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ADVANCES IN HEMOGLOBINOPATHIES RESEARCH

β-THALASSEMIA IN TURKEY: A REVIEW OF THE CLINICAL, EPIDEMIOLOGICAL, MOLECULAR, AND EVOLUTIONARY ASPECTS

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INTRODUCTION

β-Thalassemia (thal) is one of the most frequent monogenic disorders in the world and the first disease to be characterized at the DNA level. It is an autosomal recessive disorder characterized by microcytosis and hemolytic anemia, and it accounts for thousands of childhood deaths per year, primarily in regions of the world in which malaria used to be endemic (1). β-Thal results from a variety of molecular defects that reduce (β^+) or abolish (β^0) the normal synthesis of the β-globin chains of hemoglobin (Hb) (2).

STUDIES ON THE CLINICAL ASPECTS OF β-THALASSEMIA

β-Thal was first recognized in Turkey in 1941 when two patients were identified as having the severe form of the disease, thalassemia major or Cooley's anemia (3). However, the importance of β-thal as a health problem was brought to the attention of physicians only in the late 1950s (4). Common problems encountered in Turkish β-thal major patients include below-average height, growth retardation (mainly in patients of 10 years of age or more), delay in bone maturity, and delayed puberty (5,6). Growth and endocrine disturbances

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have significant negative effects in the quality of life of Turkish thalassemia patients; the main cause of death being heart failure (N. Akar, personal communication).

The first 11 Turkish patients with thalassemia intermedia were identified in 1970. Five of them were more than 20 years of age and all had moderate clinical manifestations and hematological findings (7). A large number of cases of thalassemia intermedia were observed thereafter. There is significant variation in the degree of clinical manifestation in thalassemia intermedia largely reflecting the heterogeneity of β^+-thal mutants and the variation in the level of the fetal hemoglobin (Hb F) synthesis (8).

DISTRIBUTION OF β-THALASSEMIA IN TURKEY

The first report on the prevalence of β-thal carriers in Turkey was in 1971 and was reported to be 2.1% (9). Dinçol and colleagues (10) were the first investigators to report regional differences in the prevalence of β-thal trait in Turkey and the presence of both β^+- and β^0-thal genes in the country. Studies conducted between 1985–1997 confirmed this observation and noted a frequency variability ranging between 0.6–11.7% (Table 1). Because of this relatively high incidence of β-thal in the Turkish population, the presence of patients co-inheriting β-thal along with another congenital disorder is not surprising. So far, there have been several reports about the co-inheritance of β-thal together with hereditary persistence of fetal Hb (HPFH) (26,27), α-thal (28,29), $\delta\beta$-thal (27), Immerslund Grasbeck syndrome (30), Fanconi anemia (31), familial Mediterranean fever (32), and α or β Hb variants such as Hb H (β_4), Hb Knossos [β27(B9)Ala $\rightarrow$ Ser], Hb City of Hope [β69(E13)Gly $\rightarrow$ Ser], Hb Strumica [β112(G19)His $\rightarrow$ Arg] (33–38). The co-inheritance of β-thal and Hb S (Hb S/β-thal disease) is observed in one out of 1000 Turkish newborns (24). In Turkey, a Hb S/β-thal compound heterozygosity is expressed mainly as a severe type of disease, because β-thal mutations frequently found in Turkey are severe in nature, and Hb S is associated with severe Benin haplotype. The gradual decrease of Hb F values by age can also not be neglected (11,39,40).

β-THALASSEMIA RESEARCH IN TURKEY

The initiation of molecular based analyses in Turkey led to great progress in β-thal research. In 1987, while screening for the three β-thal mutations common in Mediterranean populations, namely codon 39 (C $\rightarrow$ T), IVS-I-6 (T $\rightarrow$ C), and IVS-I-110 (G $\rightarrow$ A), Akar and colleagues identified only the latter mutation in patients of Turkish descent (41). Further advances in the methodologies of analyzing mutations in the β-globin gene has made it possible to conduct large-scale, country-wide, studies that characterized many other

Table 1. The Distribution of the β-Thalassemia Gene in Different Regions and Some Towns of Turkey

Area/Town	# of Individuals	β-Thalassemia Frequency (%)	Reference
Turkey (overall)	(–)	2.10	9
	3140	2.26	11
Thrace Region	102	10.80	12
	79	10.12	11
Marmara Region			
Mustafakemalpaşa/Bursa	894	2.78	13
Aegean Region	1124	2.67	14
Nazili	1428	4.20	15
Denizli	(–)	3.00	16
	1006	3.67	11
Mediterranean Region			
Antalya	1095	2.00	11
	(–)	6.40	16
	1616	10.20	17
	9130	11.73	18
Manavgat/Serik/Boztepe	135	6.70	19
Central Anatolia Region			
Ankara	(–)	2.00	16
Kirşehir	(–)	3.60	16
Southeast Anatolia Region	1223	3.40	20
Çukurova	(–)	3.50	21
Hatay	27232	1.56	22
Kahramanmaraş	439	0.92	23
Elbistan/Kahramanmaraş	1109	0.90	11
Eti-Turks (Arab speaking)	651	1.23	24
	(–)	1.20	16
Kurdish Speaking Groups	1223	7.10	20
East Anatolia Region	4258	0.61	11
Van Lake Area	1014	0.88	25

mechanisms responsible for the occurrence of -thal in Turkey (8,36,42–48). These studies showed that, in contrast to many other populations, β-thal is quite heterogeneous in the Turkish population (Table 2), and that more than 40 mutations account for the great variability in clinical expression of this disorder; thus, one in every five mutations described in the world is present in the Turkish population (Table 3). The seven most common mutations account for only about 72% of the total β-thal alleles in the Turkish population [IVS-I-110 (G → A) (41%), IVS-I-6 (T → C) (13%), IVS-II-1 (G → A) (8%), frameshift codon 8 (−AA) (6%), IVS-I-1 (G → A) (5%), IVS-II-745 (C → G) (5%)] (36). In other Mediterranean countries six or fewer mutations comprise over 90% of the β-thal determinants (70).

Molecular studies of β-thal in 47 countries (19,459 chromosomes) have shown that a large diversity of mutations is found in regions where malaria and β-thal are less common. In contrast, in regions where malaria was hyperendemic in the past, only a small number of alleles account for most of the β-thal genes.

Table 2. β-Thalassemia Alleles Described in the Turkish Population

Mutation	Refs.	Mutation	Refs.
−101, C → T	49	IVS-I-116, T → G	45
−87, C → G	42	IVS-I-130, G → A	56
−30, T → A	50	IVS-I-130, G → C	44
−28, A → C	44	Codons 36/37, −T	54
5′UTR +22, G → A	51	Codon 37, G → A	36
Codon 5, −CT	44	Codons 37/38/39, −7 bp	57
Codon 6, −A	43,44	Codon 39, C → T	42
Codon 8, −AA	52	Codon 44, −C	55
Codons 8/9, +G	43,44	Codons 7/75, −C	58
Codon 15, T*G*G → T*A*G	43	IVS-II-1, G → A	42
Codons 22/23/24, −AAGTTGG	53	IVS-II-654, C → T	59
Codon 26, G → A (Hb E)	36	IVS-II-745, C → G	42
Codon 27, G → T (Hb Knossos)	8	IVS-II-848, C → A	36
Codon 30, G → C	54	3′UTR + 1565 to + 1577 (−13 bp)	60
IVS-I-1, G → A	42	Poly A, AA*T*AAA → AA*C*AAA	61
IVS-I-1, G → C	55	Poly A, AATAA*A* → AATAA*G*	36
IVS-I-1, G → T	36	Poly A, AAT*A*AA → AAT*G*AA	48
IVS-I-5, G → A	44	290 bp deletion	62
IVS-I-5, G → C	42	7.6 kb deletion	63
IVS-I-5, G → T	44	12 kb deletion	59
IVS-I-6, T → C	42	30 kb deletion	64
IVS-I-110, G → A	41		

Table 3. Expansion of β-Thalassemia Mutations Reported in the World and in Turkey Between the Years 1981–1999 (Compiled from References 65–69)

Year	# of Mutations in the World	# of Mutations in Turkey
1981	7	1
1982	15	1
1983	21	1
1984	32	1
1985	35	1
1986	37	1
1987	43	3
1988	54	11
1989	71	14
1990	93	22
1991	120	26
1992	131	28
1993	150	30
1994	159	32
1995	171	38
1996	181	39
1997	197	41
1998	–	42
1999	–	43

A comparison of the different regions of Turkey shows that the distribution of β-thal alleles displays a decreasing gradient of mutational heterogeneity from East to West Anatolia. Thus, fewer mutations account for the β-thal in West compared to East Anatolia, where there is higher molecular heterogeneity and lower frequencies of β-thal; the regional β-thal trait frequency gradient increases from East (3.4%) to West Anatolia and Thrace (11%) (Table 1). Eastern Anatolia is mainly a rugged region with many lofty ranges of altitudes higher than 5000 meters, not an environment conducive to the proper development of the malarial parasite. On the opposite side, the landscape of Thrace and Western Anatolia is simpler and more suitable for the transmission of malaria. Thrace is composed of several low plateaus, and Western Anatolia includes many depressed floors with many valleys leading from the Aegean Sea up to the borders of Central Anatolia.

In addition to the results of population studies, many reports of single cases of Turkish β-thal patients, either living in Turkey or abroad, contributed to the wealth of information about the presence of many rare and several novel β-globin mutations in the Turkish population (49,51,53–58,60–62,71–75). Several other papers described uncommon forms of molecular alterations leading to β-thal, such as a deletion/inversion rearrangement of the β-globin gene cluster (64,76,77) and homozygosity for two consecutive 7.6 bp deletions of the ψβ and β genes leading to a severe form of β-thal (63).

PREVENTION OF β-THALASSEMIA IN TURKEY

At present, more than 2000 β-thal patients are followed in 10 centers in Turkey. These patients receive free medical treatment including regular blood transfusions, desferrioxamine infusions, as well as social and psychological services. However, there is no ultimate cure for the disease yet and, at present, prevention is the best option for families at risk. Prenatal diagnosis was first introduced into Turkey in 1983 at the Prenatal Diagnosis Unit at Hacettepe University (Ankara) using fetal blood sampling and *in vitro* Hb synthesis (36). The introduction of DNA techniques greatly enhanced the efficiency and accuracy of the prenatal diagnosis of this disorder. Chorionic villi sampling followed by the application of DNA techniques such as allele specific oligonucleotide (ASO) hybridization, the amplification refractory mutation system (ARMS), restriction endonuclease digestion analysis, and DNA sequencing, were adopted in prenatal diagnosis in specialized centers in Turkey starting in 1990 (78). Despite the difficulties imposed by the presence of various kinds of mutations leading to β-thal in Turkey, prenatal diagnosis is feasible when methods of early fetal sampling are combined with the advent of polymerase chain reaction (PCR)-based techniques (79), especially the reverse dot-blot hybridization technique (80). At present, prenatal diagnosis of β-thal is performed in several centers in Turkey including Hacettepe University in Ankara (432 cases dealt with between 1983–1998), Çukurova University in

Adana (319 cases dealt with between 1992–1998), Boğaziçi University in Istanbul (90 cases dealt with between 1991–1998), and Ankara University (nine cases dealt with between 1990–1992) (36,78,79, N. Akar, personal communication).

Consanguineous marriage appears to contribute significantly to the frequency of affected births in Turkey. The overall consanguinity in Turkey is about 21%, but rates may be as high as 46–63%. First-cousin marriages are the most frequent type of consanguineous mating (81). The rate of consanguinity among parents of a large number of β-thal patients studied between 1990 and 1996 ranged from 35–65%. Surprisingly, 9–30% of the consanguineous couples carried two different β-thal mutations, in spite of the fact that these couples came mostly from small villages, providing further evidence for the high diversity of mutations in Turkey (36,79).

Besides the high rate of consanguineous marriages, Turkey is one of the countries showing the highest rate of population increase in the world (36:1000; Census 1994). Both factors appear to contribute drastically to the frequency of affected births. The expected number of infants born with β-thal in Turkey annually have been calculated to be approximately 150 to 200. Hence, approximately 800 pregnant women should seek prenatal diagnosis each year (16). Unfortunately, the total number of prenatal diagnoses performed in all operating centers barely exceeds one-eighth of the expected value each year (36). This stresses the need for implementing a comprehensive genetic prevention program in Turkey like those currently applied in many Mediterranean countries. This could be performed either by screening of couples at their reproductive age when they register for marriage, or by educating the population at risk and their physicians (79). In a pilot study of premarital screening for hemoglobinopathies in Turkey in which 2,113 couples volunteered to participate, a high frequency of carriers was observed and elective abortion was performed in all pregnancies with affected fetuses, and two planned carrier marriages were canceled (82). On the other hand, the number of prenatal diagnoses performed in Turkey between 1990 and 1998 increased from 30 to 135 cases per year (N. Akar, personal communication). These observations indicate that the intensive involvement of the population by community education and informed genetic counseling is an important prerequisite of a successful prevention program (79).

It is important to note that the large migration flows of Turkish citizens to European countries during the last 35 years, primarily to Germany, contributed to the sudden appearance of β-thal in such non-malarial countries (59). Socio-medical surveys conducted in Turkish societies living in those countries demonstrated that the knowledge and perception of thalassemias were extremely limited. This makes large-scale screening programs a difficult task, especially when taking into account the cultural and educational conditions of the Turkish minority. However, members of these Turkish communities were in favor of termination of pregnancy after early diagnosis, despite the new therapeutic

approaches that have significantly improved the outlook for patients with β-thal disease (83).

THE ORIGIN OF β-THALASSEMIA IN TURKEY

The distribution of β-thal in the world corresponds to regions where malaria is currently, or was until recently, endemic (1). This correlation led Haldane (84) to propose that heterozygous carriers of β-thal are less susceptible to severe malarial infection. The age of β-thal as a human health problem is expected to be closely related to the evolution of malaria.

The malarial parasite must have been with modern humans for a long time, perhaps for the entire existence of the genus *Homo* (85), but malaria may have started spreading about 10,000 years ago in the Levant and parts of Asia, because of a less marked drop of temperature during the last glaciation (86). Before the advent of agriculture, roughly 7,000–9,000 years ago, it is unlikely that humans were exposed to large malarial outbreaks; this stage was probably only reached when Anatolian farmers settled in mosquito-infested soft and marshy soil (6500–2000 BC) (87). It is at this time that large malarial outbreaks occurred in Anatolia (5,000 years ago) (86,88) and imposed a positive selection effect on heterozygous carriers of the oldest β-thal alleles in the region [*e.g.*, IVS-I-110 (G → A) as shown by β-globin sequence haplotype analysis] (70). In 2000 BC, a group of Indo-Europeans, probably Nordic, settled in Central Anatolia and reorganized the population under the Hittite Empire. By 1500 BC, this dominion expanded to cover Anatolia, upper Mesopotamia, present-day Armenia, Syria, and Lebanon, carrying the selected β-thal mutations out of Anatolia (*e.g.*, IVS-I-110) (70). From that time to 1100 BC, close relationships developed among Hittites and their neighboring Phoenicians. The distribution pattern of the IVS-I-110 allele correlates with the early migrations of Phoenicians and Carthaginians, who may have spread the β-thal allele(s) in the Mediterranean Basin (85). By 546 BC, Anatolia fell under Persian domination, and it was to remain as such until 334 BC. Although Persians had very little impact on the structure of the Anatolian population, the development of extensive trade routes at that time might have facilitated the introduction of β-thal mutations common in Asian-Indian populations into Anatolia through its Eastern parts [*e.g.*, frameshift codons −8/9 (+G), IVS-I-5 (G → C), codon 15 (T*G*G → T*A*G)].

Through the Ionian, Doric, Roman, and Byzantine settlements, the racial make-up of the original population inhabiting Anatolia was only slightly altered [*e.g.*, the introduction of codon 39 (C → T)]. The arrival of the Turks of the Western-Central Asiatic steppe in 1070 AD, who were physically similar to the early inhabitants, served to strengthen the original stock in Anatolia and might have introduced newer β-thal mutations uncommon to the region [*e.g.*, frameshift codon 8 (−AA)]. In the 13th century AD, Anatolia witnessed an incomparable spread of agricultural prosperity that led to a major malarial recrudescence (88,89).

It is probably at this time that all β-thal mutations present in Anatolia were selected and brought to frequencies close to what is observed at present. This may also include isolated rare alleles favored by the geography of the region. The expansion of the Ottoman Empire towards Eastern Europe, Northern Africa, and Central Asia permitted further spread of β-thal mutations in and out of Anatolia making it a melting-pot of a large number of alleles. The migration of many Muslim groups living in former Turkish territories in Southeastern Europe during the decline of the Ottoman Empire (starting from 1914 AD) contributed more to the racial admixture [*i.e.*, the introduction of IVS-II-745 (C → G), −87 (C → G), and IVS-II-654 (C → T) mutations).

With the knowledge that a significant amount of genetic information can be recovered from ancient skeletal remains (90), analysis of genetic markers in "ancient DNA" has become an important research tool in archeology. The examination of modern and ancient human remains suggestive of hereditary anemias resulted in well-described guidelines to distinguish bone deformities caused by iron deficiency syndrome from hereditary anemias (91). In 1995, Filon et al (92), presented the first proof of the occurrence of an inherited anemia in the archeological remains of a child with severe bone pathology consistent with β-thal. The remains came from a grave in Northern Israel, thought to date from the Ottoman period, sometime between the 16th and the 19th centuries. DNA analysis has shown that the child was homozygous for the frameshift mutation at codon 8 (−AA) as well as the polymorphism (C → T) in the second codon of the β-globin gene (92). Thus, we are beginning to realize the promise of ancient DNA analysis to experimentally answer previously unapproachable questions regarding the genetics of ancient populations.

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