History and Origin of β-Thalassemia in Turkey: Sequence Haplotype Diversity of β-Globin Genes

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Abstract In the present study we report the sequence haplotypes associated with 22 β-globin gene mutations present in Turkey. Nine nucleotide polymorphisms and an $(AT)_xT_y$ motif located at the 5′ end of the β-globin gene form the sequence haplotypes that were investigated in 204 unrelated β-thalassemia and wild-type chromosomes from Turkey. Twelve sequence haplotypes were observed in the chromosomes analyzed and haplotypic heterogeneity was found in the wild-type β-globin genes. Samples from the Black Sea region demonstrated a remarkable level of haplotypic heterogeneity in contrast to the homogeneity present in Central Anatolian samples. Of the 22 β-globin mutations analyzed, 18 were related with single sequence haplotypes. This simple association led to the attempt to determine the origin of these mutations by comparing their frequencies in Turkey with those in other countries and/or the world distribution of the haplotypes carrying them. However, the presence of several exceptions for the "one haplotype/one mutation" rule showed that the β-globin gene cluster is far from static. Each of the IVS-I-110 (G→A), Cd 39 (C→T), IVS-I-6 (T→C), and –30 (T→A) β-globin mutations was associated with a minimum of two sequence haplotypes. This fact is best explained by the likelihood of strong recombination mechanisms taking place, rather than by assuming multiple origins for each of these alleles. According to our results, malarial selection for the oldest β-thalassemia allele in Anatolia (i.e., IVS-I-110 G→A) may have occurred between 6500 and 2000 B.C. From that date on, most of the common β-thalassemia mutations in Turkey were established, and by the 13th century A.D. most of them were brought to frequencies close to those observed at present.

β-thalassemia is an autosomal recessive disorder characterized by microcytosis and hemolytic anemia resulting from a variety of molecular defects that intervene with the normal synthesis of the β-globin chains of hemoglobin (Weatherall and Clegg 1981). β-thalassemia constitutes one of the most serious health problems

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worldwide, primarily in regions of the world endemic for malaria (WHO 1983). The high prevalence of β-thalassemia in malaria-infested regions led Haldane (1949) to propose that heterozygous carriers for this disorder are less susceptible to severe malarial infection, since cells containing abnormal hemoglobins are not very conductive to the proper development of the malarial parasite.

Research on β-thalassemia in Turkey began in 1941 (Aksoy 1991). However, it was only in 1971 that the overall prevalence of β-thalassemia carriers in Turkey was reported to be 2% (Çavdar and Arcasoy 1971). Since 1987, several studies have been conducted in order to elucidate the molecular basis of β-thalassemia in Turkey (Akar et al. 1987; Diaz-Chico et al. 1988; Gurgey et al. 1989; Schnee et al. 1989; Aulehla-Scholz et al. 1990; Oner et al. 1990; Başak et al. 1992a; Atalay et al. 1993; Altay and Başak 1995; Nisli et al. 1997; Tadmouri et al. 1998a). In a previous attempt to study the origin of β-thalassemia in Anatolia, we compared the frequencies of β-globin mutations in different regions of Turkey with those derived from neighboring countries. The analysis provided evidence for a remarkable genetic stratification of the Turkish people and for preservation of ethnic identities in the eastern parts of Anatolia (Tadmouri et al. 1998a).

In recent years, DNA sequence variation in the intergenic domain upstream of the β-globin gene has attracted increased attention. Several studies have been conducted in the last few years, most of which have attempted to deduce the possible origins of some β-globin gene mutations through the analysis of several nucleotide polymorphisms and the $(AT)_xT_y$ motif located at the 5′ end of the β-globin gene (Trabuchet et al. 1991a; Trabuchet et al. 1991b). Because of its small size and its relative variability, this polymorphic region was implemented to analyze the evolution of different β-thalassemia mutations in limited samples from Algeria, Sardinia, Sicily, and Turkey (Tadmouri et al. 1998a; Perrin et al. 1998; Tadmouri et al. 1998b; Tadmouri et al. 1999; unpublished data). To our knowledge, there has been no systematic attempt to account for the high frequencies of the different β-thalassemia alleles in Turkey using combined mutation/haplotype analysis. Here we present extensive analysis of 191 β-thalassemia and 13 wild-type chromosomes from Turkey using the above strategy. Our study provides information on the history and origin of the different β-thalassemia mutations, population movements, and the relationships between the different affected groups in the country. To improve the drawing of a geographic map, results are also compared with similar data from other countries and data from the literature in relation to the geographic and historical backgrounds of the Turkish people.

Subjects and Methods

Subjects. A total of 191 unrelated β-thalassemia chromosomes from Turkey were analyzed in this study. Most of the β-thalassemia chromosomes were previously included in a study on the regional distribution of different β-thalassemia alleles in Turkey (Tadmouri et al., 1998a). Chromosomes were grouped according to their geographical origins, which included the Marmara region (MR; 15),

Black Sea region (BSR; 19), Aegean-Mediterranean region (AM; 30), Central Anatolia (CA; 33), Southeast Anatolia (SEA; 25), East Anatolia (EA; 18). Additional groups included immigrants from the Balkan countries and Cyprus (BLK; 21) and chromosomes of undefined origins (unknown; 30). Of the 191 β-globin chromosomes investigated, 181 carry the following 19 β-thalassemia mutations: IVS-I-110 (G→A), IVS-I-6 (T→C), -30 (T→A), Cd39 (C→T), -87 (C→G), FSC-8/9 (+G), IVS-I-5 (G→C), IVS-I-1 (G→A), FSC-8 (–AA), IVS-II-1 (G→A), IVS-II-745 (C→G), Cd27 (G→T; Hb Knossos), FSC-5 (–CT), IVS-I-116 (T→G), FSC-74/75 (–C), Cd15 (G→A), IVS-II-654 (C→T), IVS-I-130 (G→A), and IVS-II-848 (C→A), as well as three abnormal hemoglobins, Hb Saskatoon, Hb S, and Hb D Los Angeles, were encountered. In the remaining 10 chromosomes the molecular defect could not be characterized. Additionally, 13 unrelated wild-type β-globin chromosomes from Turkey were also included for comparative analysis.

Molecular Analysis. The polymorphic DNA sequence located 400 base pairs (bp) upstream of the β-globin genes of homozygous thalassemia patients was amplified by the polymerase chain reaction (PCR) using primers B and D as described by Perrin et al. (1998). Each DNA was amplified twice independently. The chromosomes of heterozygous patients carrying different β-globin mutations were selectively amplified using the Amplification Refractory Mutation System (ARMS) (Newton et al. 1989) as described by Perrin et al. (1998). The resulting amplification products were purified using QIAquick PCR Purification Kit (Qiagen, Germany). Four microliters of the purified product were then subjected to manual (Sequenase Version 2.0, Amersham, USA; ^{35}S-dATPαS, Amersham, USA or Izotop, Hungary) (Perrin et al. 1998) and/or automatic fluorescent sequencing techniques (PRISM™ Ready Reaction Dye Deoxy Terminator Cycle Sequencing Kit, Applied Biosystems, Inc., USA; ABI Prism 370 DNA Sequencer, Perkin-Elmer). The 770-bp fragment (B/D) was sequenced with primers D, A (Trabuchet et al. 1991a) on one strand and with primers B, C, and Q on the other strand (Perrin et al. 1998). Nine polymorphic nucleotides and one hypervariable microsatellite of the composite structure $(AT)_xT_y$ were analyzed. Each combination of specific polymorphic sites defines a sequence haplotype.

Results

Analysis of the polymorphic region of the globin gene described above demonstrates the presence of 12 sequence haplotypes in the 204 β-thalassemia and wild-type chromosomes investigated in this study (Table 1).

Sequence Haplotypes Associated with β-Thalassemia Chromosomes. Eleven of the 12 sequence haplotypes are associated with 191 β-thalassemia chromosomes. Haplotypes *HT2, HT5, HT15, HT16,* and *HT17* have been found thus far only in β-thalassemia chromosomes (Table 1). The two most frequent sequence haplotypes, *HT1* and *HTR,* account for 47.1% and 34.6% of β-thalassemia chro-

Table 1. Sequence Haplotypes Observed in 204 β-Thalassemia and Wild-Type β-Globin Genes from Turkey Compared to the Ancestral Human Sequence Haplotype

-1069	*-989*	*-780*	*-710*	*-703*	*-551*	*-543*	*$(AT)_xT_y$*	*-521*	*-491*	*Haplotype*	*Occurrence*	
A	*C*	*T*	*T*	*T*	*T*	*T*	*?*?*	*C*	*C*	*HTAnc*	*β-*	*Wild-*
*	*G*	*A*	*	*	*	*	*8*4*	*	*	*HTBenin*	*Thal*	*Type*
G	*	A	*	*	*	C	7*7	*	A	*HTR*	+	+
*	*	A	*	*	*	C	7*7	*	A	*HT1*	+	+
*	*	A	*	C	C	C	9*5	*	A	*HT2*	+	–
G	*	A	*	C	C	C	11*3	*	A	*HT3*	+	+
G	*	A	*	C	C	C	7*7	*	A	*HT4*	+	+
G	*	A	*	C	*	C	7*7	*	A	*HT5*	+	–
*	*	A	*	*	C	C	7*7	*	A	*HT7*	+	+
G	*	A	*	C	C	C	9*5	*	A	*HT8*	+	+
G	*	A	*	*	C	C	9*5	*	A	*HT9*	–	+
*	G	A	*	*	*	C	8*4	*	*	*HT15*	+	–
G	*	A	*	*	*	C	8*4	T	A	*HT16*	+	–
G	G	A	*	C	C	C	9*5	*	A	*HT17*	+	–

Note: HTAnc is the ancestral human sequence haplotype and HTBenin is the HB S Benin sequence haplotype, both described by Trabuchet et al. (1991a). HTR is the sequence haplotype corresponding to the reference sequence described by Poncz et al. (1983). The (*) symbol indicates homology with the corresponding ancestral haplotype position (HTAnc). (+) and (–) symbols indicate the presence or absence of the sequence haplotype in β-thalassemia and wild-type chromosomes.

mosomes, respectively, for a total of 81.7% (Table 2). Haplotype *HTR* is associated with the largest variety of β-globin gene mutations (11; Table 3). Sequence haplotypes *HT4* and *HT1* are associated with five and four different β-thalassemia mutations, respectively. Each of the haplotypes *HT2, HT3, HT5, HT8, HT15, HT16,* and *HT17* is associated with one mutation. The IVS-I-110 (G→A) mutation is linked to six haplotypes. Each of the mutations Cd 39 (C→T), IVS-I-6 (T→C), and –30 (T→A) is related to two haplotypes.

The remaining 18 mutations have associations with only a single haplotype at a time (Table 3). Each of five mutations has its private genetic background; the remaining 13 mutations share similar sequence haplotypes. Hence, this type of "one mutation/one haplotype" association demonstrates that none of these mutations has witnessed a recombination event since it was introduced in the Turkish population.

Sequence Haplotypes Associated with Wild-Type Chromosomes. The 13 wild-type chromosomes analyzed in this study exhibit seven sequence haplotypes. The four most frequent sequence haplotypes (*HTR, HT1, HT4,* and *HT7*) account for 77.1% of wild-type chromosomes, *HTR* being the most common with

Table 2. Regional Distribution of Sequence Haplotypes Present in 191 Turkish β-Thalassemia Chromosomes

Haplotype	*BLK*	*MR*	*BSR*	*AM*	*CA*	*SEA*	*EA*	*Unknown*	*No. of Chromosomes*
HT1	11 (52.3)	5 (33.3)	5 (26.3)	16 (53.3)	22 (66.7)	11 (44)	7 (38.9)	13 (43.3)	90 (47.1)
HTR	7 (33.3)	6 (40)	6 (31.6)	10 (33.3)	9 (27.3)	11 (44)	6 (33.3)	11 (36.7)	66 (34.6)
HT4	1 (4.8)	2 (13.3)	1 (5.3)				4 (22.2)	4 (13.3)	12 (6.3)
HT17			2 (10.5)	1 (3.3)	1 (3)	1 (4)	1 (5.6)	2 (6.7)	8 (4.2)
HT3			2 (10.5)		1 (3)				3 (1.6)
HT7	1 (4.8)			1 (3.3)		1 (4)			3 (1.6)
HT8			3 (15.8)						3 (1.6)
HT15									2 (1.0)
HT16				2 (6.7)					2 (1.0)
HT2	1 (4.8)								1 (0.5)
HT5						1 (4)			1 (0.5)
Total	21 (11.0)	15 (7.9)	19 (10.0)	30 (15.7)	33 (17.2)	25 (13.1)	18 (9.4)	30 (15.7)	191 (100)

Note: BLK, Balkan countries and Cyprus; MR, Marmara region; BSR, Black Sea region; AM, Aegean-Mediterranean region; CA, Central Anatolia; SEA, Southeast Anatolia; EA, East Anatolia. Numbers in parentheses indicate percentages.

Table 3. β-Thalassemia Mutations in Turkey, Corresponding Sequence Haplotypes, and Regional Distribution

Mutation	*Haplotype*	*BLK*	*MR*	*BSR*	*AM*	*CA*	*SEA*	*EA*	*Unknown*	*Total*
IVS-I-110 (G-A)	*HT1*	8	3	4	16	20	9	7	10	77 (87.5)
	HTR			1	1	1	1			4 (4.6)
	HT3			2		1				3 (3.4)
	HT4								2	2 (2.3)
	HT5						1			1 (1.1)
	HT7	1								1 (1.1)
IVS-I-6 (T-C)	*HTR*	3	4		4	3	4	2	1	21 (91.3)
	HT15		2							2 (8.7)
Cd39 (C-T)	*HT1*	1	2			2	1		3	9 (56.2)
	HTR	2		1					4	7 (43.8)
-30 (T-A)	*HTR*			4	2			2	1	9 (90)
	HT1						1			1 (10)
IVS-II-1 (G-A)	*HT17*			2	1	1	1	1	2	8
IVS-II-745 (C.-G)	*HTR*					2	4			6
FSC-8 (-AA)	*HTR*				2	2		1		5
IVS-I-1 (G-A)	*HTR*	1	1			1			2	5
FSC-8/9 (+G)	*HT4*		2					1		3
-87 (C-G)	*HT8*			3						3
IVS-I-116 (T-G)	*HT1*	2								2
IVS-I-5 (G-C)	*HT16*				2					2
FSC-5 (-CT)	*HT7*				1					1
Hb D Los Angeles	*HT7*						1			1
Cd 15 (G-A)	*HT4*			1						1
HB E Saskatoon	*HT4*								1	1
IVS-I-130 (G-A)	*HT4*	1								1
IVS-II-654 (T-C)	*HT2*	1								1
IVS-II-848 (C-A)	*HTR*								1	1
FSC-74/75 (-C)	*HTR*							1		1
Cd 27 (G-t)	*HTR*	1								1
HB S	*HTR*						1			1
Unknown	*HTR*		1		1		1		2	5 (50)
	HT4							3	1	4 (40)
	HT1			1						1 (10)
Total		21	15	19	30	33	25	18	30	191

Note: BLK, Balkan countries and Cyprus; MR, Marmara region; BSR, Black Sea region; AM, Aegean-Mediterranean region; CA, Central Anatolia; SEA, Southeast Anatolia; EA, East Anatolia. Numbers in parentheses indicate percentages.

30.9% (Table 4). Sequence haplotype *HT9* is exclusively observed in wild-type β-globin chromosomes (Table 1).

Geographical Distribution of Sequence Haplotypes. Geographically speaking, samples from the Black Sea region present seven sequence haplotypes. Turkish chromosomes from the Balkan countries and Cyprus exhibit six sequence

Table 4. Regional Distribution of Sequence Haplotypes Present in 13 Turkish Wild-Type β-Globin Chromosomes

Haplotype	*BLK*	*MR*	*BSR*	*EA*	*Unknown*	*Total*
HTR	3 (60)				1 (20)	4 (30.9)
HT1	1 (20)				1 (20)	2 (15.4)
HT4				1 (50)	1 (20)	2 (15.4)
HT7					2 (40)	2 (15.4)
HT9			1 (100)			1 (7.7)
HT3	1 (20)					1 (7.7)
HT8		1 (100)				1 (7.7)
Total	5	1	1	1	5	13 (100)

Note: BLK, Balkan countries and Cyprus; MR, Marmara region; BSR, Black Sea region; EA, East Anatolia. Numbers in parentheses indicate percentage frequency of the corresponding haplotype.

haplotypes. Samples from the Marmara region, the Aegean-Mediterranean region, Southeast Anatolia, and samples of unknown origin each exhibit five different sequence haplotypes. Samples from East and Central Anatolia present four sequence haplotypes for each region (Table 2, Table 4).

Discussion

By April 1999, the total number of β-thalassemia alleles described in the Turkish population was ~40 (Tadmouri 1999), and this number can be considered as a testimony of past settlements in Asia Minor. The comparison of regional patterns of these mutations served to throw light on the relationship between residents of different regions of Turkey (Tadmouri et al. 1998a). The present study combines the frequency data of β-globin mutations and their sequence haplotypes among Turkish people, providing insights into the distribution, history, and origin of β-thalassemia in Turkey.

Common Mutations Associated with a Single Sequence Haplotype. The origin of these mutations can be assigned by (1) comparing their frequencies in Turkey with those in other countries, and/or (2) examining the world distribution of the haplotypes carrying them.

- The mutation IVS-II-1 (G→A) occurs on the private sequence haplotype *HT17* (Table 3). This mutation is observed at variable frequencies in many countries of the world with a fairly homogenous distribution (Tad-

mouri 1999; Huisman et al. 1997). Data from restriction fragment length polymorphism (RFLP) haplotypes indicate the association of this mutation with at least four backgrounds in different populations. This observation lead Chifu et al. (1992) to suggest multiple origins for this mutation. Similarly, the IVS-I-5 (G→C) mutation, a common β-thalassemia allele in Southern Asia and associated with nearly a dozen RFLP haplotypes (Flint et al. 1993), occurs in Turkey on the private sequence haplotype *HT16*, not seen in European and Mediterranean populations (unpublished data). RFLP analysis of the present IVS-II-1 and IVS-I-5 chromosomes should shed more light on the nature of the RFLP/sequence haplotype association.

- The FSC-8 (–AA), IVS-I-1 (G→A), IVS-II-745 (C→G), IVS-II-848 (C→A), FSC-74/75 (–C), and Cd27 (G→T) mutations share the same sequence haplotype *HTR*. As in Turkey, *HTR* is the most common sequence haplotype in many wild-type chromosomes of Mediterranean origin (unpublished data). The restricted geographical distribution of these mutations, found mostly in the Eastern Mediterranean (Huisman et al. 1997), favors a recent unicentric origin for each of them, the origin being confined to the areas where they occur most frequently.
- The high occurrence of the FSC-8/9 (+G) in Eastern Anatolia may be because of its introduction to Turkey from Southern Asia (Tadmouri et al. 1998a; Huisman et al. 1997). The same route might have been followed by the Cd15 (G→A) mutation, since it carries the same sequence haplotype as FSC-8/9 and occurs in patients of Eastern Asian ancestry (Huisman et al. 1997).
- The –87 (C→G) promoter mutation occurs on a sequence haplotype that is restricted to normal β-globin chromosomes from Turkey and Algeria. Frequency distribution data of this mutation favor a local origin for the mutation in Black Sea populations (Tadmouri et al. 1998a; Huisman et al. 1997; Petkov et al. 1990).
- The distribution of FSC-5 (frequent in Palestine, Turkey, Bulgaria, Tunisia, and United Arab Emirates) and Hb D Los Angeles (seen in sporadic cases all over the world) mutations favors an Eastern Mediterranean origin (Tadmouri et al. 1998a; Huisman et al. 1997). These two mutations occur on sequence haplotype *HT7*, commonly observed in wild-type chromosomes from Greece, Cyprus, and Turkey (Table 4).
- Most of the Hb S genes in Turkey occur in an Arab-speaking group (Eti-Turks) that lives in Southeast Anatolia where carrier frequencies of 22.8% are recorded (Kocak et al. 1995). It is commonly known that Hb S genes in Turkey are of the "Benin" type that is associated with specific RFLP (Benin) and sequence haplotypes (*HT1*; Trabuchet et al. 1991a; Aluoch et al. 1986; Table 1). However, the uncommon presence of a Turkish Hb S chromosome on haplotype *HTR* (Table 3) might demonstrate a new variant for this hemoglobinopathy that may be specific for

Turkey. Analysis of a large number of Turkish Hb S chromosomes and of those from neighboring countries would clarify this issue.

Mutations Associated with Multiple Sequence Haplotypes.

- The IVS-I-110 (G→A) mutation is related to six sequence haplotypes in Turkish people, whereas only two sequence haplotypes were found associated with Algerian IVS-I-110 chromosomes (Perrin et al. 1998). Approximately 87% of Turkish IVS-I-110 chromosomes occur on sequence haplotype *HT1* (Table 3). Preliminary data of a detailed sequence haplotype analysis for this mutation in 12 Mediterranean and European populations, to be presented elsewhere, suggest a probable Anatolian origin for this allele that goes back to the time of early agricultural practice in the region.
- In accordance with other Mediterranean populations (Perrin et al. 1998), the nonsense mutation at codon 39 (C→T) occurs on two sequence haplotypes in Turkey: *HT1* (~56%) and *HTR* (~44%; Table 3). Analysis of Cd39 chromosomes from Algeria demonstrated the relation of this mutation to four sequence haplotypes (Perrin et al. 1998). Studies of RFLP haplotypes associated with this same mutation have shown its linkage to 14 haplotypes, six of which were described in Tunisians (Flint et al. 1993). These two independent observations strengthen the hypothesis of a West Mediterranean origin for this mutation (Perrin et al. 1998).
- The IVS-I-6 mutation, present at equal frequencies in the Mediterranean, occurs in Turkey on sequence haplotype *HTR* (91.3%) and the private sequence haplotype *HT15* (8.7%). Of the six RFLP haplotypes associated with this mutation in the Mediterranean, five are described in Lebanese and Turkish people (Flint et al. 1993), hence demonstrating an older origin for this wild mutation in the Eastern Mediterranean.
- The –30 (T→A) promoter mutation, which occurs at high frequencies in East Anatolia (7%–9%) (Tadmouri et al. 1998a), is associated with two sequence haplotypes: *HTR* (90%) and *HT1* (10%; Table 3). The mutation is rarely observed in Mediterranean populations. The association of the –30 mutation with *HT1* might favor a considerably old age for this allele, strongly arguing for a Turkish origin. Low frequencies of this mutation in the Mediterranean may be explained by a recent spread out of Anatolia, and its presence in Tunisians (6.8%) (Huisman et al. 1997) could be linked to the Ottoman influence in the region between the 16th and 19th centuries.

Collectively, the multiple associations observed in the IVS-I-110, Codon 39, IVS-I-6, and –30 mutations can be explained by recombination events rather than by independent multiple origins for each mutation. Perrin et al. (1998) have suggested that a variety of gene conversion and recombination mechanisms are

possible factors leading to the heterogeneity of the mutation/haplotype associations. Some of these mechanisms include the conversion of –200 base pairs of DNA sequence including parts of the first two exons of the β-globin gene, thus shifting any possible mutation to a new sequence haplotype. Other mechanisms involve single recombinations that occur at the sequences upstream of the β-globin gene or within the first exon of the gene.

Population Genetics of the Turkish People. World data indicate that in regions where malaria and β-thalassemia are less common a variety of β-thalassemia mutations is found, whereas in regions with long histories of exposure to malaria only a small number of alleles account for most of the β-thalassemia genes (Huisman et al. 1997). The geographical distribution of β-thalassemia mutations in different regions of Turkey reflects a decreasing gradient of mutation numbers when moving from East to West Anatolia (Tadmouri et al. 1998a) (Figure 1). East Anatolia is a rugged region not conducive to the proper development of the malarial parasite. In contrast, the landscape of Thrace and West Anatolia is simpler and more suitable for the development and transmission of malaria. Interestingly, this mutation/malaria gradient can also be mirrored by the results of surveys conducted in Turkey between 1980 and 1995 that noted a regional β-thalassemia trait frequency gradient increasing from East (3.4%) to West Anatolia and Thrace (11%) (Kocak et al. 1995; Aksoy et al. 1980; Aksoy et al. 1985; Kürkçüoglu et al. 1986; Bircan et al. 1993).

Several mutations described in eastern Turkey are thought to be of Asian Indian origin (e.g., FSC-8/9, IVS-I-5, Cd15, and others). Throughout history, this

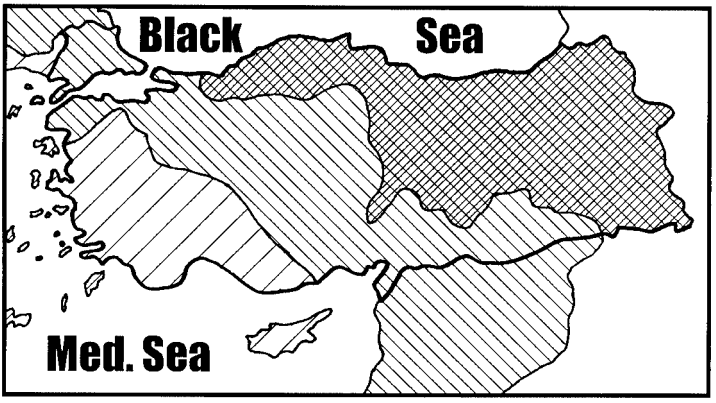

Figure 1. Differences in β-thalassemia mutation heterogeneity in Turkey and neighboring countries (mutation/chromosome ratio). Note the decreasing heterogeneity from the eastern to western regions of the country. Adapted from data of Kattamis et al. (1990); Petkov et al. (1990); Baysal et al. (1992); Adekile et al. (1994); El-Hazmi et al. (1995); Traeger-Synodinos et al. (1998); Tadmouri (1998); Tadmouri et al. (1998a). ⊠ : 1 mutation/<6 chromosomes (more heterogeneous). ▧ : 1 mutation/6–10 chromosomes. ◰ : 1 mutation/>10 chromosomes (less heterogeneous).

area has acted as a buffer zone separating the Caucasoid and Indian groups since their divergence 10,000 to 35,000 years ago (Cavalli-Sforza et al. 1996). When these groups witnessed the appearance of their specific types of β-thalassemia mutations, these alleles diffused slowly from east and west through the rugged land of East Anatolia to form the mosaic presently observed (Tadmouri et al. 1998a).

Sequence haplotype analysis in the present study exhibits a different picture for the Turkish population with a discontinuous gradient of sequence haplotype diversity observed in the different regions of the country (Figure 2). Central Anatolia demonstrates a relative haplotypic homogeneity. This region is mainly a plateau that is bordered on the north, south, and east by high mountains that may have isolated Central Anatolians and conserved them from admixture with different migratory groups that passed by the coastal areas of Turkey. On the other hand, the heterogeneous composition of subjects originating from the Black Sea region may be explained by the fact that since A.D. 1849 people from different parts of Anatolia have increasingly moved to mining centers in the area. This flow was fortified by the introduction of genetic elements from former Turkish territories around the Black Sea during the decline of the Ottoman Empire early in this century (The New Encyclopaedia Britannica 1991; Tadmouri 1999). In between these two extremes lie the rest of the Turkish groups, residents of Thrace, Marmara, East, Southeast, and West Anatolia.

A Brief History of Anatolia and β-Thalassemia. Anatolia has a very long and complex history. The early inhabitants of Anatolia were physically very sim-

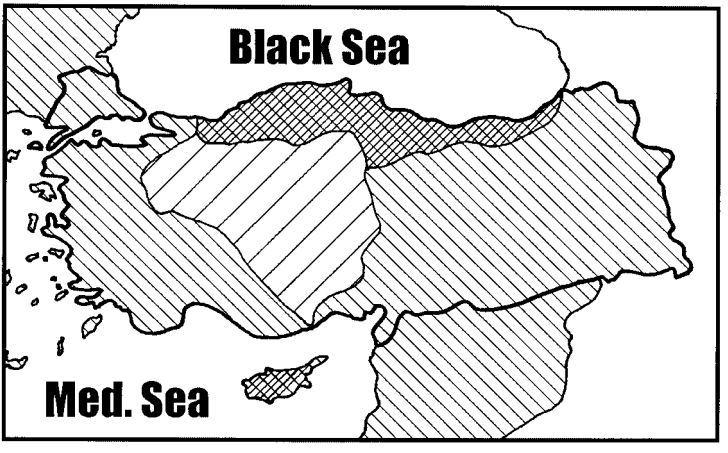

Figure 2. Differences in sequence haplotype heterogeneity in β-thalassemia and wild-type β-globin chromosomes from Turkey and neighboring countries (haplotype/chromosome ratio). Note the haplotype heterogeneity in the Black Sea Region (Northern Turkey) and in the island of Cyprus. ⊠ : 1 haplotype/1–3 chromosomes (more heterogeneous). ⧅ : 1 haplotype/4–6 chromosomes. ⧄ : 1 haplotype/>6 chromosomes (less heterogeneous).

ilar to the Alpine subrace of Europe, akin to the Sumerians of Mesopotamia and the Turanian Turks of Central Asia (The New Encyclopaedia Britannica 1991). When Anatolian farmers settled in mosquito-infested soft and marshy soil (6500–2000 B.C.; Scarre 1998), large malarial outbreaks occurred in Anatolia (Angel 1966; de Zulueta 1994) and could have imposed a positive selection effect on heterozygous carriers of the oldest β-thalassemia alleles in the region (e.g., IVS-I-110 G→A). Between 2000 B.C. and A.D. 1100, Anatolia served as the ground for several civilizations (Hittite, Persian, Greek, Roman, Byzantine, and Seljuk), resulting in an intensive flow of mutations into and out of present-day Turkey. In the 13th century A.D., Anatolia witnessed an incomparable spread of agricultural prosperity that gave rise to a major malarial recrudescence (Angel 1966; Grmek 1994). It is at this time, probably, that most of the β-thalassemia mutations present in Anatolia were selected and brought to frequencies close to what is observed at present (Filon et al. 1995). All these episodes have genetically influenced the resident population of today's Turkey; molecular analyses conducted over several other genes in Turkey are unceasingly proving the validity of this assumption (Onay et al. 1998).

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