The Frequency and Distribution Pattern of ß-Thalassemia Mutations in Turkey

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ABSTRACT

ß-thalassemia, a-thalassemia and sickle cell anemia are the three most common hemoglobinopathies in Turkey. ß-thalassemia major makes up 73%, sickle cell anemia 23% and Hb H disease 4% of total patients with hemoglobinopathy. The overall frequency of ß-thalassemia is 2%. However, the frequency shows regional variations and in some areas it is as high as 13%. Molecular studies indicated the presence of more than 35 different mutations associated with ß-thalassemia. In this study it was shown that five different mutations, namely IVSI-110 (G-A), IVSI-6 (T-C), IVSII-1 (G-A), IVSII-745 (C-G) and IVSI-1 (G-A) make up 71% of all ß-thalassemia mutations. The rate of consanguineous marriage in families with thalassemia major is %63. However, in 11% of these families, parents carry two different thalassemia mutations. The IVSI-1 (GÆA) mutation is most prevalent in the Aegean Region and it seemed that this mutation moved from this region toward Marmara, Black Sea, Middle, East and South-eastern Anatolia. The IVSII-745 (C-G) is most prevalent in the Mediterranean region and it moved toward Central Anatolia, Black Sea and South-eastern Anatolia. Contrary to these two mutations FSC8 (-AA), IVSII-1 (G-A) and -30 (T-A) mutations are most prevalent in Eastern Anatolia and they moved from this region to South-eastern Anatolia, Mediterranean Region, Central Anatolia and Aegean Region. The frequency of various mutations in Central Anatolia is very close to the mean figures given for Turkey indicating that these mutations are well mixed in this region. Sickle cell anemia and sickle cell ß-thalassemia are almost exclusively seen in eastern coast of Mediterranean Sea and in Thrace.

In Turkey, ß-thalassemia mutations are very heterogeneous although frequencies of these mutations show some regional differences there is no specific that ß-thalassemia mutation or distribution pattern that would aid in the identification of any ethnic background.

Key Words: ß-thalassemia, Turkey.

Turk J Haematol 2002;19(2):309-315

INTRODUCTION

Although the overall frequency of ß-thalassemia in Turkey is 2%, there are some significant regional differences. The highest frequencies are observed in Antalya and Thrace (10% for both) and Mugla (4.8%)[1,2]. Both Antalya and Mugla are located in the western Mediterranean coast and Thrace is located in Northwest[1,2]. The frequency of ß-thalassemia is lowest in

eastern and northern Anatolia. ß-thalassemia mutations are very heterogeneous[1,3-8]. In a previous study it was shown that 7 mutations outline only 67% of all ß-thalassemia mutations[1,6].

Regional differences in the frequency of various mutations may give some clues regarding the migration patterns and the ethnic background about the population studied[6].

MATERIALS and METHODS

A total of 412 chromosomes (206 patients) with ß-thalassemia major and 222 chromosomes (111 patients) with ß-thalassemia intermedia and 430 heterozygotes diagnosed at Hacettepe Ihsan Dogramaci Children's Hospital are the subjects of this study. None of these subjects were relatives. Routine hematological analysis was performed by conventional methods[9,10]. DNA analyses were performed using PCR and oligoprimers (ARMS technique). Sequence analysis was applied for DNA samples when no mutation was detected with ARMS[11,12].

RESULTS

The list and frequencies of the most common mutations seen in ß-thalassemia major and ß-thalassemia intermedia and distrubution pattern of the mutations are given in Tables 1,2,3 and 4.

DISCUSSION

ß-thalassemia, abnormal hemoglobins and HbH disease are present in Turkey[1,13] (Figure 1). According to Association of Turkish Society of Hemoglobinopathies there are 2733 registered patients. Seventy three percent has ß-thalassemia major or ß-thalassemia Intermedia, 23% other hemoglobinopathies and 3.6% HbH disease[14] (Figure 1). Majority of the patients with abnormal hemoglobin either has sickle cell anemia or sickle cell/ß-thalassemia disease[14]. The overall frequency of ß-thalassemia trait was reported to be 2% in Turkey[1]. However, the frequency and the number of patients vary among different geographical areas[1,2]. The highest frequency is found in the western part of Mediterranean Sea (10-13%) and the lowest in Eastern Anatolia (0.2%)[1,2]. The total number of ß-thalassemia mutations diagnosed in the Turkish ß-thalassemia major and ß-thalassemia intermedia patients are more than 35[1,3-8]. The rate of consanguineous marriage is found to be 63% which is slightly lower than the previously reported. In 11% of these families compound heterozygosity for ß-thalassemia mutations was present.

The most common 5 ß-thalassemia mutations are IVSI-110(G-A) which comprises 41% of the total mutations, is followed by IVSI-6 (T-C) (10.3%), IVSII-1 (G-A) (8.1%), IVSII-745 (C-G) (6.2%) and IVSI-1 (G-A) (5.7%), (Table 1). These 5 mutations make up 71% of total mutations. This figure is slightly higher than

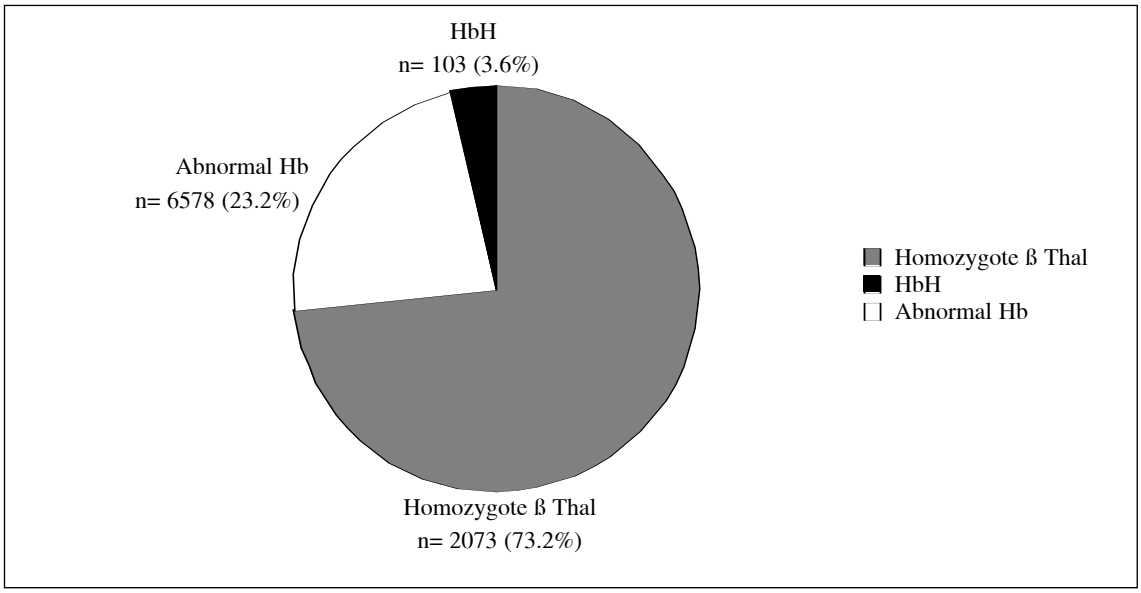

Figure 1. Hemoglobinopathies in Turkey (n= 2833).

the figure found in a previous study[1]. According to Weatherall in Mediterranean and Middle Eastern countries, 4 mutations [ß 39 (C-T), IVSI-110 (G-A), IVSI-6 (T-C) ; IVSI-1 (G-A)] make up 75% of the total ß-thalassemia determinants[15]. An examination of patients as two distinct groups of thalassemia major and thalassemia intermedia significantly effects the distribution frequencies of the mutations (Tables 2,3). Frequencies of mutations reported in previous studies and that of found in this study are quite similar[16](Table 4). Although IVS-110 mutation is the most common mutation in all of these regions, the frequency varies from 47% in Mediterranean region to 27.5% in Marmara and 30% in Eastern Anatolia (Table 4). The second most common mutation in overall Turkey is IVSI-6 (T-C) mutation which is also the second most common mutation in Marmara, Mediterranean, Central Anatolia and Black Sea region while it is the 4th common mutation in Agean Region. The second most common mutation in Agean Region is IVSI-1. It seems that this mutation moved towards east but not towards Mediterranean region (Table 4). The IVSII-745 (C-G) is the third most common mutation in the Mediterranean region. It seems that this mutation spread from this region to other regions of the country. IVSII-1 (G-A), FSC8 (-AA) and -30 (T-A) mutations probably originated from Eastern Anatolia and moved towards Southe-eastern and Central Anatolia and then to Mediterranean and Aegian Regions (Table 4).

Table 1. Frequencies of various mutations in ß-thalassemia major and intermedia

Mutation		%
1	IVSI-110 (G-A)	40.88
2	IVSI-6 (T-C)	10.33
3	IVSII-1 (G-A)	8.08
4	IVSII-745 (C-G)	6.20
5	IVSI-1 (G-A)	5.72
6	FSC8 (-AA)	4.69
7	-30 (T-A)	4.22
8	CD39 (C-T)	2.66
9	FSC 8,9	2.63
10	Cd44	1.78
Others		12.56
Total		100.00

Table 2. Frequencies of various mutations in ß-thalassemia major

Mutation		%
1	IVSI-110 (G-A)	58.72
2	IVSI-1 (G-A)	5.58
3	IVSII-745 (C-G)	5.09
4	IVSI-6 (T-C)	4.61
5	CD39 (C-T)	4.12
6	FSC8 (-AA)	3.39
7	FSC5 (-CT)	1.69
8	-30 (T-A)	1.21
9	IVSI-130 (G-C)	1.21
10	Others	11.40
Total		100.00

Table 3. Frequencies of various mutations in ß-thalassemia intermedia

Mutation		%
1	IVSI-6 (T-C)	26.57
2	IVSII-1 (G-A)	17.11
3	-30 (T-A)	12.16
4	FSC8 (-AA)	11.71
5	Delta/beta	9.03
6	IVSI-110 (G-A)	5.40
7	+22 (G-A)	1.35
8	-28	0.90
9	PolyA	0.90
10	-87	0.90
Others		13.97*
Total		100.00

*%3.6 alfa triplication.

Except for very rare individual mutations, majority of the mutations have been encountered in all regions. However, the total number of the mutations per region varies. The number of mutations is 16 in Mediterranean, 21 in Aegean Region, 15 in Marmara and, 15 in Central Anatolia, 11 in Black Sea and 9 in Eastern

Table 4. Frequencies of various mutations found in this study in various geographical parts of Turkey*

Mutation	Total number of chromosomes	%**	Marmara	Mediter-Aegean	Black ranean	Central Sea	Eastern Anatolia	East Anatolia	South-Anatolia
IVSI-110 (G-A)	435	40.88	27.5	43.47	47.17	30.76	43.42	30.0	41.17
IVSI-6 (T-C)	110	10.33	22.5	6.08	12.82	20.0	9.71		3.36
IVSII-1 (G-A)	86	8.08	7.5	4.34	7.69	10.0	3.42	6.66	12.60
IVSII-745 (C-G)	66	6.20	2.5	6.52	10.25	7.5	9.71		2.52
IVSI-1 (G-A)	61	5.73	10.0	13.47	2.56	5.0	5.14	3.3	2.52
FSC8 (-AA)	50	4.69	2.5	2.17	2.56	2.5	8.0	16.6	6.72
-30 (T-A)	45	4.22		1.30	1.53		4.57	13.3	11.76
CD39 (C-T)	31	2.91	2.5	4.34	1.53	2.5	5.71		
FSC8,9 (+G)	28	2.63	2.5	4.78	1.02		3.42	13.3	3.36
Cd44 (-C)	19	1.78		1.30	2.05	5.0	1.14	10.0	5.88
FSC5 (-CT)	13	1.22	5.0	0.43	1.53	5.0	1.14		0.84
IVSI-130 (G-C)	9	0.84		0.86	1.53				
FSC6 (-AA)	7	0.64		0.43	0.51		1.71		
IVSI-116 (T-G)	6	0.56	2.5	0.86			0.57		
PolyA	6	0.56	2.5			5.0			0.84
IVSI-5 (G-C)	6	0.56	5.0	2.17	0.51				1.68
+22 (G-A)	5	0.46	2.5	0.43					
IVSII-848 (C-A)	5	0.46		0.43					1.68
FSC36-37	4	0.37		0.86					
IVSI-5 (G-T)	3	0.28							
-87 (C-T)	2	0.18							1.68
IVSI-1 (G-T)	2	0.18							
-28 (A-C)	2	0.18							1.68
FSC15 (G-A)	2	0.18					1.14		
FSC74,75 (-C)	2	0.18							1.68
-101 (C-T)	1	0.09	2.5						
IVSI-5 (G-A)	1	0.09		0.43					
IVSI-130 (G-A)	1	0.09						3.3	
CD37 (G-A)	1	0.09	2.5						
Hb knossos	2	0.18		0.43	0.51				
Delta beta	22	2.06		1.30	1.02	5.0	4.57		
Del-N-Del	2	0.18						6.6	
Unknown	29	2.72		3.47	5.12		1.14		
Total	1064	100	100	100	100	100	100	100	100

* One subject from each family.

** Present study

Table 5. Frequencies of various ß-thalassemia mutations found in neighbouring countries*

Mutation	Number of the chromosomes	%**	Greece	Italy	Iran	Syria	Azerbaijan	Macedonia	Bulgaria
IVSI-110 (G-A)	435	40.88	58.32	11.21	6.81	44.4	20.20	47.30	24.75
IVSI-6 (T-C)	110	10.33	7.08	7.54	6.06		7.07	18.56	7.35
IVSII-1 (G-A)	86	8.08	1.18	1.48	15.90	2.7	21.21	0.59	1.71
IVSII-745 (C-G)	66	6.20	4.72	2.78	8.33	16.6	3.03	2.99	4.16
IVSI-1 (G-A)	61	5.73	10.1	3.9	3.5	16.6	2.1	8.9	3.2
FSC8 (-AA)	50	4.69	0.65	0.05	3.03		21.2	1.19	1.22
-30 (T-A)	45	4.22	0.06	-			2.02	1.19	0.24
CD39 (C-T)	31	2.91	12.57	66.84	5.30	11.1	2.02	2.99	24.26
FSC8,9 (+G)	28	2.63	0.06	-	3.78		2.02		0.40
Cd44 (-C)	19	1.78	-	0.11	2.27		3.03		0.49
FSC5 (-CT)	13	1.22	0.35	0.03				2.99	7.10
IVSI-130 (G-C)	9	0.84	-	0.08					
FSC6 (-AA)	7	0.64	1.71	1.9				4.19	5.88
IVSI-116 (T-G)	6	0.56	-	0.05					
PolyA	6	0.56	0.06	-				2.39	0.24
IVSI-5 (G-C)	6	0.56	-	0.11	6.06		1.01		
+22 (G-A)	5	0.46	-	-			1.01		0.49
IVSII-848 (C-A)	5	0.46	0.12	-				1.19	
FSC36-37 (-T)	4	0.37	-	-	1.51		2.02		0.24
IVSI-5 (G-T)	3	0.28	-	-					
-87 (C-T)	2	0.18	-	0.02					
IVSI-1 (G-T)	2	0.18	-	-					
-28 (A-C)	2	0.18	-	-			1.01		
FSC15 (G-A)	2	0.18	-	-					
FSC74,75 (-C)	2	0.18	-	-					
-101 (C-T)	1	0.09	0.18	0.25	0.75				0.24
IVSI-5 (G-A)	1	0.09	10.09	0.05					
IVSI-130 (G-A)	1	0.09	-	-					
CD37 (G-A)	1	0.09	-	-					
Hb knossos	2	0.18	-	-					
Delta beta	22	2.06	0.12	-					
Del-N-Del	2	0.18		-					
Unknown	29	2.72	1.30	1.24	31.81	8.3	0	2.99	7.84
Total	1064	100	1694	5940	132	36	99	167	408

* See reference 16 for countries other than Turkey.

** Present study

Anatolia (Table 4).

Comparison of the frequencies of several mutations to those in some of the neighboring countries shows that the IVSI-110 (G-A) emerges as the most common mutation in Syria, Macedonia, Greece and Bulgaria. The order of frequency for other mutations, however, is different[15,16](Table 5). Interestingly, -30 (T-A) mutation which is more common in Eastern and Southeastern Anatolia, is present in small numbers in Macedonia, Bulgaria and Greece but not in Iran or Syria[15,16](Table 5). The IVSI-1 (G-A) mutation, which is highly prevalent in Marmara and Aegian Sea regions in Turkey is also one of the 4 most common mutation present in Mediterranean and Middle Eastern countries[15](Table 4,5). The IVSI-130 (G-C) mutations which are exclusively seen in subjects originating from the city of Burdur in Mediterranean region has not been reported from any other countries.

The most common ß-thalassemia mutations associated with ß-thalassemia intermedia are IVSI-6 (T-C) (26%), IVSII-1 (G-A) (17%), -30 (T-A) (12.2%), FSC8 (-AA) (12%) and dß-thalassemia (9%) (Table 3). Association of one of the ß-thalassemia mutations and triplication of a-gene is one of the common causes of ß-thalassemia intermedia in Turkey[17](Table 3). The frequencies of these mutations show some variations in the seven geographical regions of Turkey. IVSII-1 (G-A) and FSC8 (-AA) both are ß°-thalassemia mutations associated with mild thalassemia. These two mutations are the two most common mutations of Azerbaijan[15,16] (Table 5).

Sickle cell anemia is the second most common hemoglobinopathy in Turkey. Although almost all of the ßS mutations are exclusively associated with haplotype 19 (Benin) type, in HbS/ß-thalassemia disease revealed the presence of more than 9 different ß-thalassemia mutations in trans of HbS mutation[18-20] (Table 6). This indicates that although HbS mutation is present in a closed population, gene exchanges with other groups had occurred in the past in this population. Presence of six different types of dß-thalassemia and 4 different types of d-thalassemia further support the notion of heterogeneity in thalassemia (Tables 7,8).

CONCLUSION

This study supports the previous observations that the frequency of several mutations varies from one region to another. ß-thalassemia mutations are very heterogeneous and there is no specific distribution pattern that would aid in the identification of any ethnic background.

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Table 6. Genotype of the patients with Sickle ß-thalassemia

Genotype	Number of the patients	%
IVSI-110/S	20	42.55
IVSI-1 (G-A)/S	6	12.76
IVSII-745/S	6	12.76
IVSII-1/S	3	6.38
IVSI-6/S	2	4.25
Cd39/S	2	4.25
Cd44/S	2	4.25
-30/S	2	4.25
FSC8/S	1	2.12
?/S	3	6.38
Total	47	100

Table 7. Molecular pathology of dß-thalassemias in Turkey

1-GgA g (dß)°-thalassemias
Sicilian (deltabeta)°-thal (ª13.37 bp)*
Turkish (deltabeta)°-thal (ª30 kb)
Turkish (del-inver-del) (11.5 kb, 7.6 kb, 1.6 kb)
2-Gg (^{A}gdß)°-thalassemias
Turkish Gg (^{A}gdß)°-thal (ª36 kb)

Table 8. Delta-thalassemia mutations

1- Codon 27 (GCC- > TCC)
2- IVSI-2 (T- > C)
3- Codon 59 (-A)
4- 7.2 kb Corfu deletion

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