β-Thalassemia mutations and hemoglobinopathies in Adana, Turkey: results from a single center study

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Abstract

Introduction: β-Thalassemia and hemoglobinopathies are common genetic disorders in Turkey and in this retrospective study our aim was to determine the frequency of β-thalassemia and hemoglobinopathies in Adana, which is one of the biggest cities located in the southern part of Turkey.
Material and methods: Data from 3000 individuals admitted to Seyhan Hereditary Blood Disorders Center in Adana were evaluated. The blood samples were collected into EDTA-containing tubes and hematological parameters were analyzed using an automatic cell counter. High performance liquid chromatography technique was used to determine the type of hemoglobin. Molecular screening of the β-globin gene was performed with β-Globin StripAssay.
Results: Of 3000 cases, 609 were diagnosed as β-thalassemia or hemoglobinopathy. We have found that the rates of occurrence of β-thalassemia and hemoglobinopathies are 13.46% and 6.83% respectively in this area. We have identified 18 different β-thalassemia mutations and three separate abnormal hemoglobins: HbS, HbD Los Angeles, and HbE. In molecular analyses, β-thalassemia gene mutations of IVSI.110 (G>A), codon 8 (–AA), IVSI.1 (G>A), IVSI.6 (T>C), –30 (T>A), IVSII.1 (G>A), codon 39 (C>T), codon 44 (–C), IVSI.5 (G>C), codon 5 (–CT), codon 8/9 (+G), IVSII.745 (C>G), codon 22 (7bp del), –101(C>T), codon 36/37 (–T), IVSI.15 (T>G), codon 6 (–A), –88 (G>A) were detected.
Conclusions: Considering the high incidence of mutations that we have found, β-thalassemia and hemoglobinopathies still seem to be a public health problem in Adana.

Key words: β-Globin StripAssay, β-thalassemia, hemoglobinopathy, mutation, sickle cell anemia.

Introduction

β-Thalassemia (β-thal) syndromes, among the most common hereditary diseases worldwide, are characterized by a genetic deficiency in the synthesis of β-globin chains [1]. The β-thal results from mutations that cause reduced (β^+ type) or absent (β^0 type) β-globin chain synthesis [2]. A total of 393 thalassemia mutations have been reported so far [3].

Hemoglobinopathy is a genetic defect that results in abnormal structure of one of the globin chains of the hemoglobin molecule [4]. Hemoglobin S (HbS, β6 Glu-Val) results from a single base mutation in the β-globin gene, and is the most prevalent abnormal hemoglobin in Turkey [5]. It has been reported that Hemoglobin D (β121 Glu-Gln) is the second most common hemoglobinopathy in Turkey and is distinguished from HbS by its normal solubility and its failure to produce sickling in a deoxygenated state. Hemoglobin E (β26 Glu-Lys), the third most commonly seen abnormal hemoglobin in Turkey, is a thalassemic hemoglobinopathy characterized by microcytosis and hypochromia [5-7].

The β-thal is widespread throughout the Mediterranean Region, in Africa, the Middle East, the Indian subcontinent and Burma, Southeast Asia and Indonesia [8]. Similarly, hemoglobinopathies are common in different ethnic groups and in a broad geographic area including Equatorial Africa, Southern Turkey, Saudi Arabia, Southern India, and Greece [1, 2].

Adana, which is the biggest city located in the Cukurova Region in the southern part of Turkey, has been shown to be one of the top cities in terms of the frequency of β-thal and hemoglobinopathies. Mersin and Antakya, two other cities located in the Cukurova Region, also have a high rate of β-thal and hemoglobinopathies. Following Aksoy *et al*., who initiated scientific investigations on abnormal hemoglobins in Turkey in 1955, data from several studies have been published and the prevalence of sickle cell trait (Hb AS) has been found to be 8.2% in the Cukurova Region [9, 10]. The rate of occurrence of β-thal has been reported to be approximately 4.3% in Turkey [11]. However, in some parts, the rate increases to as high as 10% to 13% [8, 11].

Table I. Frequencies of β-thalassemia mutations in Adana

Mutation	Type	No. of cases	Percentage
IVSI.110 (G>A)	β^+	142	35.14
Codon 8 (–AA)	β^0	37	9.15
IVSI.1 (G>A)	β^0	35	8.66
IVSI.6 (T>C)	β^+	31	7.67
–30 (T>A)	β^+	30	7.42
IVSII.1 (G>A)	β^0	26	6.43
Codon 39 (C>T)	β^0	24	5.94
Codon 44 (–C)	β^0	20	4.95
IVSI.5 (G>C)	β^+	15	3.71
Codon 5 (–CT)	β^0	12	2.97
Codon 8/9 (+G)	β^0	10	2.47
IVSII.745 (C>G)	β^+	9	2.22
Codon 22 (7bp del.)	β^0	4	0.99
–101 (C>T)	β^+	3	0.74
Codon 36/37 (–T)	β^0	3	0.74
IVSI.116 (T>G)	β^0	1	0.25
Codon 6 (–A)	β^0	1	0.25
–88 (G>A)	β^+	1	0.25
Total		404	100

In this retrospective study, we report the number and the type of β-thal mutations and hemoglobinopathies detected in individuals admitted to Seyhan Hereditary Blood Disorders Center (SHBDC) in Adana with a complaint of anemia. Molecular analysis of the β-globin gene was performed using a β-Globin StripAssay. We have identified 18 different β-thal mutations and three different abnormal hemoglobins: HbS, HbD Los Angeles, and HbE.

Material and methods

Data from 3000 individuals, including premarital couples and patients with symptoms of anemia, admitted to SHBDC were restrospectively analyzed. Of these cases, 609 were found to be diagnosed as β-thal or hemoglobinopathy.

Blood samples were collected in EDTA containing tubes and high performance liquid chromatography (HPLC) technique was used to confirm the diagnosis. To perform molecular diagnostic tests, DNAs were isolated from white blood cells by using conventional methods. Mutation analyses for the β-globin gene were evaluated with β-Globin StripAssay (ViennaLab cat. no. 4-120, Austria), which is used for detection of the 22 most common β-thalassemia and hemoglobinopathy mutations in the Mediterranean Region. The StripAssay is based on the reverse-hybridization technique. The β-globin genes of isolated DNAs are amplified *in vitro* by multiplex PCR and labeled with biotin in a single reaction. Subsequently, the amplification products are hybridized to oligonucleotide probes (wild and mutant type) containing the test strip.

Approval from the institutional Ethics Committee of Seyhan Hereditary Blood Disorders Center was provided for this study.

Results

DNA samples from 609 individuals with β-thal or hemoglobinopathies were analyzed and 18 different β-thal mutations and also HbS, HbD Los Angeles, and HbE were identified. Molecular features of the patients are detailed in Tables I–III.

The most frequent mutation of β-thal was found to be IVSI.110 (G>A) in Adana followed by codon 8 (–AA), IVSI.1 (G>A), IVSI.6 (T>C), –30 (T>A), IVSII.1 (G>A), codon 39 (C>T), codon 44 (–C), IVSI.5 (G>C), codon 5 (–CT), codon 8/9 (+G), IVSII.745 (C>G), codon 22 (7bp del), –101 (C>T), codon 36/37 (–T),

Table II. Frequency of hemoglobinopathy mutations in Adana

Mutation	Type	No. of cases	Percentage
HbS	β^S	192	93.65
HbD Los Angeles	β^D	7	3.42
HbE	β^E	6	2.93
Total		**205**	**100**

IVSI.15 (T>G), codon 6 (–A), and –88 (G>A) respectively (Table I). The overall rate of occurrence of β-thal mutations in our group was 13.46% (n = 404).

We detected 205 cases with abnormal hemoglobins out of 3000 individuals (6.83%). One hundred and ninety-two of 205 subjects with abnormal hemoglobins were heterozygous HbS (93.65%) whereas seven and six of these subjects were identified as HbD Los Angeles (3.42%) and HbE (2.93%) respectively (Table II). Therefore, the frequency of HbS heterozygotes among 3000 subjects admitted to our center was found to be 6.4%. The frequency of HbD Los Angeles and HbE in the whole group was 0.23% and 0.2% respectively.

There were a total of 56 cases who were either homozygous or compound heterozygous amongst our subjects. While 40 of 56 cases were diagnosed as sickle cell homozygotes (71.43%), 10 cases were β-thal major (17.87%), 5 cases were compound heterozygotes (8.93%) and 1 subject was HbS/HbD (1.79%) (Table III). In summary, the rate of sickle cell homozygote cases among our group was 1.33%.

Table III. The number and percentage of homozygous and compound heterozygous genotypes in Adana

Genotype	Number	Percentage
HbS/HbS	40	71.43
IVSI.110/HbS	4	7.14
IVSI.6/IVSI.6	3	5.36
IVSII.1/IVSII.1	2	3.57
–30/IVSI.6	2	3.57
Codon 8/HbS	1	1.79
IVSI.1/IVSI.1	1	1.79
–30/–30	1	1.79
IVSI.110/IVSI.110	1	1.79
HbS/HbD Los Angeles	1	1.79
Total	**56**	**100**

Discussion

The β-thals were among the first human genetic diseases to be analyzed by recombinant DNA technology. Today, the molecular pathology of disorders resulting from mutations in the β-globin gene region has been well defined, and point mutations have been found to be the main cause of molecular defects in β-thals [12, 13]. Adana is one of the oldest settlements in Turkey. The first settlers arrived in this region during the Paleolithic period, that is at least 3000 years ago. Since then, Arab, Byzantine, Sumerian, Hittite and Turkish societies have lived here and played important roles in the history of the region, subsequently leading to a complex ethnic structure.

Currently the majority of the population in Adana is of Arab origin, known as "Eti-Turks", whose ancestors immigrated from Syria and Egypt centuries ago. Antakya and Mersin are neighbor cities of Adana and most of the people who live there are also of Arab origin. Therefore, the prevalence of HbS has been found to be considerably high in these areas compared to other parts of Turkey. In addition, consanguineous marriages are seen in a high incidence in these provinces, which also contributes to the increased frequency of HbS and HbE heterozygotes [14, 15].

Aksoy reported the first case with HbS trait in Southern Turkey in 1955 [9]. The first study regarding β-thal was also published by Aksoy *et al*. in 1985 [16]. The prevalence of β-thals varies greatly between different parts of Turkey, however; the overall percentage has been reported to be 4.3% [8, 11, 17, 18]. A screening study conducted by Yüregir *et al*. revealed that the incidences of β-thals and HbS trait were 3.7% and 8.2% respectively in the Cukurova Region [15].

In our study, we performed a screening program mostly for premarital couples at SHBDC to determine the frequency of β-thal and hemoglobinopathies in Adana. We have detected a total of 609 cases with β-thal or hemoglobinopathies. Abnormal hemoglobins consisting of HbS trait in 192 samples (6.4%), 7 of HbD Los Angeles (0.23%) and 6 of HbE (0.2%) and 404 patients with heterozygote thalassemia (13.46%) were found. Although the rate of occurrence of HbS trait that we found is consistent with previous studies, the prevalence of β-thal reported in our study is higher than the results revealed from other studies [6, 7]. Since the SHBDC solely focuses on performing molecular analysis on β-thal and abnormal hemoglobins in Adana, our study population consists of individuals with a family history or high risk of β-thal and hemoglobinopathies. Thus, the high rate of β-thals should not be considered contradictory with previous studies [7, 19].

In Adana, we have identified 18 different β-thal mutations in 404 cases, whereas Altay and Tadmouri reported more than 30 mutations of the globin gene in Turkey [8, 17]. The nine most prevalent β-thal mutations were noted in 89% of our cases (Table I). These results confirm that β-thal mutations are highly heterogeneous, attributed to the ethnic characteristics in different parts of Turkey.

In our study, we have found that the most common β-thal mutation is IVSI.110 (G>A), similar to the data published by Altay [8]. IVSI.110 (G>A) mutation was identified in 142 cases (35.14%) in our cohort, which appears to be related to consanguineous marriages in Adana. We noted that codon 8 mutation, which is present at high frequencies in Azerbaijan [20], is the second most commonly seen defect of β-thal in Adana. This result is found to be different from two large studies performed by and Başak *et al*. [17, 18]. The mutations of –30 and codon 39, commonly encountered in Balkan countries [4, 21, 22], showed rates of 7.48% and 5.98% respectively in this study.

Although HbE is the second most common hemoglobinopathy worldwide, HbD Los Angeles has been reported to be the second most prevalent abnormal hemoglobin seen among Eti-Turks, with a frequency of 0.16-2.4% in our country [23]. Our results with 0.23% of HbD Los Angeles are consistent with those of Güler *et al*. [24].

In conclusion, a variety of PCR-based methods are currently in use for detection of the most common β-thal mutations. We have observed that β-Globin StripAssay provides a simple, rapid and sensitive method to identify β-thal mutations and hemoglobinopathies even in large numbers of cases for screening programs.

Although the Deniz Database in Bogazici University has been used to collect data regarding β-thal mutations from different countries, a national database needs to be established in order to compose a map of the β-globin gene in our country.

Our results indicate that β-thal and hemoglobinopathies still appear to be an important public health problem in Adana. Due to the fact that consanguineous marriages are very common in this area, genetic counseling should be provided for premarital couples to prevent homozygote births.

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