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Molecular and Population Genetic Analyses of β-Thalassemia in Turkey

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In this report we describe the molecular analysis of 795 chromosomes derived from unrelated Turkish β-thalassemia and sickle cell anemia carriers identified in hematology clinics in Istanbul, Ankara, Izmir, Adana, and Antalya. The determination of the molecular pathology of 754 β-thalassemia and 42 abnormal hemoglobin genes and analysis of the frequency distribution in six distinct regions of Turkey was accomplished. The experimental strategy, based on PCR amplification of the β-globin gene, included dot-blot hybridization with 18 probes specific for the Mediterranean populations, denaturing gradient gel electrophoresis, and genomic sequencing. When the regional results are compared with the overall frequency of mutations in the country, it is observed that the frequencies in the western and southern parts of Turkey are in good accordance with the overall distribution, whereas the northern and eastern parts have a more region/population-specific profile with some rare mutations having a significantly high occurrence in these regions. Further evaluation of the data with respect to region- or population-dependent differences will contribute to a better understanding of the mechanisms leading to the marked genetic heterogeneity in Turkey, but could also be extremely valuable in facilitating rapid identification of mutations in families at risk for different hemoglobinopathies. Am. J. Hematol. 57:215–220, 1998. **© 1998 Wiley-Liss, Inc.**

Key words: Turkey; β-thalassemia; sickle cell anemia

INTRODUCTION

β-Thalassemia is an autosomal recessive disorder characterized by microcytosis and hemolytic anemia, which is a result of the reduced synthesis of the β-globin chains of hemoglobin [1]. The disorder affects about 150 million people in the Mediterranean, West Africa, and large parts of Asia [2]. Although there are now more than 180 known β-thalassemia mutations worldwide [3], a smaller collection of alleles accounts for the inactivation of most β-globin genes in each population or ethnic group [4].

Turkey is one of the largest countries of the Middle East occupying the whole of classical Asia Minor (Anatolia) and a small portion of Eastern Thrace in Europe. As a crossroad between Asia and Europe, such a position has allowed extensive intermingling of racially and culturally distinct groups since early prehistoric times.

β-Thalassemia, represented with a gene frequency of 2% in Turkey, is reflected by a wide spectrum of clinical manifestations ranging from β-thalassemia intermedia to severe, transfusion-dependent β-thalassemia major [5]. Recent molecular studies on Turkish β-thalassemia genes revealed the presence of more than 30 different mutations associated with the disorder [6]. Such a great

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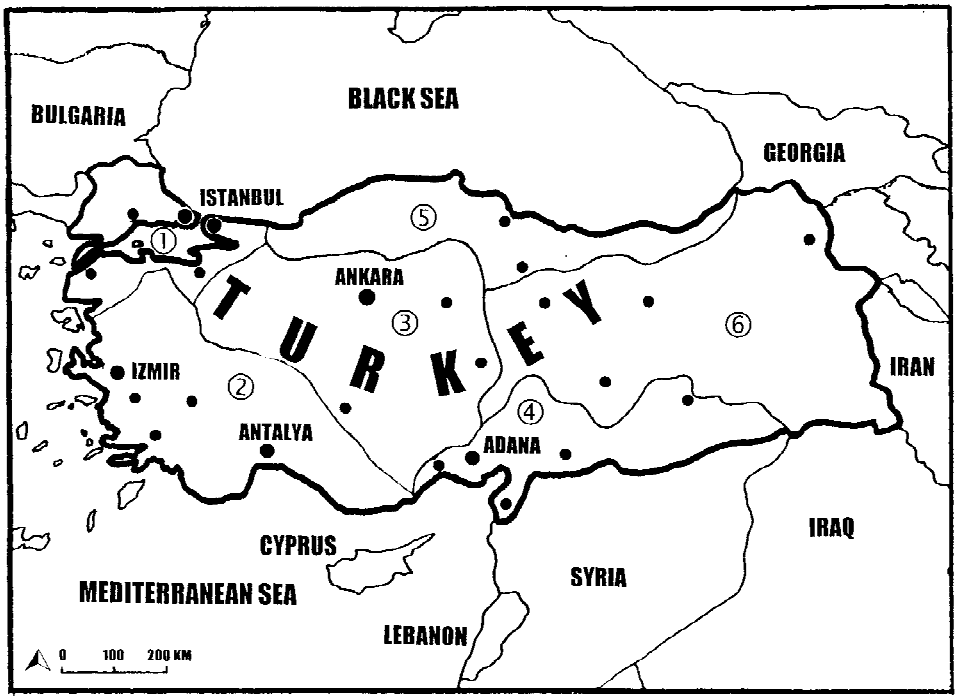

Fig. 1. Map of Turkey showing the six regions studied: ① Marmara region, ② Aegean and Mediterranean region, ③ Central Anatolia, ④ South Eastern Anatolia, ⑤ Black Sea region, ⑥ East Anatolia.

mutational diversity poses rather large problems in designing prevention programs.

This study, including detailed family histories from β-thalassemia and sickle cell anemia (HbS) carriers, aims to trace the precise geographic origins of the various alleles identified in Turkey. We have investigated the molecular pathology of 795 β-thalassemia genes from six different regions of Turkey (Fig. 1) to study the geographic distribution of mutations.

MATERIALS AND METHODS

Probands

A total of 795 randomly selected Turkish β-thalassemia and HbS chromosomes from carriers, who attended different hospitals in Istanbul, Ankara, Izmir, Adana, and Antalya, participated in this study. These medical centers with fully equipped hematology clinics are considered to be largely representative for whole Turkey. The majority of individuals selected were from one hundred different towns in Turkey; the rest consisted of immigrant Turks from the Balkan countries, Cyprus, and the Near East. Special care was given to ensure that the patients were neither pre-selected nor related.

DNA Isolation and PCR Amplification

Blood samples were collected in EDTA-containing tubes. DNA was extracted from white blood cells according to two different methods [7,8]. In vitro amplification of genomic DNA [9] was performed using different sets of primers: one extending from −140 nt 5′ to the gene to IVS-II-70, and the other from IVS-II-597 to 199 nt 3′ to the gene. The resulting segments, 704 and 560 nt, respectively, contain most of the known mutant sites specific for the Mediterranean populations.

Analysis of Mutations

Dot-blot hybridization was performed as described by Diaz-Chico et al. [10]. The oligonucleotide probes used in this study were designed to define the most common mutations known to be present in the Mediterranean Basin and the Near/Middle East. Some of the mutations were detected by either using the amplification refractory mutation system (ARMS; [11]) or the denaturing gradient gel electrophoresis (DGGE) analysis [12]. DNA samples showing migration patterns different from the ones displayed by the common β-thalassemia mutations were subjected to genomic sequencing using the asymmetric amplification procedure [13,14].

RESULTS

In this report we describe the molecular analysis of 795 unrelated chromosomes derived from Turkish β-thalassemia and HbS carriers. Hybridization of DNA samples with 18 oligonucleotide probes and DGGE analysis combined with genomic sequencing allowed the detection of 31 different mutations in the chromosomes investigated. The overall distribution and frequencies of

TABLE I. Frequency and Regional Distribution of β-Thalassemia Mutations and Some Abnormal Hemoglobins in Turkey*

Mutation	Type	Marmara region	Aegean and Mediterranean region	Central Anatolia	South Eastern Anatolia	Black Sea region	East Anatolia	Immigrant Turks	Unknown origin	Overall distribution in Turkey
IVS-I-110 (G-A)	β^+	30 (34.1)	84 (42.4)	58 (52.3)	31 (26.4)	17 (31.0)	16 (27.1)	29 (47.7)	47 (44.4)	312 (39.3)
IVS-I-6 (T-C)	β^+	13 (14.8)	25 (12.6)	8 (7.2)	10 (8.5)	6 (10.9)	6 (10.2)	6 (9.8)	6 (5.7)	80 (10.1)
FSC-8 (-AA)	β^0	7 (8.0)	9 (4.6)	7 (6.3)	5 (4.3)	1 (1.8)	5 (8.4)	4 (6.6)	5 (4.7)	43 (5.5)
IVS-I-1 (G-A)	β^0	8 (9.1)	13 (6.6)	5 (4.5)	1 (0.9)	1 (1.8)	3 (5.1)	6 (9.8)	3 (2.8)	40 (5.0)
IVS-II-745 (C-G)	β^+	4 (4.6)	9 (4.6)	8 (7.2)	7 (6.0)	6 (10.9)	1 (1.7)	4 (6.6)	1 (0.9)	40 (5.0)
IVS-II-1 (G-A)	β^0	3 (3.4)	11 (5.6)	6 (5.4)	3 (2.6)	3 (5.5)	2 (3.4)	—	9 (8.5)	37 (4.7)
Cd 39 (C-T)	β^0	4 (4.6)	5 (2.5)	4 (3.6)	1 (0.9)	2 (3.6)	2 (3.4)	4 (6.6)	8 (7.6)	30 (3.8)
−30 (T-A)	β^+	—	5 (2.5)	2 (1.8)	8 (6.7)	5 (9.1)	5 (8.5)	—	—	25 (3.1)
FSC-5 (-CT)	β^0	3 (3.4)	3 (1.5)	2 (1.8)	4 (3.4)	1 (1.8)	—	—	4 (3.8)	17 (2.2)
FSC-8/9 (+G)	β^0	2 (2.3)	—	2 (1.8)	—	1 (1.8)	3 (5.1)	—	2 (1.9)	10 (1.3)
FSC-44 (-C)	β^0	1 (1.1)	—	2 (1.8)	3 (2.6)	—	1 (1.7)	—	3 (2.8)	10 (1.3)
IVS-I-5 (G-C)	β^+	—	4 (2.0)	3 (2.7)	1 (0.9)	—	1 (1.7)	—	—	9 (1.1)
−87 (C-G)	β^+	1 (1.1)	1 (0.5)	—	—	4 (7.3)	—	—	—	6 (0.8)
Poly A (TAA-TGA)	β^+	—	—	—	1 (0.9)	1 (1.8)	1 (1.7)	—	1 (0.9)	4 (0.5)
FSC-6 (-A)	β^0	1 (1.1)	—	—	1 (0.9)	—	—	—	1 (0.9)	3 (0.4)
IVS-II-848 (C-A)	β^+	1 (1.1)	—	—	1 (0.9)	—	—	1 (1.6)	—	3 (0.4)
IVS-I-116 (T-G)*	β^0	1 (1.1)	—	—	—	—	—	1 (1.6)	—	2 (0.2)
FSC-74/75 (-C)**	β^0	—	—	—	—	—	1 (1.7)	—	—	1 (0.1)
−101 (C-T)*	β^+	1 (1.1)	—	—	—	—	—	—	—	1 (0.1)
−28 (A-C)*	β^+	—	1 (0.5)	—	—	—	—	—	—	1 (0.1)
Cd 15 (G-A)*	β^0	—	—	—	—	1 (1.8)	—	—	—	1 (0.1)
Cd 27 (G-T)*	β^+	—	—	—	—	—	—	1 (1.6)	—	1 (0.1)
3′-UTR (−13 bp)**	β^+	—	—	1 (0.9)	—	—	—	—	—	1 (0.1)
FSC 22–24 (−7 bp)**	β^0	—	—	—	1 (0.9)	—	—	—	—	1 (0.1)
FSC 36/37 (-T)*	β^0	—	—	—	—	—	1 (1.7)	—	—	1 (0.1)
IVS-I-130 (G-A)*	β^0	—	—	—	—	—	—	1 (1.6)	—	1 (0.1)
−290 bp deletion*	β^0	—	—	—	—	—	1 (1.7)	—	—	1 (0.1)
HbS	β^S	—	5 (2.5)	—	28 (23.8)	1 (1.8)	1 (1.7)	1 (1.6)	3 (2.8)	39 (4.9)
HbD Los Angeles	β^D	—	—	—	1 (0.9)	—	—	—	—	1 (0.1)
HbE Saskatoon	β^E	—	1 (0.5)	—	—	—	—	—	—	1 (0.1)
δβ-Thalassemia	δβ	—	—	—	—	—	—	1 (1.6)	—	1 (0.1)
Unknown		8 (9.1)	22 (11.1)	3 (2.7)	10 (8.5)	5 (9.1)	9 (15.2)	2 (3.3)	13 (12.3)	72 (9.1)
Total		88 (11.1)	198 (24.9)	111 (13.9)	117 (14.7)	55 (6.9)	59 (7.5)	61 (7.7)	106 (13.3)	795 (100.0)

*Values indicate the number of chromosomes and values in parentheses indicate percentages. Mutations followed by (*) mark are rare and by (**) are novel.

these mutations are shown in Table I. The IVS-I-110 (G-A) mutation is the most common β-thalassemia defect in Turkey, followed in decreasing order by IVS-I-6 (T-C), frameshift codon 8 (-AA) [FSC-8 (-AA)], IVS-I-1 (G-A), IVS-II-745 (C-G), IVS-II-1 (G-A), Cd 39 (C-T), −30 (T-A), FSC-5 (-CT) lesions, all of which have frequencies above 2%. The β-thalassemia and HbS mutations, identified in the framework of this study, comprise 85.7 and 4.9% of the diseased globin genes we identified in Turkey, respectively, adding up to 90.6%. Three single cases of HbD Los Angeles, HbE Saskatoon, and δβ-thalassemia are also shown to be present (0.3%), which were initially diagnosed on the basis of their hematology. HbD and HbE Saskatoon were confirmed by EcoRI digestion and direct sequencing, respectively. The mutations in 72 out of 795 chromosomes (9.1%) could not be identified so far.

Table I also lists the relative frequencies of the 31 mutations, classified to six different regions of Turkey. With respect to our total number of identified abnormal chromosomes the percentage in each region is: Marmara Region (11.0%), Aegean/Mediterranean Region (24.9%), Central Anatolia (13.9%), Southeastern Anatolia (14.7%), Black Sea Region (6.9%), and East Anatolia (7.5%). Turks from Balkan countries, Cyprus, and the Near East are considered as a seventh distinct group (7.7%). The 106 chromosomes whose origins could not be traced are displayed in a separate column (13.3%; Table I).

DISCUSSION

Consistent with the history of Turkey, which is situated at the meeting point of three continents and stands as

TABLE II. Comparison of the Percentage of the Most Common β-Thalassemia Mutations in Several Populations of the Eastern Mediterranean and Continental Areas

Mutation	Greece	Bulgaria	Turkey	Azerbaijan	Iran	Lebanon	Cyprus
IVS-1-110 (G-A)	42.4	24.2	39.3	20.2	4.7	48.5	79.7
IVS-I-6 (T-C)	7.2	10.2	10.1	7.1	9.4	6.9	6.2
FSC-8 (-AA)	0.6	5.5	5.5	21.2	—	3.4	0.2
IVS-I-1 (G-A)	13.2	3.1	5.0	2.0	4.7	9.8	5.9
IVS-II-745 (C-G)	6.9	10.2	5.0	3.1	4.7	3.4	5.5
Cd 39 (C-T)	17.0	21.9	3.8	2.0	1.6	1.5	2.4
FSC-8/9 (+G)	0.3	5.5	1.3	2.0	7.8	—	—
Others	12.4	19.4	30.0	42.4	67.1	26.5	0.1
References	[16]	[17]		[18]	[19]	[20–23]	[24]

a crossroad between Asia and Europe, the country has attracted migrations of different populations; thus it has an ethnic diversity unparalleled in any other country of the Mediterranean region. This admixture is the probable cause of the large number of β-thalassemia mutations observed in Turkey.

Since β-thalassemia is the most common single gene defect in Turkey, many studies have been conducted to investigate its molecular basis [6]. Previously, only one study was known to aim at defining the regional distribution of β-thalassemia mutations in Turkey [15]. However, that study did not reveal a notable region-specificity, most probably due to the arbitrary division of the map of Turkey, which was not according to the more usual regional demarcations. In addition, the number of chromosomes analyzed was only 139, and only some of the most common mutations were considered.

In this study the IVS-I-110 (G-A) substitution, a severe β^+ mutation, was the most frequent allele in all regions. However, notable occurrences concerning the frequency of this mutation in the different regions studied are observed. The highest frequencies were recorded in the central (52.3%) and western regions of Turkey (34.1–42.4%), whereas these figures decline to their lowest values in eastern Turkey (26.4–26.6%). The IVS-I-110 mutation is known to be an eastern Mediterranean defect (Table II); this fact may explain why the frequency of this mutation tends to be low in the eastern inlands of Turkey. The IVS-I-6 (T-C) mutation is equally distributed in the different Turkish regions discussed. This is in complete accordance with the data known about the countries neighbouring Turkey (Table II). The dinucleotide deletion at codon 8, originally detected in a Turkish patient [25], is observed to occur mostly in East Anatolia (8.3%) and the Marmara region (8.0%). Interestingly, this mutation is present at high frequencies in Azerbaijan [18], which could account for its strong presence in East Anatolia. The recent general pattern of migration from the rural eastern provinces towards the coal and metallurgical districts in the Northwest may also explain the relatively high presence of the same mutation in the Marmara region. The Cd 39 (C-T) mutation is mainly found in Turkish citizens residing in the Marmara region (4.6%) as well as in those originating from the Balkan countries, namely Greece, Bulgaria, Romania, former Yugoslavia, and Albania (6.6%). Less frequent incidences were observed elsewhere in Turkey (Table I). This may be due to the fact that this mutation is primarily a western Mediterranean abnormality [26]. A similar observation is noted for the G-A substitution at IVS-I-1, seen mainly in the belt extending from Czech/Slovakia [27] to Egypt [28] through Hungary [29], former Yugoslavia [30], Greece [16], and Cyprus [24]. This, most likely, accounts for the higher representation of this mutation in the Marmara (9.1%), Aegean and Mediterranean regions (6.6%) and in Turkish people originating from the Balkan countries (9.8%; Table I). The severe β^+ mutation at IVS-II-745 (C-G) is mainly confined to the Turkish coasts extending from the Black Sea, Marmara Sea, Aegean Sea, to the Mediterranean Sea (Table I). The presence of the IVS-II-745 mutation in these regions reflects the route that was followed during the 12th and 13th centuries by the hundreds of thousands of Crusaders heading to Jerusalem, who may have introduced new genetic elements in these parts of the country. Another interesting observation is the significant concentration of the rare β^+ promoter mutation T-A at position −30 in the Eastern and Northern regions of Turkey where it is observed in frequencies ranging from 6.7–9.1%, a figure not observed anywhere else in the world. This suggests that this mutation may have originated in this region. The prevalence of the FSC 8/9 (+G) mutation in East Anatolia (5.0%), however, may be a result of it being present proximal to what was known as the Great Silk Road, which extends from Xian in China through the Indian Subcontinent to Iran. In addition to the common mutations, three novel and eight rare lesions were detected in the framework of this study, using genomic sequencing (Table I) [31–33]. Approximately 9% of the chromosomes investigated in this study remained unidentified. Their analysis will be the topic of another publication.

Most of the diagnosed HbS genes belong to the South East Anatolia region where the gene occurs at a frequency of 23.8%. A certain part of this region is mainly

inhabited by a population who can speak Arabic; the high incidence of HbS disease among Eti-Turks living in the South of Turkey was first shown by Aksoy [34] who had speculated that Eti-Turks had immigrated into Turkey from Syria and Egypt [35]; 25 years later, this hypothesis was proven to be valid [36].

A comparison of the different regions of Turkey shows that the distribution of mutant alleles differs within each geographic area with marked local variations in their prevalence. When the regional results are compared with the overall frequency of mutations in the country, it can be noticed that the western and southern parts of Turkey, occupied by at least 50% of Turkey's population, are in good accordance with the overall distribution, whereas the northern and eastern parts have a more region/population-specific profile. The ethnic identities of the latter regions seem to be more preserved than the western and southern coastal parts of the country, which display a greater heterogeneity. On the other hand, although less heterogeneous, the northern, eastern, and southeastern parts of Turkey seem to have their own battery of mutations, e.g., −30, −87, FSC-8/9, and IVS-II-745 have a higher occurrence in these regions. Another notable feature of these three regions, especially of East and Southeastern Anatolia, is the relatively high number of unidentified mutations; this may be explained by the possible presence of rare and novel lesions in these isolated areas, in which consanguinity is practiced extensively also.

Human settlement in Anatolia has been traced back well before 7,000 BC. It was penetrated, settled, or ruled by Hittites, Phrygians, and Gauls from the North and Northwest, by Greeks and Macedonians from the West, and by Parthians and Mongols from the East. The most decisive influence was the incursion of the Ottomans from the East who introduced a new element of mixed Mediterranean-Mongoloid origin into the country's ethnic composition. They further contributed to the racial mixture, particularly during their Empire's decline, when many Muslim groups living in former Turkish territories in Southeastern Europe and in countries around the Black Sea migrated to the home country. Throughout the history, the waves of all of the above-mentioned immigrants, doubtless, have genetically influenced the resident population of today's Turkey.

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