Research Article

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Analysis of beta globin gene mutations in Diyarbakir

DİYARBAKIR'DA BETA GLOBİN GEN MUTASYONLARININ ANALİZİ

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Abstract

Objectives: Hemoglobin disorders are quite heterogeneous in the Turkish population. Up to now, more than forty different beta thalassemia mutations and 60 hemoglobin variants have been characterized in the country. The aim of this study was to investigate genetic heterogeneity of HBB gene mutations in patients and their parents at Southeastern Anatolia in Turkey.

Methods: Genomic DNA was isolated from 145 thalassemic patients' blood samples and their parents in this study. Ten different HBB gene mutations HBB:c.-80T>A, HBB:c.17_18delCT, HBB:c.25_26delAA, HBB:c.92+1G>A, HBB:c.92+5G>C, HBB:c.92+6T>C, HBB:c.93-21G>A, HBB:c.135delC, HBB:c.315+1G>A, HBB:c.316-106C>G were screened by amplification refractory mutation system. Four Hb variants and some rare beta thalassemia mutation were characterized by DNA sequencing.

Results: In this study, 97 homozygous and 48 compound heterozygous thalassemic patients were diagnosed by molecular genetic analyses. As a results, 18 β-thalassemia mutations and four abnormal hemoglobins; HBB:c.20A>T, HBB:c.364G>C, HBB:c.34G>A and HBB:c.208G>A were detected at Dicle University Hospital.

Conclusions: In the results, HBB:c.93-21G>A is the most common mutation in the region. Three mutations [(HBB:c.93-21G>A), (HBB:c.25_26delAA) and (HBB:c.135delC)] account for about 58 per cent of all the point mutations. Except HBB:c.20A>T and HBB:c.364G>C, two silent Hb variants (HBB:c.34G>A and HBB:c.208G>A) were detected in this study. Hb Hamilton [β11 (GTT>ATT) Val>Ile] was seen first time in Turkey.

Keywords: β-thalassemia; HBB:c.208G>A; HBB:c.34G>A; southeastern Anatolia region.

Amaç: Hemoglobin hastalıkları Türk halkında oldukça çeşitlidir. Bu güne kadar ülkede 40 dan fazla beta talasemi mutasyonu, 60 dan fazla da Hb varyantı karakterize edilmiştir. Bu çalışmanın amacı Güneydoğu Anadolu Bölgesindeki hasta ve ebeveynlerinin HBB gen mutasyonlarının genetik çeşitliliğini araştırmaktı.

Gereç ve Yöntem: Bu çalışmada 145 talasemi hastası ve onların ebeveynlerinin kan örneklerinden genomik DNA izole edildi. On farklı HBB gen mutasyonu [HBB:c.-80T>A, HBB:c.17_18delCT, HBB:c.25_26delAA, HBB:c.92+1G>A, HBB:c.92+5G>C, HBB:c.92+6T>C, HBB:c.93-21G>A, HBB:c.135 delC, HBB:c.315+1G>A, HBB:c.316-106C>G] Amplification Refractory Mutation System ile tarandı. Dört Hb varyantı ve bazı nadir görülen beta talasemi mutasyonları DNA dizi analizi ile karakterize edildi.

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Bulgular: Bu çalışmada 97 homozigot ve 48 birleşik heterozigot beta talasemi hastası DNA analizi ile teşhis edildi. Sonuç olarak, Dicle Üniversitesi hastanesinde 18 farklı beta talasemi mutasyonu ile four anormal hemoglobin (HBB:c.20A>T, HBB:c.364G>C, HBB:c.34G>A ve HBB:c.208G>A) molekülü belirlendi.
Sonuç: Güneydoğu Anadolu Bölgesindeki bulgular içinde en sık görülen mutasyon HBB:c.93-21G>A dır. Mutasyonlardan üçü (HBB:c.93-21G>A, HBB:c.25_26delAA ve HBB:c.135delC) tüm sonuçların % 58'ini içermekteydi. HBB:c.20A>T ve HBB:c.364G>C'den başka iki sessiz Hb varyantı (HBB:c.34G>A ve HBB:c.208G>A) daha belirlendi. Türkiyede HBB:c.34G>A varyantı [β11(GTT>ATT) Val>Ile] ilk kez görüldü.

Anahtar kelimeler: β-talasemi; HBB:c.34G>A; HBB:c.208G>A; Güneydoğu Anadolu Bölgesi.

Introduction

The thalassemias are a diverse group of genetic disorders characterized by a microcytic, hypochromic anemia and an imbalance in the synthesis of globin chains. Alpha and beta thalassemias are common disorders and seen at high frequencies in countries where malaria is endemic such as Southeast Asia, Middle Eastern and Mediterranean countries [1, 2].

Beta thalassemia is a group of genetic disorders of hemoglobin synthesis characterized by reduced (β^+) or total absence of HBB (β^0) chains. The two main types of beta thalassemia (β^+ or β^0) are inherited in the heterozygous state, the homozygous state, or in various compound heterozygous states [3–5]. Beta thalassemia syndromes can be diagnosed by clinical and hematological examination. Patients with classical homozygous beta thalassemia are expected to have marked degree of anemia, which is typically hypochromic and microcytic with low MCH and MCV values, respectively. As a result, destruction of red blood cells and the subsequent increase in bilirubin concentration ensues.

The clinical picture of beta thalassemia syndromes ranges from the asymptomatic carrier states, through intermediate to beta thalassemia major. The latter are transfusion-dependent starting from the first year of life and even with an appropriate transfusion regimen, most of them die in the second or third decade of life if iron chelation therapy is not offered. Beta thalassemia intermedia status is used to designate a broad spectrum of clinical phenotypes varying the asymptomatic beta thalassemia trait to the transfusion-dependent thalassemia major [5]. The patients are just able to keep Hb levels about 6–8 g/dL without transfusion. The molecular basis of beta thalassemia intermediate may explain this large spectrum, but in general it involves the interaction between different molecular defects and factors that partially correct the globin chain imbalance.

Hemoglobin disorders have become of particular interest in recent years because they were the first group of diseases to be characterized by DNA technology. The frequency of β-thalassemia trait is 2.1% in Turkey. The incidence of carriers reaches as high as 13% in some of the cities such as Antalya. Beta thalassemia mutations are very heterogeneous in our population [6, 7]. Using molecular biology techniques, β-thalassemia has been prevented by screening of carriers and prenatal diagnosis in many countries for 2–3 decades. In the last decade, premarital screening program and prenatal diagnosis have been implemented in Turkey [8–15]. The number of the affected births has been decreased considerably recently.

Although both alpha and beta thalassemia are highly heterogeneous, screening survey results have shown so far that 10 different beta thalassemia mutations are common in Turkish population [-30 (T>A) HBB:c.-80T>A, Fsc5 (-CT) HBB:c.17_18delCT, Fsc8 (-AA) HBB:c.25_26delAA, IVS I-1 (G>A) HBB:c.92+1G>A, IVS I-5 (G>C) HBB:c.92+5G>C, IVS I-6 (T>C) HBB:c.92+6T>C, IVS I-110 (G>A) HBB:c.93-21G>A, Fsc44 (-C) HBB:c.135delC, IVS2-1 (G>A) HBB:c.315+1G>A, IVS 2-745 (C>G) HBB:c.316-106C>G] as well as five alpha globin gene deletions; α-thal-1 (–17.4 kb, –26.5 kb and –20.5 kb) and α-thal-2 (–3.7 kb and –4.2 kb) [16–18]. Sickle cell anemia and beta thalassemia are considered to be a serious health problem in various parts, especially in Çukurova Region of Turkey [19–23]. The aim of this study was to investigate genetic heterogeneity of HBB gene mutations in patients and their parents at Southeastern Anatolia in Turkey.

Material and methods

This study was conducted on registered patients and their parents included in routine work at the pediatric hematology department of Dicle University. The project was approved by the Ethics Committee of Dicle University. Written informed consent was obtained from all the patients or parents. One hundred 45 patients and 200 of their parents (father or mother, or both) were analyzed in genetic laboratory at the Dicle University Hospital in Diyarbakir. Venous blood sample was taken in tube with EDTA as anticoagulant. Hematological and hemoglobin analyses; complete blood count (CBC) were carried out by cell counter (Sysmex XT2000i, Kobe, Japan). The hemoglobin variants were characterized by HPLC; High Performance Liquid Chromatography (VARIANT II, Bio-Rad Laboratories, Hercules, CA, USA). Genomic DNA was isolated by Zinexts from white blood cells (MagPurix Blood DNA Extraction Kit, Taiwan). Common HBB gene mutations were screened by Amplification Refractory Mutation System (ARMS). ARMS-PCR were set up in two separate tubes for each sample. One test tube for the amplification of the normal ARMS primer and the second for the amplification of the mutant ARMS primer (Table 1). A total of 20 μL of final PCR reaction volume was used for this purpose.

The reaction volume was composed of 0.5 µg of the DNA template, 0.01 µg of each of the four primers (2 control primers 5′-CAA TGT ATC ATG CCT CTT TGC ACC-3′ and 5′-GAG TCA AGG CTG AGA GAT GCA GGA-3′, one common primer 5′-ACC TCA CCC TGT GGA GCC AC-3′ or 5′-CCC CTT CCT ATG ACA TGA ACT TAA-3′, and 1 mutant/normal ARMS primer for the normal/mutant allele), 0.5 unit Taq DNA polymerase (Sigma D 6677), and 0.2 mM of each dNTP (Sigma D-4788, D-4913, D-5038, T-9656) in a solution of 10 mM Tris-HC1 (Amresco 0324), 50 mM $MgCl_2$, and 1 mM spermidine (Sigma S-0266). The thermal cycling was set for 5 min initial denaturation at 94 °C, followed by 25 cycles at 94 °C for 1 min, at 65 °C 1 min, and 72 °C for 1 min 30 s, and the final extension at 72 °C for 6 min. Fifteen microliters of the PCR products were mixed with 3 µL of a loading buffer and then loaded on a 2% agarose gel (Sigma A-0169). The gel was set at 100 V for 1 h and then stained with ethidium bromide (Sigma E 8751). After staining, the bands could be seen under UV light. A rare β-thalassemia mutations and Hb variants were characterized by DNA sequencing. Amplification of β-globin gene was carried out using specific primer set (forward: 5′-GCC AAG GAC AGG TAC GGC TGT CAT C-3′) and (reverse: 5′-CCC TTC CTA TGA CAT GAA CTT AAC CAT-3′) using Platinum Taq DNA Polymerase (Invitrogen, Brazil) in 25 µL reaction volume. Thermocycling conditions consisted of 1 denaturing cycle at 96 °C for 5 min followed by 35 cycles of denaturing at 94 °C for 30 s, annealing at 62 °C for 40 s, and extension at 72 °C for 20 s. Final extension was at 72 °C for 10 min using SimpliAmp Thermal Cycler (Applied Biosystems). Amplicons were electrophoresed on 2% agarose gel. Nucleotide Sequencing was done using a BigDye Terminator cycle sequencing kit and an ABI PRISM 310 Genetic analyzer (Applied Biosystems, USA) [23–25].

Results

A total of 345 patients and their parents were screened. The results of 145 affected thalassemic patients were shown in Tables 2 and 3. Ninety seven were observed to be homozygous β-thalassemia and 48 patients were observed to be compound heterozygous for beta thalassemia mutations. Fifty-five affected infants were born in Diyarbakır at 2018. Only six patients were observed to be co-inherited β-thalassemia mutations and abnormal hemoglobins, HbS [β6 (GAG>GTG) Glu>Val] (HBB:c.20A>T) and Hb Hamilton [β11 (GTT>ATT) Val>Ile] (HBB:c.34G>A).

In this study, 18 different beta thalassemia mutations were detected. Furthermore, two hemoglobin variants, HbD-Punjab [β121 (GAA>CAA) Glu>Gln] (HBB:c.364G>C) and

Table 2: β-thalassemia homozygous patients in the region.

No	Mutations	Substitution	Case number
1	HBB:c.93-21G>A	G>A	46
2	HBB:c.92+1G>A	G>A	10
3	HBB:c.135delC	-C	10
4	HBB:c.25_26delAA	-AA	8
5	HBB:c.-80T>A	T>A	7
6	HBB:c.92+6T>C	C>T	4
7	HBB:c.112delT	-T	3
8	HBB:c.48G>A	G>A	2
9	HBB:c.*110T>C	T>C	1
10	HBB:c.315+1G>A	G>A	1
11	HBB:c.118C>T	T>C	1
12	HBB:c.-78A>C	A>C	1
13	HBB:c.93-1G>C	G>C	1
14	HBB:c.251delG	-G	1
15	HBB:c.20A>T	A>T	1
Total			97

Table 1: The sequences of primers used for common beta thalassemia mutations.

Mutations	Primers for mutant and normal alleles
HBB:c.93-21G>A	M: 5′-CTGATAGGCACTGACTCTCTCTGCCTGTTA-3′
IVS 1-110 (G>A)	N: 5′-ACCAGCAGCCTAAGGGTGGGAAAATACACC-3′
HBB:c.92+1G>A	M: 5′-TTAAACCTGTCTTGTAACCTTGATACGAAT-3′
IVS 1-1 (G>A)	N: 5′-TTAAACCTGTCTTGTAACCTTGATACGAAC-3′
HBB:c.118C>T	M: 5′-CAGATCCCCAAAGGACTCAAAGAACCTGTA-3′
Cod 39 (C>T)	N: 5′-TTAGGCTGCTGGTGGTCTACCCTTGGTCCC-3′
HBB:c.92+6T>C	M: 5′-TCTCCTTAAACCTGTCTTGTAACCTTCATG-3′
IVS1-6 (C>T)	N: 5′-TCTCCTTAAACCTGTCTTGTAACCTTCATA-3′
HBB:c.25_26delAA	M: 5′-ACACCATGGTGCACCTGACTCCTGAGCAGG-3′
FSC 8 (-AA)	N: 5′-ACACCATGGTGCACCTGACTCCTGAGCAGA-3′
HBB:c.-80T>A	M: 5′-GCAGGGAGGGCAGGAGCCAGGGCTGGGCAA-3′
-30 (T>A)	N: 5′-GCAGGGAGGGCAGGAGCCAGGGCTGGGCAT-3′
HBB:c.315+1G>A	M: 5′-AAGAAAACATCAAGGGTCCCATAGACTGAT-3′
IVS2-1 (G>A)	N: 5′-AAGAAAACATCAAGGGTCCCATAGACTGAC-3′
HBB:c.316-106C>G	M: 5′-TCATATTGCTAATAGCAGCTACAATCGAGG-3′
IVS2-745 (C>G)	N: 5′-TCATATTGCTAATAGCAGCTACAATCGAGC-3′
HBB: c.92+5G>C	M: 5′-CTCCTTAAACCTGTCTTGTAACCTTGTTAG-3′
IVS1-5(G>C)	N: 5′-CTCCTTAAACCTGTCTTGTAACCTTGTTAC-3′
HBB: c.27dupG	M: 5′-CCTTGCCCCACAGGGCAGTAACGGCACACC-3′
FSC8/9 (+G)	N: 5′-CCTTGCCCCACAGGGCAGTAACGGCACACT-3′

Table 3: Compound heterozygote patients in the region.

No	Combination of β-globin gene mutations		Case number
1	HBB:c.25_26delAA	HBB:c.93-21G>A	15
2	HBB:c.135delC	HBB:c.93-21G>A	8
3	HBB:c.92+1G>A	HBB:c.93-21G>A	4
4	HBB:c.118C>T	HBB:c.93-21G>A	2
5	HBB:c.251delG	HBB:c.93-21G>A	1
6	HBB:c.17_18delCT	HBB:c.-80T>A	1
7	HBB:c.92+6T>C	HBB:c.-80T>A	1
8	HBB:c.135delC	HBB:c.-80T>A	1
9	HBB:c.315+1G>A	HBB:c.-80T>A	1
10	HBB:c.92+6T>C	HBB:c.92+5G>C	2
11	HBB:c.316-106C>G	HBB:c.92+5G>C	2
12	HBB:c.315+1G>A	HBB:c.92+1G>A	2
13	HBB:c.25_26delAA	HBB:c.92+6T>C	2
14	HBB:c.93-21G>A	HBB:c.20A>T	2
15	HBB:c.48G>A	HBB:c.20A>T	2
16	HBB:c.-80T>A	HBB:c.20A>T	1
17	HBB:c.93-21G>A	HBB:c.34G>A	1
Total			48

Hb City of Hope [β69 (GGT>AGT) Gly>Ser] (HBB:c.208G>A) were seen as heterozygotes.

Two hundred parents of patients were diagnosed as beta thalassemia trait. The mutation points and chromosome numbers were presented in Table 4. The HBB:c.93-21G>A was the most common mutation in the region. Three mutations (HBB:c.93-21G>A, HBB:c.25_26delAA and Fsc 44 (-C) HBB:c.135delC) account for about 58% of all the mutations.

Table 4: Distribution of the β-globin gene mutations in the region.

No	Mutations	Case number	%
1	HBB:c.93-21G>A	70	35.0
2	HBB:c.25_26delAA	24	12.0
3	HBB:c.135delC	22	11.0
4	HBB:c.92+1G>A	16	8.0
5	HBB:c.315+1G>A	15	7.5
6	HBB:c.-80T>A	9	4.5
7	HBB:c.17_18delCT)	8	4.0
8	HBB:c.316-106C>G	5	2.5
9	HBB:c.112delT	5	2.5
10	HBB:c.92+5G>C	3	1.5
11	HBB:c.93-1G>C	3	1.5
12	HBB:c.-78A>C	3	1.5
13	HBB:c.251delG	3	1.5
14	HBB:c.92+6T>C	3	1.5
15	HBB:c.*110T>C	1	0.5
16	HBB:c.20A>T	6	3.0
17	HBB:c.364G>C	2	1.0
18	HBB:c.208G>A	1	0.5
19	HBB:c.34G>A	1	0.5
Total		200	100

Discussion

Hemoglobin disorders are very heterogeneous in Mediterranean populations. More than 42 point mutations in HBB gene and 55 abnormal hemoglobins have been detected in Turkey [7, 16, 23, 25]. Ten point mutations on HBB gene account for 90% of the all of the mutations in the country. HBB:c.20A>T is very common in Çukurova region (Adana, Mersin and Antakya). In addition, there are some rare abnormal hemoglobin such as HbC (HBB:c.19G>A), HbD (HBB:c.364G>C), HbE (HBB:c.79G>A), HbO-Arab (HBB:c.364G>A) and Hb Adana (HBA2:c.179G>A or HBA1) [25–27]. Due to molecular diversity, sometimes prenatal diagnosis takes time in some couples at risk for hemoglobinopathies. For getting quick result, it is important that mutations should be detected before pregnancy [22, 25].

Turkish government issued a law for eradication of genetic diseases in 1993. Premarital screening laboratories were set up by law in 40 provinces for detection of the carriers. They have played a major role for decreasing the rate of affected births in 40 cities [8–15]. In 2018, Turkish Ministry of Health has enlarged premarital screening program to all around the Anatolia. Ideally the screening program should be conducted by family medicine. Physicians are responsible for their registered families about hemoglobinopathies. Blood samples should be taken during the early stage of pregnancy. Complete blood count and HbA2 levels of couples should be checked carefully. The couples at risk for hemoglobinopathies are registered and followed up for pregnancy to prevent hemoglobin disorders in Turkey.

Pregnant women at risk for beta thalassemia have been directed to local hospitals for prenatal diagnosis by physicians [19–23, 28]. Many fetuses have been diagnosed annually. However, some of the mother has not understood the implications of genetic counseling very well or they neglected to go to university hospitals for prenatal diagnosis. Consequently, around 55 affected babies are born in Diyarbakır at 2018. This was exacerbated by consanguineous marriages which are very common in Anatolia. Although, the average rate of consanguinity is about 21% this rates may reach 63% in some regions of Turkey [25, 28]. The high number of patients in Southeastern Anatolia was due to that area having the highest rates of consanguineous marriage and fertility [29].

Although, Dicle University results are limited compared to four University Hospitals (Akdeniz, Boğaziçi, Çukurova and Hacettepe) percentages of first mutation is similar (Table 5). Two frameshift mutations [Fsc36/37 (HBB:c.112delT) and Fsc82/83 (HBB:c.251delG)] are seen

Table 5: Comparison of β-thalassemia mutations in five University Hospitals in Turkey.

No	β-thalassemia mutations	Dicle[a] (n: 200)	Akdeniz[b] (n: 411)	Bogaziçi[c] (n: 140)	Çukurova[d] (n:714)	Hacettepe[e] (n: 1114)
1	HBB:c.93-21G>A	35.0	42.3	37.1	50.6	49.0
2	HBB:c.25_26delAA	12.0	3.2	5.7	1.8	7.6
3	HBB:c.135delC	11.0	3.2	1.4	1.8	3.2
4	HBB:c.92+1G>A	8.0	5.1	7.1	8.1	7.9
5	HBB:c.315+1G>A	7.5	8.8	5.7	4.2	5.9
6	HBB:c.-80T>A	4.5	3.4	0.7	4.2	1.4
7	HBB:c.17_18delCT	4.0	3.4	2.8	6.0	2.5
8	HBB:c.316-106C>G	2.5	6.8	3.5	3.5	7.0
9	HBB:c.92+6T>C	1.5	7.0	7.1	4.2	4.6
10	HBB:c.92+5G>C	1.5	1.5	1.4	2.7	1.0
11	HBB:c.93-1G>C	1.5	0.7	–	0.2	0.1
12	HBB:c.112delT	2.5	–	–	–	–
13	HBB:c.251delG	1.5	–	–	–	–

[a]This study, [b]Mendilcioğlu et al. [21], [c]Tüzmen et al. [20], [d]Çürük et al. [23], [e]Beksaç et al. [28].

only Southeastern Turkey. Three frameshift mutations (HBB:c.112delT, HBB:c.135delC and HBB:c.251delG) are considered to be specific for Southeastern Anatolia, Iran and Azerbaijan [16, 30]. Two silent Hb variants (HBB:c.34G>A and HBB:c.208G>A) were detected in this study. Hb Hamilton [β11 (GTT>ATT) Val>Ile] was observed for the first time in Turkey. This HBB variant was discovered in 1984 and it was reported that the substitution did not change the functional properties of the HBB chain [31]. A Val>Ile substitution at position *β*11 should not have much effect on the tertiary or quaternary structure of the hemoglobin molecule; and Hb Hamilton heterozygotes do not present any significant hematological abnormalities. Our case with the combination of Hb Hamilton and β^{+}-thalassemia (HBB:c.93-21G>A) behaves as a β-thalassemia trait with no evidence of anemia. We report a case of HBB:c.208G>A. It is an uncommon and silent Hb variant. The glycine residue at β69(E13) is external to the active center and is not involved in the α1β1 or α1β2 interactions in normal tetramers, and has no direct contact with the heme group. Hb City of Hope [β69 (GGT>AGT) Gly>Ser] was first time reported in combination with β-thalassemia mutations in a Turkish patient in 1989 [32].

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