Only in cases with HBB: c.*233G>C heterozygous, HbA2 was <3.5% in 15 cases, while in 1 patient, HbA2 was >3.5%. Data could not be obtained for 6 patients. Two cases were homozygous for the HBB:c.*233G>C variant. Homozygous cases and 19 heterozygous cases were found to be compound with other HBB gene mutations.

Discussion

Hemoglobin disorders that affect the structure or function of hemoglobin are among the most common monogenic disorders in the world, and there are approximately 270 million thalassemia carriers worldwide (13). Thalassemia syndromes, including α-thalassemia, β-thalassemia, and hemoglobin-E disease, lead to a critical public health problem due to their high prevalence considering. Mersin province receives a lot of immigration due to its geographical location. Due to the high thalassemia carrier rate in Mersin, determining the mutation diversity and common mutations is very important in terms of informing the public, genetic counseling and public health. In short, it is important to identify regional mutations to create effective treatment and prevention programs.

In this study we detected 32 different β-globin gene mutations, 4 of which are abnormal Hb variants; HbE, Hb D-Los Angeles (Hb D-LA), Hb O-Arabic and Hb Zurich-Langstrasse (Table1).

HBB:c.93-21G>A mutation is reported at varying rates in numerous studies including different regions In Turkey, the HBB:c.93-21G>A mutation is seen with a frequency of nearly 52.3% in Central Anatolia Region Turkey (7). However, in the eastern and south-eastern Anatolian regions of Turkey, the frequency of the HBB:c.93-21G>A mutation has been shown to be 25-30% (14). Ince et al. reported the frequency of HBB:c.93-21G>A mutation as 27.8% in a study conducted in Diyarbakır province located in the southeastern region of Turkey (15). Beksaç et al. found it to be 49.01% in a study conducted in Ankara province located in the Central Anatolia region (16). In our study, as in other geographical regions of Turkey, the most frequent mutation is HBB:c.93-21G>A and constitutes 25.2% of all mutant alleles which was consistent with other studies.

The HBB:c.*233G>C variant has been temporarily included in the HbVar database based on a study conducted on Palestinians with β-

thalassemia disease or carrier status in the Gaza Strip (17). In their the study by of Smith et al. the allele frequency of the HBB:c.*233G>C variant was found to be 9.1% and they showed that this variant is a common benign polymorphism (18). In a study conducted in Malaysia, HBB: c.*233G>C (3' UTR+101 G>C) was reported to have the same protective role (19). In our study, we detected compound heterozygotes in 18 patients (13.3%). HBB: c.*233G>C (3' UTR+101 G>C) variation was not shown in the study conducted by Tadmuri et al. in our country (20). In our study, the HBB: c.*233G>C variant was the second most frequent variant with a frequency of 23.1% and was the most frequent compound heterozygous variant. HBB:c.*233G>C variation heterozygous have average HbA2 level of 23 cases was found to be 2.6 (±0.4) and HbF was 6.1 (±7.7). Parameters determined by whole blood count analysis were calculated with red the cell distribution width (RDW) index (MCVxRDW/RBC) formula. RDW index of <220 was interpreted in favor of thalassemia carrier status. The RDW index was <220 in 11 of 20 cases with the heterozygous HBB:c.*233G>C variation. In 2 cases with heterozygous HBB:c.*233G>C and heterozygous HBB:c.25-26delAA, RDW index was <220. In cases with heterozygous HBB:c.25-26delAA mutation, the RDW index was <220. In cases with homozygous HBB:c.92+6T>C mutation, the RDW index was >220. The RDW index was <220 in the case with homozygous HBB:c.92+6T>C and homozygous HBB:c.*233G>C and also in the case with heterozygous HBB:c.92+6T>C and heterozygous HBB:c.*233G>C. HBB: c.*233 G>C mutation has been reported as benign in previous studies. Therefore, these results show us that more detailed information about HBB:c.*233G>C is needed.

The reason why HBB:c.*233G>C was detected more in our study group may be because we used the DNA sequencing method. Furthermore, it may not have been detected in other groups due to differences in study methods or may not have been reported by laboratories because the HBB:c.*233G>C mutation is considered benign. It would be appropriate to evaluate this variation in more detail with clinical and laboratory data of patients in future studies. Also, any nucleotide changes (mutations or polymorphisms) found must be reported to the Hemoglobin Variant Database.

Turkey is one of them hotspots for variations in the globin genes. HBB:c.315+74T >G is one of the most frequently characterized polymorphic regions on the β-globin gene. Among the healthy controls, HBB:c.315+74T >G was commonly found in multiple investigations and was recognized as a polymorphism (21). Hocaoğlu et al. reported in their study that a HBB:c.315+74T >G homozygous change may be sufficient to cause the β-thalassemia carrier phenotype (21). It is thought that functional characterization of variants and elucidating their roles in the progression of the disease may provide a great advantage in designing appropriate treatment.

According to the Turkish registry study, HBB:c.92+6T>C (7.5%), which is linked to moderate β^{+}-thalassemia mutations, was the third most prevalent mutation in pediatric β-thalassemia major patients in Turkey (22). In our study, we detected the third most common HBB:c.92+6T>C mutation (5.6%), consistent with this study.

The HBB:c.92+1G>A mutation, which is very common in the Aegean and Marmara regions of Turkey, is one among the four most common mutations in the Mediterranean and Middle Eastern countries (23). Throughout the European countries, the HBB:c.92+1G>A mutation is observed with a frequency of 52.9% in the Czech Republic (24), and approximately 30% in Spain (25). Karaer et al. and Güvenç et al. reported that this variation 6.83% and 8.66%, respectively (26,27). The 4th most common mutation in our study was HBB:c.92+1G>A and the allele frequency was 4.7%.

In studies conducted in our country, HBB:c.135del mutation was found to be 3.23% by Karaer et al. and 4.7% in our study (26). While Güvenç et al. reported that HBB:c.25-26delAA mutation was 9.1% in Adana (27), Karaer et al. reported 4.9% in Southeastern Turkey (26). In our study, we reported the allele frequency of the HBB:c.25-26delAA mutation to be 4.2%.

In a study conducted in Syria, HBB:c.92+5G>C (4.1%) was reported as one of the common β-globin gene mutations (28). In our study, we found HBB:c.92+5G>C mutation (1%).

In our study, the β-globin gene mutation HBB: c.92+5G>A, which has not been previously reported in Turkey, was detected with a rate of 0.5%. Jarjour et al. reported the frequency of HBB:c.92+5G>A mutation as 3.2% in 189 Syrian β-thalassemia patients and carriers (29). There may also be co-inheritance of different mutations involving the β-thalassemia gene, which include gene structural variants that cause genotypic and phenotypic symptoms such as HbS-β-thalassemia (sickle-β-thalassemia) or HBB:c.79G>A-β-thalassemia (HbE-β-thalassemia or E-β-thalassemia). In our study, the group with HbS was not evaluated because it was excluded from this study. In several Asian nations, hemoglobin E (HbE), a structural hemoglobin variation, is prevalent and occurs at high frequency. HbE; the β chain is produced at a lower rate, causing a mild β thalassemia phenotype. While HbE by itself does not result in major clinical issues, it interacts with different types of α and β thalassemia to produce a variety of clinical syndromes that are co-inherited with β-thalassemia and range in severity. This condition, called HbE β-thalassemia, is the most common form of severe β-thalassemia in Asia and accounts for approximately 50% of clinically

severe β-thalassemia disorders worldwide (30). Güvenç et al. was detected the frequency of HbE to be 0.2% in Adana (27). In our study, we detected heterozygous HbE variation in 2 patients (1.1%). Although HbE is the second most common hemoglobinopathy globally, HBB:c.364G>C (HbD-Los Angeles) has been reported as the second most common abnormal hemoglobin among the Eti-Turks in Turkey, with a prevalence of 0.16-2.4% (31). While HbD-Los Angeles is more common in the Eastern Mediterranean and Southeastern Anatolia, is more common Hb E in the Western Mediterranean region and HBB:c.364G>A (Hb O Arab) is more common in the Thrace region (32). According to the researches, while HbD-Los Angeles was determined as 0.2% in Turkey, this abnormal hemoglobin is in the first place with a frequency of 57.8% in Denizli province (31,33). In our study, the frequency of HbD-Los Angeles was 3.6%. In the presence of Hb O-Arab, a clinical picture ranging from normal phenotype to mild anemia is observed. Hb O-Arab heterozygous variation does not show any clinical symptoms, whereas Hb O-Arab homozygous show mild hemolysis and mild splenomegaly. Co-inheritance of the hemoglobin O-Arab mutation in sickle cell disease patients causes a severe hemoglobinopathy with clinical and hematological features similar to sickle cell disease and may require frequent blood transfusions (33-36). Canatan et al. reported the HbO-Arab frequency as 0.4% in 2016 (32). In our study, this rate was 1.5%. Therefore, close follow-up of these patients in the clinic is important and people carrying this mutation should be recommended to undergo screening programs before marriage. Given that Turkish people have a wide spectrum of thalassemia syndromes and abnormal hemoglobin, all these results increase the importance of detecting Hb variant status.

HBB:c.315+1G>A is one of the mutations frequently detected in Arab countries, particularly in Erbil-Northern Iraq and Iran, Saudi Arabia, and Kuwait (29,37,38). Karaer et al. reported the prevalence of HBB:c.315+1G>A between 1-11% in an article in which they compared it with studies conducted in other regions of Turkey and Syria (26). In the current research, we observed the HBB:c.315+1G>A mutation as heterozygous in 6 patients (3.1%). The 5 most frequently inherited compound heterozygous variations in our research were HBB:c.*233G>C/HBB:c.92+6T>C/A (27.4%); HBB:c.25-26delAA/HBB:c.*233G>C (18.2%); HBB:c.*233G>C/HBB:c.316-106C>G/HBB:c.-31C>T (9.2%); HBB:c.135delC/HBB:c.*233G>C (9.2%); HBB:c.93-21G>A/HBB:c.*233G>C (4.5%).

In a study conducted in 1998, a total of 42 mutations were determined in Turkey (20). We identified a total of 32 different β globin gene mutations (4 of them are abnormal Hb variants), and 12 compound heterozygous variants. The molecular heterogeneity of Mersin province may be explained by its geographical location. In the study Abbasali et al. reported that the phenotype of heterozygous cases with the HBB: c*96T>C mutation was compatible with the silent carrier of β-thalassemia (39). In our study, we noted the HBB:c*96T>C mutation as heterozygous in one patient. Although this change is quite rare, it is a clinically significant β-globin gene mutation. This mutation, which can be categorized as a silent β-thalassemia defect and contributes to a decrease in β-globin chain synthesis, results in non-transfusion-dependent β thalassemia in compound heterozygosity. Even if one of the parents has a silent mutation such as the heterozygous HBB:c*96T>C gene mutation, screening the other one of the parents β-globin gene mutation carrier is essential in terms of the compound heterozygosity of future generations (40).

In our region, where the carrier rate is high, it is very important to first evaluate the complete blood count and perform hemoglobin electrophoresis to detect patients and carrier individuals. It is necessary to make a differential diagnosis of β thalassemia from diseases such as iron deficiency anemia, alpha thalassemia trait and anemia of chronic disease. In this study, the use of DNA sequencing was increased the detection percentage of undetectable mutations.

A multi-center study conducted by the Turkish Pediatric Hematology Association in 2018 demonstrated that the count of families with more than one affected child was high, the consanguineous marriage of the parents was high and, preventive measures were not implemented even in families where the risk. As an outcome of this the research carried out in 2018, Aydinok et al. express that the majority of families did not undergo premarital screening, and that prenatal diagnosis was either not offered to families at risk or was not accepted by the families (22).

Conclusion

The main purpose of molecular diagnosis in β-thalassemia is to identify mutation profiles before marriage, and it is thought to be useful for prenatal molecular diagnostic tests and genetic counselling. Knowing the spectrum of prevalent and rare mutations in the national population is of great significance in terms of the identification of new mutations that causing a clinically significant disease, genetic counselling, informing the society and prenatal diagnosis, together with effectively controlling the disease.

Limitations

In this study, patients who underwent hemoglobinopathy screening in Mersin province during a certain period (2017-August 2019) were included in a cross-sectional design. Since our study was retrospective, laboratory parameters such as

hematological parameters, complete blood count and hemoglobin A2 levels could not be obtained in all cases in which *HBB* gene sequence analysis was performed. The results of our study reflect the genetic characteristics of this region and cannot be generalized to the entire population in Turkey.

Conflict of interest statement

No potential conflict of interest was reported by the author(s)

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