Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2024;49(1):40-46
DOI: 10.17826/cumj.1394315

RESEARCH

Genotype-phenotype correlation and mutation spectrum of *HBB* gene in the Hatay province of Turkey

Türkiye'nin Hatay ilinde *HBB* geninin genotip-fenotip korelasyonu ve mutasyon spektrumu

Murat Kaçmaz[1], Semih Aşıkovalı[2]

[1]Diyarbakır Gazi Yaşargil Training and Research Hospital, Diyarbakır, Turkey
[2]Hatay Mustafa Kemal University, Hatay, Turkey

Abstract

Purpose: Thalassemia carriage and hemoglobinopathies are quite common disorders in Turkey, especially in the Çukurova region, including Hatay province. Due to the high case population and genetic diversity in our region, this study aimed to investigate the genotype-phenotype correlation in the *HBB* gene.

Materials and Methods: The data of patients who applied to Hatay Mustafa Kemal University Medical Genetics or Hematology Clinic between January 2010 and November 2022 were evaluated retrospectively.

Results: A total of 40 (100%) cases, comprising 25 (62.5%) homozygous and 15 (37.5%) compound heterozygous genotypes, were included in the study based on the mutation profiles in the *HBB* gene. In the analysis of the cases, it was seen that there were 17 different variants and 22 distinct genotypes. The three most common variants identified in this study were IVS-I-6 (T>C), IVS-I-1 (G>A), and IVS-II-848 (C>A). Of the cases with homozygous genotypes, 13 (52%) had the IVS-I-6 (T>C) variant. The most frequent genotypes observed in cases with compound heterozygous genotype were IVS-I-6 (T>C)/IVS-I-110 (G>A), IVS-I-6 (T>C)/Hb Knossos, and IVS-I-110 (G>A)/-101 C>T, each in 2 (13%) cases.

Conclusion: This study provides information on the phenotypic characteristics of very rare genotypes. We think that this information will be very beneficial, especially for clinicians interested in prenatal diagnosis, preimplantation genetic diagnosis, and postnatal genetic counseling.

Keywords: Hemoglobinopathies, hemoglobin knossos, genetic counseling

Öz

Amaç: Talasemi taşıyıcılığı ve hemoglobinopatiler, aralarında Hatay ilininde bulunduğu Çukurova bölgesi başta olmak üzere Türkiye'de oldukça yaygın görülen rahatsızlıklardır. Bölgemizdeki vaka popülasyonunun ve genetik çeşitliliğin fazla olması nedeniyle bu çalışma *HBB* genindeki genotip-fenotip korelasyonunu araştırmayı amaçlamaktadır.

Gereç ve Yöntem: Hatay Mustafa Kemal Üniversitesi Tıbbi Genetik veya Hematoloji Kliniğine Ocak 2010 ve Kasım 2022 arasında başvuran hastaların verileri retrospektif olarak değerlendirildi.

Bulgular: Bu çalışmaya *HBB* genindeki mutasyon profillerine göre 25'i (%62,5) homozigot ve 15'i (%37,5) birleşik heterozigot genotipten oluşan toplam 40 (%100) olgu dahil edildi. Olguların analizinde 17 farklı varyant ve 22 farklı genotip olduğu görüldü. En sık saptanan üç varyant IVS-I-6 (T>C), IVS-I-1 (G>A) ve IVS-II-848 (C>A) idi. Homozigot genotipli olguların 13'ünde (%52) IVS-I-6 (T>C) varyantı vardı. Birleşik heterozigot genotipli olgularda en sık görülen genotipler, her biri 2 (%13) olguda görülmek üzere IVS-I-6 (T>C)/IVS-I-110 (G>A), IVS-I-6 (T>C)/Hb Knossos ve IVS-I-110 (G>A)/-101 C>T şeklindeydi.

Sonuç: Bu çalışmada oldukça nadir görülen genotiplerin fenotipik özelliklerine dair bilgiler mevcuttur. Bu bilgilerin özellikle doğum öncesi tanı, preimplantasyon genetik tanı ve doğum sonrası genetik danışmanlıkla ilgilenen klinisyenler için oldukça faydalı olacağı görüşündeyiz.

Anahtar kelimeler: Hemoglobinopatiler, hemoglobin knossos, genetik danışmanlık.

Address for Correspondence: Murat Kaçmaz, Diyarbakır Gazi Yaşargil Training and Research Hospital, Department of Hematology, Diyarbakır, Turkey E-mail: dr_muratkacmaz@hotmail.com
Received: 22.11.2023 Accepted: 29.01.2024

INTRODUCTION

Beta thalassemia (β-thal) is an inherited blood disorder characterized by abnormal or reduced production of hemoglobin (Hb). The severity of the disease can vary, ranging from severe anemia to asymptomatic individuals[1]. The Hemoglobin Subunit Beta (*HBB*) gene, located on chromosome 11, is responsible for encoding the beta-globin chain of hemoglobin. Mutations in this gene are the primary cause of β-thal, with over 350 mutations identified so far[2]. These mutations are classified based on their clinical severity, with β^+ indicating milder mutations that result in a relative reduction of β-globin synthesis, and β^0 indicating severe mutations that can lead to the complete absence or inadequate of the β-globin chain. Individuals who are homozygous or compound heterozygous for β-thal mutations clinically present as either β-thalassemia major (BTM) or intermedia (BTI). BTM patients typically manifest severe anemia and require regular blood transfusions from an early age, while BTI patients present later in life with mild to moderate anemia[2,3].

Turkey is one of the countries with a high prevalence of thalassemia carriers and hemoglobinopathies, particularly in the Çukurova region, which includes Hatay province. It is estimated that there are approximately 1.5 million thalassemia carriers in Turkey[4,5]. A significant portion of the population has the thalassemia carrier, which emphasizes the importance of awareness and screening programs. Also, different regions in Turkey show diverse frequencies of different β-thal genotypes. The Çukurova region, where we are located, stands out with a higher case population in terms of thalassemias compared to other regions in Turkey[6-8]. For example, the β-thal carrier prevalence was found to be 13% in the study conducted by Guvenc et al. in the Adana province of Çukurova region, compared to the expected prevalence of 2.1% for the entire country[9,10]. Hatay province, where the study was conducted, is located in the southwest of Turkey, and is known for its various ethnic origins. Additionally, the region has been affected by migration due to wars, which can contribute to the emergence of new genetic profiles[11,12].

In this study we aim to investigate the phenotypic characteristics of the *HBB* genotypes in the Hatay province, taking into consideration its unique geographical location and the high frequency and diversity of mutations. In this way, the phenotypic characteristics of the rare genotypes that we think we will detect in our study have been revealed and we think that they will contribute to the literature. We also believe that our study results can be used in genetic counseling, prenatal diagnosis, and patient management.

MATERIALS AND METHODS

Study design

This study was conducted as a retrospective and descriptive single-center laboratory archive review in the Department of Medical Genetics and Hematology, at the Hatay Mustafa Kemal University between January 2010 and November 2022. Patients with homozygous or compound heterozygous *HBB* gene mutation were included in the study. All age and gender groups were included in the study. Patients with sickle cell disease (SCD) who had either homozygous or compound heterozygous c.20A>T mutations in the *HBB* gene were excluded from the study. This exclusion is due to the severe clinical course of SCD, which can complicate the analysis or skew the results. Also, cases with homozygous HbC and HbE diseases were excluded from the study. After this study design, genotype information of a total of 65 cases was obtained, and after the inclusion criteria were applied, the data of 40 cases were analyzed.

Demographic and medical data of the cases were collected from our center records. Data collection included patient age, gender, diagnosis, laboratory data (hemogram and HPLC results), age at first blood transfusion, history of splenectomy or splenomegaly, disease initiation, serum ferritin level, genetic variants in the *HBB* gene, and other complications. This study was conducted in accordance with the ethical principles set forth in the Declaration of Helsinki. For this study, ethical approval was obtained from the Clinical Research Ethics Committee of Hatay Mustafa Kemal University with a decision dated 06.10.2022 and numbered 05.

Hematological analysis

Complete blood counts of RBC, Hb, Hct, MCV, MCH, and RDW parameters were performed for all samples using an automatic hematology analyzer (Mindray BC 6800). Hemoglobin electrophoresis was performed using HPLC method with Biorad Variant

II autoanalyzer. Serum ferritin levels were measured as ng/mL by immunoassay method.

DNA analysis

Genomic DNA was extracted from peripheral blood leukocytes using an isolation kit (Macherey-Nagel GmbH & Co. KG, Germany) according to the manufacturer's protocol. All coding exons, introns, 3' UTR, 5' UTR, and promoter regions of the *HBB* gene (NM_000518.5) were amplified with appropriate primers.

PCR amplicons were sequenced using an ABI PRISM BigDye Terminator Cycle Sequencing Ready reaction kit (Applied Biosystems, Foster City, CA, USA). Genetic sequence analysis was performed using CLC Genomics Workbench (Qiagen). Hbvar, HGMD, ClinVar, and ExAC databases were used to analyze mutations. Allelic segregations were confirmed for the compound heterozygous mutations.

Statistical analysis

The statistical analyses for this study were performed using IBM SPSS Software (Version 26.0 Armonk, NY). Descriptive statistics were used to summarize the data. Categorical data were expressed as numbers (n) and percentages (%), and numerical data as median and mean ± standard deviation.

RESULTS

According to the mutation profile in the *HBB* gene, a total of 40 (100%) cases were in the study, of which 25 (62.5%) were homozygous and 15 (37.5%) were compound heterozygous mutations. The median age was 21 years (min-max: 2-74 years) and 26 (65%) cases were female. The majority of the study group consisted of cases with BTI 18 (45%) and BTM 16 (40%) phenotypes. Among all the cases, 17 (42.5%) had no transfusion history, 15 (37.5%) had regular transfusions due to BTM, and 2 (5%) had regular transfusions due to BTI. Additionally, 6 (15%) cases had intermittent transfusions due to BTI. Among all the cases, 13 (32.5%) had splenomegaly, 8 (20%) had undergone a splenectomy, and 2 (5%) cases had a normal spleen volume. However, spleen information was not available for 17 (42.5%) cases. Characteristic and demographic features of the cases are presented in Table 1.

Table 1. Characteristic and demographic features of the cases

Variable	
Number of patients, n (%)	40 (%100)
Age, median (min-max)	21 (2-74)
Gender (M/F)	14 (%35) / 26 (%65)
Genotype (*HBB* mutation profile)	
Homozygous	25 (%62.5)
Compound Heterozygous	15 (%37.5)
Phenotype or HPLC diagnosis	
BTM	16 (%40)
BTI	18 (%45)
β-thal trait	4 (%10)
Silent β-thal	2 (%5)
Transfusion and clinical course	
BTM, regular transfusion	15 (%37.5)
BTI, no history of transfusion	10 (%25)
BTI, intermittent transfusion	6 (%15)
BTI, regular transfusion	2 (%5)
Clinically normal (one patient had ASCT due to BTM)	7 (%17.5)
Spleen	
Splenomegaly	13 (%32.5)
Splenectomized	8 (%20)
Normal	2 (%5)
Unknown	17 (%42.5)

HBB, Hemoglobin Subunit Beta; HPLC, high-performance liquid chromatography; BTI, β-thalassemia intermedia; BTM, β-thalassemia major; ASCT, allogeneic stem cell transplantation.

A total of 22 different *HBB* genotypes accompanied by 17 different variants were found in the cases included in the study. The two most common variants detected in this study were IVS-I-6 (T>C) and IVS-I-1 (G>A) with frequencies of 41% and 10% respectively. Among cases with homozygous *HBB* genotypes, the most common variants were IVS-I-6 (T>C), IVS-I-1 (G>A), and IVS-II-848 (C>A), with frequencies of 52% (n:13), 12% (n:3), and 8% (n:2), respectively. In addition to the three most common variants, seven different variants were detected in cases with homozygous *HBB* genotypes, including IVS-I-5 (G>C), Codon 39 (C>T), IVS-II-1 (G>A), 5'UTR; +22 (G>A), 5'UTR; +26 (T>C), 3'UTR +1536 (A>G), and -101 C>T. Among cases with compound heterozygous *HBB* genotypes, the most common genotypes were IVS-I-6 (T>C)/IVS-I-110 (G>A), IVS-I-6 (T>C)/Hb Knossos Codon 27 (G>T), and IVS-I-110 (G>A)/-101 C>T, each occurring in 2 (13%) cases. Apart from the most

common compound heterozygous genotypes, there were nine different genotypes identified, each occurring once in the remaining cases. The *HBB* genotypes, hemoglobin electrophoresis data, clinical and laboratory findings of the cases are shown in Tables 2 and 3.

Table 2. Characteristics of cases with homozygous *HBB* genotype

Allel	Phenotype or HPLC	Sex (M/F)	Age year (min-max)	Hb g/dL (min-max)	Ferritin ml/ng (min-max)	HbA (%) (min-max)	HbA2 (%) (min-max)	HbF (%) (min-max)	Clinical course
IVS-I-6 (T>C) (HBB:c.92+6T>C)	BTI (n:10)	M:3 F:7	37.1 (9-74)	8.3 (6.7-9.8)	794 (167-3683)	80 (64.5-89.4)	6.6 (6.2-7.1)	9 (2.7-18.2)	Tx Regularly: 2 Occasionally: 3 Spleen Splenectomized: 3 Splenomegaly: 5 Unknown: 2
	BTM (n:3)	M:1 F:2	23.3 (9-33)	8.5 (7.9-9.6)	1877 (767-2824)	71.1 (70.2-72)	6.1 (6-6.3)	18.2 (17.8-18.6)	Tx Regularly: 3 Spleen Splenectomized: 2 Unknown: 1
IVS-I-1 (G>A) (HBB:c.92+1G>A)	BTI	F	26	8.7	98	80,9	6.7	12.6	Tx: Independent Splenomegaly
	BTM	M	2	8.3	1428	-	-	-	Tx: Regularly Spleen: Unknown
	BTM	M	21	9.2	1415	80	2.9	17	Tx: Regularly Splenectomized
IVS-II-848 (C>A) (HBB:c.316-3C>A)	BTM	F	5	6.5	761	-	-	65.6	Tx: Regularly Splenomegaly
	BTM	M	16	14.3	2120	-	-	-	ASCT
IVS-I-5 (G>C) (HBB:c.92+5G>C)	BTM	F	22	8.1	408	65.4	2.9	15.6	Tx: Regularly Splenomegaly
Codon 39 (C>T) (HBB:c.118C>T)	BTM	F	18	8.1	-	94.2	2.9	1.9	Tx: Regularly Splenomegaly
IVS-II-1 (G>A) (HBB:c.315+1G>A)	BTI	M	13	9.4	381	0.2	1	96.6	Tx: Independent Spleen: Unknown
5'UTR; +22 (G>A) (HBB:c.-29G>A)	BTI	F	21	8.8	32	85	6.5	8.3	Tx: Independent Splenomegaly
5'UTR;+26 (T>C) (HBB:c.-25T>C)	Silent β-thal	F	39	13.3	46	95	2.5	0.2	Clinically normal
3'UTR +1536 (A>G) (HBB:c.*62A>G)	β-thal trait	M	16	12.4	-	95	2.7	0.2	Clinically normal
-101 C>T (HBB:c.-151C>T)	β-thal trait	F	17	11.6	31	82.2	4.7	6.4	Clinically normal

HBB, Hemoglobin Subunit Beta; HPLC, high-performance liquid chromatography; Hb, hemoglobin; BTI, β-thalassemia intermedia; BTM, β-thalassemia major; Tx, transfusion; ASCT, allogeneic stem cell transplantation.

Table 3. Characteristics of cases with a compound heterozygous *HBB* genotype

Allel 1	Allel 2	Phenotype or HPLC	Gender (M/F)	Agey ear	Hb g/dL	Ferritin ml/ng	HbA (%)	HbA2 (%)	HbF (%)	Clinical course
IVS-I-6 (T>C) (HBB:c.92+6T>C)	IVS-I-110 (G>A)(HBB:c.93-21 G>A)	BTM	F	42	9.8	>1650	83.6	4	13.1	Tx: RegularlySplenectomizedNot alive
		BTM	M	36	8.2	532	77.6	3	10.3	Tx: RegularlySplenectomized
IVS-I-6 (T>C) (HBB:c.92+6T>C)	Hb Knossos (HBB:c.82G>T)	BTI	F	8	6.6	254	91	5.4	2.1	Tx: OccasionallySpleen: Unknown
		BTI	M	10	6.1	387	92.1	5.9	1	Tx: OccasionallySpleen: Unknown
IVS-I-110 (G>A) (HBB:c.93-21G>A)	-101 C>T(HBB:c.-151C>T)	BTI	F	3	9.2	38	52.5	4.9	31.2	Tx: IndependentSplenomegaly
		BTI	F	6	9.8	91	90	4.5	2	Tx: IndependentSpleen: Unknown
IVS-I-6 (T>C) (HBB:c.92+6T>C)	Codon 5 (-CT) (HBB:c.17-18delCT)	BTM	F	5	8.6	1190	-	2.6	26.8	Tx: RegularlySpleen: Unknown
IVS-I-6 (T>C) (HBB:c.92+6T>C)	IVS-II-1 (G>A)(HBB:c.315+1G >A)	BTM	M	10	7.1	2786	-	-	-	Tx: RegularlySpleen: Unknown
IVS-I-6 (T>C) (HBB:c.92+6T>C)	IVS-II-727 (A>T) (HBB:c.316-124A>T)	β-thal trait	M	26	11.7	24	-	-	-	Clinically normal
IVS-I-1 (G>A) (HBB:c.92+1G>A)	IVS II-821 (A>C)(HBB:c.316-30 A>C)	BTI	F	24	10.7	72	-	-	-	Tx: OccasionallySpleen: Unknown
IVS-I-110 (G>A) (HBB:c.93-21G>A)	Codon 39 (C>T) (HBB:c.118C>T)	BTM	F	12	10.7	1334	-	-	-	Tx: RegularlySpleen: Unknown
IVS-II-1 (G>A) (HBB:c.315+1G>A)	IVS-I-110 (G>A)(HBB:c.93-21G>A)	BTM	M	7	7	1485	54.3	2.6	35.9	Tx: RegularlySplenomegaly
IVS-II-848 (C>A) (HBB:c.316-3C>A)	IVS-I-1 (G>A) (HBB:c.92+1G>A)	BTM	F	11	7.6	3749	12.1	3	-	Tx: RegularlySplenomegaly
IVS II-821 (A>C) (HBB:c.316-30A>C)	-101 C>T (HBB:c.-151C>T)	Silent β-thal	F	29	12	20	-	-	-	Clinically normal
HbE(HBB:c.79 G>A)	-101 C>T (HBB:c.-151C>T)	β-thal trait	F	25	12.4	22	60.7	-	-	Clinically normal

HBB, Hemoglobin Subunit Beta; HPLC, high-performance liquid chromatography; Hb, hemoglobin; Tx, transfusion; BTM, β-thalassemia major; BTI, β-thalassemia intermedia.

DISCUSSION

In this study, we presented results of the genotype-to-phenotype relationship in homozygous or compound heterozygous *HBB* gene mutations in Hatay. Our study contains significant information that will contribute to the genotype-phenotype correlation in β-thal cases with the contribution of the high case population and genetic diversity in our region. We detected a total of 17 different *HBB* gene variants in 22 distinct genotypes. The most detected *HBB* gene variants were IVS-I-6 (T>C) and IVS-I-1 (G>A).

In our study, the most common homozygous *HBB* genotypes were IVS-I-6 (T>C) in 13 cases, IVS-I-1 (G>A) in 3 cases, and IVS-II-848 (C>A) in 2 cases. Although the homozygous IVS-I-110 (G>A) genotype is typically more common in our region and country, this genotype was less frequent in our

study[9,13]. Instead, IVS-I-6 (T>C) was the most common genotype, and this finding is similar to previous research in Palestine and Israel[14-16]. Differently, the presence of a transfusion history among individuals with homozygous IVS-I-6 (T>C) genotype in our study was 61%, which was lower than the 81% reported in the Palestine study[16]. In a previous study on the homozygous IVS-I-1 (G>A) genotype, which we detected with the second frequency in our study, it was reported that the cases were transfusion dependent[15]. However, in our study, whereas two of the three homozygous IVS-I-1 (G>A) genotypes had a history of regular transfusion, one case was followed up independently of transfusion. In our study, another common homozygous IVS-II-848 (C>A) genotype is observed quite rarely to our knowledge. In a previous study involving 146 cases, the homozygous IVS-II-848 (C>A) genotype was found in only one case and was reported as transfusion dependent[15,16]. We identified two cases with the homozygous IVS-II-848 (C>A) genotype. These two cases exhibited the BTM phenotype, and one of them had received an allogeneic stem cell transplant. We think that one of the most important findings of our study is related to the clinical significance of the 5'UTR; +26 (T>C) variant. In a case, we discovered a homozygous 5'UTR; +26 (T>C) genotype, which is reported in the ClinVar database as a variant of unknown significance[17]. Since the clinical and laboratory data of our patient were completely normal, we interpreted this variant as a polymorphism.

In our study, we obtained some results that we believe are important in cases with compound heterozygous genotypes. One of them is the IVS-I-1 (G>A)/IVS II-821 (A>C) genotype. In the study of Azimi et al., the IVS II-821 (A>C) variant was defined as pathogenic despite its classification as both likely benign and of unknown significance in the ClinVar database[18]. The case in our study had BTI phenotype with intermittent transfusion, therefore we think that the IVS II-821 (A>C) variant is probably likely pathogenic. Since IVS-I-6 (T>C) variant was found more frequently in our province, clinical findings of rare genotypes with this variant were reported in this study. We detected IVS-I-6 (T>C) and/with IVS-II-1 (G>A), Codon 5 (-CT), IVS-II-727 (A>T), and Hb Knossos variants. In a previous study involving 52 cases, the IVS-I-6 (T>C)/IVS-II-1 (G>A) genotype was found in one case, and the only reported clinical information was anemia[14]. In another previous study involving 146 cases, a case with the IVS-I-6 (T>C)/Codon 5 (-CT) genotype was reported as transfusion dependent[15]. In our study, our cases with both IVS-II-1 (G>A) and Codon 5 (-CT) genotypes along with IVS-I-6 (T>C) were observed to have BTM phenotype and required regular transfusions. However, a patient with the IVS-I-6 (T>C)/IVS-II-727 (A>T) genotype had a completely normal phenotype in terms of clinical and laboratory findings. Thus, we suggest that the IVS-II-727 variant should also be considered as a polymorphism. Additionally, to our knowledge, this is the first article describing the phenotypic features of the IVS-I-6 (T>C)/Hb Knossos genotype. The two cases with this genotype were found to have BTI phenotype and required intermittent transfusion.

There are some limitations in our study. Firstly, the patients in this study did not have any results regarding *HBA* gene deletions in terms of alpha thalassemia. It is known that *HBA* gene deletions affect the phenotypes in β-thal[19]. However, in a series of 270 cases of β-thal, Saha et al. reported that *HBA* gene deletions in patients with β+/β0 genotype improved the phenotype, but alpha gene deletions alone could not explain the clinical severity in some patients with β0-β0 genotype[20]. Secondly, genotype information of our patients regarding the changes in the Krüppel-like factor 1 (KLF1) gene and XmnI polymorphism, which are expected to affect the clinic in β-thal patients, especially in sickle cell disease, were not available. However, Kumar et al. reported that KLF1 single nucleotide polymorphisms and XmnI polymorphism had no statistically significant association with HbF levels and sickle cell disease-related morbidities in a study including 180 sickle cell patients[21]. Finally, some phenotypic information was missing in our study because some of our patients were not followed up regularly.

In conclusion, in this study, phenotypic characteristics of 22 different *HBB* genotypes in a total of 40 β-thal cases were investigated. To our knowledge, this is the first study in which detailed clinical findings for genotypes with compound heterozygosity for Hb IVS-I-6(T>C)/Knossos and homozygous 5'UTR; +26 (T>C). Additionally, this study included information on the extremely rare homozygous IVS-II-848 (C>A) and compound heterozygous IVS-I-1 (G>A)/IVS II-821 (A>C) genotypes. These results suggest that the distribution of *HBB* genotypes and their association with transfusion history and clinical features can vary between different populations and regions.

Therefore, we think that these genotype-phenotype relationships reported in our study to benefit clinicians during prenatal diagnosis, preimplantation genetic diagnosis, postnatal genetic counseling, and future studies.

Author Contributions: Concept/Design: MK, SA; Data acquisition: MK, SA; Data analysis and interpretation: MK, SA; Drafting manuscript: MK, SA; Critical revision of manuscript: MK, SA; Final approval and accountability: MK, SA; Technical or material support: MK, SA; Supervision: MK, SA; Securing funding (if available): n/a.
Ethical Approval: Ethical approval was obtained from the Ethics Committee of Non-Interventional Clinical Research of Hatay Mustafa Kemal University with the decision dated 06.10.2022 and numbered 05.
Peer-review: Externally peer-reviewed.
Conflict of Interest: Authors declared no conflict of interest.
Financial Disclosure: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis. 2010;5:11.
2. Taher AT, Musallam KM, Cappellini MD. β-Thalassemias. N Engl J Med. 2021;384:727-43.
3. Steinberg MH, Forget BG, Higgs DR, Weatherall DJ. Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management. 2 ed. Cambridge: Cambridge University Press; 2009.
4. Canatan D. Thalassemias and hemoglobinopathies in Turkey. Hemoglobin. 2014;38:305-7.
5. Cürük MA, Arpaci A, Attila G, Tuli A, Kilinç Y, Aksoy K et al. Genetic heterogeneity of beta-thalassemia at Cukurova in southern Turkey. Hemoglobin. 2001;25:241-45.
6. Altay Ç. The Frequency and Distribution Pattern of ß-Thalassemia Mutations in Turkey. Turk J Haematol. 2002;19:309-15.
7. Yuzbasioglu Ariyurek S, Yildiz SM, Yalin AE, Guzelgul F, Aksoy K. Hemoglobinopathies in the Çukurova region and neighboring provinces. Hemoglobin. 2016;40:168-72.
8. Arpaci A, Gul BU, Ozcan O, Ilhan G, El C, Dirican E et al. Presentation of two new mutations in the 3'untranslated region of the β-globin gene and evaluating the molecular spectrum of thalassemia mutations in the Mediterranean region of Turkey. Ann Hematol. 2021;100:1429-38.
9. Aydınok Y, Oymak Y, Atabay B, Aydoğan G, Yeşilipek A, Ünal S et al. A national registry of thalassemia in Turkey: demographic and disease characteristics of patients, achievements, and challenges in prevention. Turk J Haematol. 2018;35:12-18.
10. Guvenc B, Canataroglu A, Unsal C, Yildiz SM, Turhan FT, Bozdogan ST et al. β-Thalassemia mutations and hemoglobinopathies in Adana, Turkey: results from a single center study. Arch Med Sci. 2012;8:411-4.
11. Canatan D, Vives Corrons JL, Piacentini G, Kara F, Keskinkılıç B, Tezel B et al. Immigration and screening programs for hemoglobinopathies in Italy, Spain and Turkey. Acta Biomed. 2021;92:e2021410.
12. Yazal Erdem A, Demir Yenigürbüz F, Pekpak E, Akıncı B, Aktekin E, Bayram C et al. Refugee children with beta-thalassemia in Turkey: Overview of demographic, socioeconomic, and medical characteristics. Pediatr Blood Cancer. 2019;66:e27636.
13. Tadmouri GO, Tüzmen S, Ozçelik H, Ozer A, Baig SM, Senga EB et al. Molecular and population genetic analyses of beta-thalassemia in Turkey. Am J Hematol. 1998;57:215-20.
14. Guzelgul F, Seydel GS, Aksoy K. β-globin gene mutations in pediatric patients with β-thalassemia in the region of Çukurova, Turkey. Hemoglobin. 2020;44:249-53.
15. Kurtoğlu A, Karakuş V, Erkal Ö, Kurtoğlu E. β-Thalassemia gene mutations in Antalya, Turkey: results from a single centre study. Hemoglobin. 2016;40:392-95.
16. Faraon R, Daraghmah M, Samarah F, Srour MA. Molecular characterization of β-thalassemia intermedia in the West Bank, Palestine. BMC Hematol. 2019;19:4.
17. Landrum MJ, Kattman BL. ClinVar at five years: Delivering on the promise. Hum Mutat. 2018;39:1623-30.
18. Azimi A, Nejati P, Tahmasebi S, Alimoradi S, Alibakhshi R. Characterization of the IVS-II-821 (A>C) (HBB: c.316-30A>C) Mutation in a β-Thalassemia Phenotype in Iran. Hemoglobin. 2019;43:23-6.
19. Traeger-Synodinos J, Harteveld CL, Old JM, Petrou M, Galanello R, Giordano P et al. EMQN haemoglobinopathies best practice meeting. EMQN Best Practice Guidelines for molecular and haematology methods for carrier identification and prenatal diagnosis of the haemoglobinopathies. Eur J Hum Genet. 2015;23:426-37.
20. Saha D, Chowdhury PK, Panja A, Pal D, Nayek K, Chakraborty G et al. Effect of deletions in the α-globin gene on the phenotype severity of β-thalassemia. Hemoglobin. 2022;46:118-23.
21. Kumar R, Yadav R, Mishra S, Singh MPSS, Gwal A, Bharti PK et al. Krüppel-like factor 1 (KLF1) gene single nucleotide polymorphisms in sickle cell disease and its association with disease-related morbidities. Ann Hematol. 2021;100:365-73.