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Full Length Research Paper

Thalassemia mutations in Gaziantep, Turkey

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Ninety-eight postnatal and six prenatal cases of thalassemia were studied by the reverse dot-blot hybridization technique in the city of Gaziantep, Turkey. We found the following mutations: IVS 1.110 (G>A) in 29.1%, IVS 2.1 (G>A) in 12.3%, IVS 1.1 (G>A) in 7.7%, Codon 8 (–AA) in 5.6%, -30 (T>A) in 4.6%, IVS 1.6 (T>C) in 4.6%, Codon 39 (C>T) in 3.6%, Codon 44 (-C) in 3.1%, IVS 2.745 (C>G) in 1.5%, Codon 8/9 (+G) in 2.1%, Codon 36/37 (-T) in 2.1%, IVS 1.5 (G>C) in 2.1%, Codon 22 (7pb del) in 0.5%, Codon 5 (-CT) in 0.5% while 20.9% were undetermined. 54 of the thalassemia patients were homozygotes, 12 were compound heterozygous and 31 were heterozygotes. In one allele of 5 thalassemia patients, α-thalassemia mutation (3.7 single gene deletions in 1 patient, anti-3.7 gene triplication in 4 patients) was determined at the same time. Finally, this is the first comprehensive study in this region and percentage of α and β- globin genes mutation is 2.6 and 79.4%, respectively.

Key words: α- thalassemia, β -thalassemia, DNA, mutation, polymerase chain reaction.

INTRODUCTION

β–Thalassemia (OMIM 141900) is one of the most common single gene defects in the world and it results from decrease in lack of β-globin chain production. It is common in the Mediterranean, Middle East, Africa, India, South Asia and South China (Kuliev, 1988; Birgens and Ljung, 2007). In β^0-thalassemia, there is no production of the β-globin chain and is an important cause of morbidity and mortality being associated with severe hypochromic hemolytic anemia, the requirement of frequent blood transfusions and lifetime iron-chelating treatment; in β^+-thalassemia, the β-globin chain is produced to a lesser extent than normal (Kuliev, 1988; Borgna-Pignatti and Galanello, 2003).

α-Thalassemia (OMIM 141800) is the most common inherited disorder of hemoglobin (Hb) synthesis in the world, with gene frequencies varying between 1 and 98% throughout the tropics and subtropics. It can occur in all ethnic groups but is more common in those of Southeast Asian descent. The American College of Obstetricians and Gynecologists recommends hemoglobinopathy screening for those of African, Southeast Asian and Mediterranean descent. More than 95% cases of recognized α-thalassemia involve deletion of one or both α-globin genes from chromosome 16p13.3 (www.labcorp.com/datasets 6300.htm - 05.08.2009).

The number of affected births is higher than expected in Turkey because of a large and young families that have many children and the number of consanguineous marriages especially first cousins are above 60% in the Eastern and Southern parts of Turkey (Basak, 2008). Genetic counseling and prenatal diagnosis programs play an important role in overcoming the problem.

We have aimed the frequency β and α-thalassemia mutations among 98 patients and 6 prenatal cases.

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MATERIALS AND METHODS

We looked for α and β-thalassemia mutations in 196 chromosomes of 98 thalassemia patients (52 male, 46 female) between the ages

Table 1. Frequency of β-thalassemia mutations (%) in Turkey.

Mutation	Gaziantep	Cukurova	Diyarbakir	Aegean	Denizli	Turkey
IVS 1.110 (G>A)	29.1	57.3	27.8	44.1	36.4	39.2
IVS 2.1 (G>A)	12.3	3.4	8.3	2.7	3.6	5.4
IVS 1.1 (G>A)	7.7	8.3	2.8	28.2	16.4	5.5
Codon 8 (–AA)	5.6	4.7	11.1	-	3.6	6.1
-30 (T>A)	4.6	5.7	2.8	-	-	3.1
IVS 1.6 (T>C)	4.6	5.7	11.1	13.3	7.3	9.5
Codon 39 (C>T)	3.6	6.4	2.8	2.4	9.1	3.8
Codon 44 (-C)	3.1	0.7	NS	-	-	1.3
IVS 2.745 (C>G)	1.5	2.8	NS	9.3	7.3	4.6
Codon 8/9 (+G)	2.1	0.4	3	-	1.8	1.5
Codon 36/37 (-T)	2.1	0.7	1.2	-	-	<1
IVS 1.5 (G>C)	2.1	0.4	NS	-	1.8	1.1
Codon 22 (7bp del)	0.5	-	-	-	-	-
Codon 5 (-CT)	0.5	-	-	-	-	-
Undetermined	20.9	?	?	?	?	?
Reference		11	12	14	13	5

-: not studied.

Table 2. Thalassemia patients who carry both α and β-globin gene mutations.

Patient Number	Gender	Age	β-globin gene mutation	α-globin gene mutation
1	Female	1	heterozygote - IVS 2.1 (G>A)	anti-3.7 gene triplication
2	Female	27	Heterozygote - IVS 1.1 (G>A)	anti-3.7 gene triplication
3	Male	34	Homozygote - Codon 8 (–AA)	anti-3.7 gene triplication
4	Female	39	Homozygote - IVS 1.6 (T>C)	anti-3.7 gene triplication
5	Male	26	Homozygote - IVS 1.110 (G>A)	anti-3.7 gene deletion

of 1 and 52 years (mean: 15.1 ± 10.9 years) and 12 chromosomes of 6 fetuses whose parents' mutations have been known to have the β-thalassemia trait. Hematological parameters were obtained with an automated cell counter. Consent from patients and family members were obtained before collection of blood and amniotic fluid samples. Genomic DNA was extracted from peripheral blood leukocyte using the salting out method techniques (Miller et al., 1988). Fetus DNA was extracted from 3 ml amniotic fluid by using NucleoSpin Isolation Kit. Detection of known β-globin gene mutations was done using a commercialized reverse dot blot platform (Strip A ViennaLab. Diagnostics, Vienna, Austria) which included the following: IVS 1.110 (G>A), IVS 1.116 (T>G), IVS 2.1 (G>A), IVS 1.2 (T>A), IVS 1.1 (G>A), -30 (T>A), IVS 1.6 (T>C), Codon 39, Codon 44, Codon 22 , Codon 8/9, IVS 1.5 (G>C) , Codon 36/37, Codon 30, IVS 2.745 (C>G), IVS 1-25 (25bp del), -87 (C>G), Codon 5, Hemoglobin C (HbC), HbS, Codon 6 and Codon 8 (Samara et al., 2007). Detection of known α-globin gene mutations was done using a commercialized reverse dot blot platform (Strip B VienneLab. Diagnostics, Vienna, Austria) which included the following mutations: 3.7 single gene deletion (SGD), 4.2-SGD, MED double gene deletion (DGD), SEA-DGD, THAI-DGD, FIL-DGD, 20.5 kb -DGD, anti-3.7 gene triplication, α1 cd 14 (TGG>TAG), α1 cd 59 (GGC>GAC) (Hb Adana), α2 init cd (ATG>ACG), α2 cd 19 (–G), α2 IVS1 (-5 nt), α2 cd 59 (GGC>GAC), α2 cd 125 (CTG>CCG) (Hb Quong Sze), α2 cd 142 (TAA>CAA) (Hb Constant Spring), α2 cd 142 (TAA>AAA) (Hb Icaria), α2 cd 142 (TAA>TAT) (Hb Pakse), α2 cd 142 (TAA>TCA) (Hb Koya Dora), α2 poly A-1 (AATAAA-AATAAG) and α2 poly A-2 (AATAAA-AATGAA) (Hadavi et al., 2007). Maternal contamination was eliminated using ChromoQuant version 2 kit (CyberGene AB, Sweden).

RESULTS AND DISCUSSION

The frequency of β-thalassemia mutations is shown in Table 1. The ratio of β^+ to β^o mutations was 0.58 (36/62). We detected 14 different mutations, of which six were present in 63.9% of the chromosomes. The IVS 1.110 mutation, the most frequent mutation, was present in 29.1% of all chromosomes. In Table 2, five thalassemia patients who carry both α and β-globin gene mutation are summarized. Also we identified the ratio of the parents' consanguinity is very high (49%).

In Turkey, however a broad range of mutations had been observed, probably because of Turkey's location at the three continents and of the influence of different cultures over the course of history. Although the mutations that we identified in Gaziantep, were similar to those seen elsewhere in our country, we detected some

Table 3. Frequency of β-thalassemia mutations in the Eastern Mediterranean.

Mutation	This study	Turkey	Cyprus	Greece	Syria	Palestine	Bulgaria	Azerbaijan	Iran	Iraq
IVS 1.110 (G>A)	29.1	39.3	79.7	42.1	24.1	17.6	24.2	20.2	4.8	1.9
IVS 2.1 (G>A)	12.3	4.7	-	3.3	4.2	2.9	-	-	33.9	18.3
IVS 1.1 (G>A)	7.7	5.0	5.9	12.8	17.0	9	3.1	2.0	2.9	8.7
Codon 8 (–AA)	5.6	5.5	0.2	0.8	0.7	-	5.5	21.2	4.5	2.9
-30 (T>A)	4.6	3.1	-	-	-	2.1	-	-	-	-
IVS 1.6 (T>C)	4.6	10.1	6.2	8.1	4.2	28.7	10.2	7.1	1.1	8.7
Codon 39 (C>T)	3.6	3.8	2.4	18.8	6.4	4.6	21.9	2.0	1.7	8.7
Codon 44 (-C)	3.1	1.3		-	0.0	-	-	-	2.6	12.5
IVS 2.745 (C>G)	1.5	5.0	5.5	6.3	-	0.3	6.9	3.1	-	-
Codon 8/9 (+G)	2.1	1.3	-	-	1.4	1.4	0.3	2.0	4.8	7.7
Codon 36/37 (-T)	2.1	0.1	-	-	-	-	-	-	-	-
IVS 1.5 (G>C)	2.1	1.1	-	-	0.0	1.1	-	-	7.6	6.7
Codon 22 (7bp del)	0.5	0.1	-	-	0.0	-	-	-	3.0	1.0
Codon 5 (-CT)	0.5	-	-	-	-	-	-	-		
Reference		15	13	17	22	18	19	20	21	22

different frequencies between the regions (Table 1) (Cavdar and Arcasoy, 1971; Gurbak et al., 2006; Curuk et al., 2001; Ince et al., 2003; Yildiz et al., 2005; Golesken et al., 2000). The IVS 2.1 (G>A) mutation is the second most frequent type (12.3%) in our study. This mutation occurs in 3.4% of the population in the Cukurova region and 8.3% in Diyarbakir (Gurbak et al., 2006; Ince et al., 2003). In Turkey as a whole, Başak et al. found this mutation frequency to be 5.4% (Basak, 2008). This mutation was the most frequent one in Iran (33.9%) and Kuwait (29.2%); however, it is difficult to explain why this mutation is frequent in Diyarbakir, Cukurova and Gaziantep as compared to Turkey in general (Table 3) (Baysal et al., 1992; Boussiou et al., 2008; Kyriacou et al., 2000; Darwish et al., 2005; Petkov and Efremov, 2007; Curuk et al., 1992; Peykar et al., 2007; Al-Allawi et al., 2006).

IVS 1.1 (G>A) is the third most frequent mutation in our study at 7.7%. It was the second most frequent mutation in Cukurova (8.3%) the Aegean (28.2%) and Denizli (16.4%) (Curuk et al., 2001; Golesken et al., 2000; Yildiz et al., 2005). It was the fourth most frequent (5.5%) mutation in Diyarbakir and in Turkey as a whole in accordance with the findings of Başak et al. (2008) and Ince et al. (2003). This mutation was seen mainly in the β-thalassemia zone, from Hungary, Yugoslavia, Greece and Cyprus to Czechoslovakia (Boussiou et al., 2008; Ringelhann et al., 1993; Dimovski et al., 1990; Efremov, 2007; Indrak et al., 1992). In Turkey, it was common in Turkish people originating from Marmara, Aegean and Balkans (Basak, 2008).

Codon 8 (-AA) was the fourth most frequent mutation in our study (5.6%) with a ratio that was lower than the studies reported from Diyarbakir. In Turkey as a whole, Başak et al. reported this mutation as the third most frequent with a ratio of 6.1 (Basak, 2008). This mutation is most frequent in East Anatolia and the Marmara region of Turkey (Basak, 2008). Among the neighboring countries, it was most frequent in Azerbaijan (21.2%) (Curuk et al., 1992). IVS 1.6 (T>C) and -30 (T>A) were the fifth most frequent mutation (4.6%) in Gaziantep. Başak et al. reported that IVS 1.6 (T>C) mutation was the second most frequent one in Turkey as a frequency of 9.5% (Basak, 2008).

The frequency of Codon 39 (C>T) were found in Gaziantep, Cukurova and Diyarbakir as 3.6, 6.4 and 2.8%, respectively (Gurbak et al., 2006; Curuk et al., 2001). It was the most frequent mutation in Italy 49%, Spain 64%, Portugal 33.5%, Bulgaria 21.9% and Tunisia 49% (Petkov and Efremov, 2007; Ferrara et al., 2001; Amselem et al., 1988; Faustino et al., 1999; Fattoum et al., 2004). Başak et al. found the average frequency in Turkey as a whole to be 3.8% (Basak, 2008). This mutation is usually the most common one that was obseved in the West Mediterranean. In contrast, it is rarely seen in Cyprus, Lebanon, Iran, Palestine and Azerbaijan (Bozkurt et al., 1992; Darwish et al., 2005; Curuk et al., 1992; Peykar et al., 2007; Chehab et al., 1987). It has been suggested that the distribution pattern of this allele correlates with the early migrations of Phoenicians and Carthaginians, who spread the β-thalassemia allele(s) in the Mediterranean Basin (Birgens and Ljung, 2007; Cavalli-Sforza et al., 1996).

This is the first study to determine the frequencies of α and β- thalassemia mutations in the city of Gaziantep. When a patient is born with β-thalassemia, there is no effective treatment option. Therefore, the best method for dealing with β-thalassemia disease is prenatal diagnosis. In the current study, six β-thalassemia families who carry the β-globin gene mutation were given genetic consultation for prenatal diagnosis based on DNA analysis. In

conclusion, after we have identified the most frequent mutations in β-thalassemia patients living in the city of Gaziantep, the frequency of prenatal diagnosis will promptly increase. Therefore, this will provide important benefits to population health and the national economy as well.

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