



Spectrum of α -thalassemia mutations including first observation of $-^{-\text{FIL}}$ deletion in Hatay Province, Turkey

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ARTICLE INFO

Article history:

Submitted 25 December 2012

Revised 22 January 2013

Available online 16 February 2013

(Communicated by J. E. Sadler, M.D., Ph.D.,
22 January 2013)

Keywords:

α -Thalassemia
Mutations
Frequency
 $-^{-\text{FIL}}$ deletion
Turkey

ABSTRACT

Alpha thalassemia (α -thal) is one of the most common genetic disorders in the world. It is characterized by the absence or reduced expression of α -globin genes. The frequency of α -thal mutations in the province of Hatay in South Turkey is unknown. Therefore, in the present study, we aimed to investigate the spectrum of α -thal mutations in this province. Three hundred and nine patients were tested for α -thal mutations by using reverse dot blot hybridization technique and nine different mutations were detected in 97 of them. Among the 9 different mutations found, the most frequent mutations were the $-\alpha^{3.7}$ (43.81%), $-\alpha 2^{-5\text{nt}}$ (6.70%), $-^{-\text{MED}}$ (5.67%) and $\alpha 2^{\text{Poly A}2}$ (2.57%). In the present study, $-^{-\text{FIL}}$ mutation was detected in a patient for the first time in Turkey. Our results indicated that α -thal mutations are highly heterogeneous and $-\alpha^{3.7}$ is the most prevalent mutation in Hatay province of South Turkey. In addition, $-^{-\text{FIL}}$ mutation was detected in a patient for the first time in Turkey. This new finding may contribute to the establishment of a national mutation database and genetic counseling.

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Introduction

α -Thal is one of the most common single gene disorders in the world, which has high frequency in the population living at tropical regions such as Africa and Asia, and Mediterranean basin [1]. α -globin gene ($\alpha 1$ and $\alpha 2$) is located at the short arm of chromosome 16 (16p13.3). In an adult, there are four α gene copies, as being 2 in each allele. There are four functional α genes, termed as $\alpha\alpha/\alpha\alpha$, in normal individuals [2]. The clinical course of α -thal is determined by the number of mutated α gene copies. Individuals with mutation in one α gene are termed silent carriers. In these asymptomatic individuals, routine hematological data such as hemoglobin, MCV and MCH are at normal levels; however, MCV and MCH levels may rarely be low. Individuals having mutation in two α genes, either at the same chromosome ($\alpha\alpha/-$) or at two distinct chromosomes ($-\alpha/-\alpha$), are termed as α -thal carriers. In these individuals who are usually asymptomatic, typical microcytosis and hypochromia are seen due to lower MCV (<80 fl) and MCH (<27 pg) levels; in addition, findings of moderate anemia can also be observed. Individuals having mutation in three α genes ($-\alpha/-$) are defined as the patients with hemoglobin H. These

patients usually have anemia, microcytosis and hypochromia in routine hematological evaluations. They have variable clinical manifestation with thalassemia intermedia. Hb H can be seen in hemoglobin electrophoresis and HPLC tests. If there is mutation in four α genes, Hb Bart's hydrops fetalis develops [3–5]. Mutations in α -globin genes are characterized by the absence or reduced expression of α -globin chains. While deletional or non-deletional α -thal forms are defined in Mediterranean populations, the most commonly observed mutations are single or double gene deletions. Gene deletions including $-\alpha^{17.4}$, $-\alpha^{26.5}$, $-\alpha^{20.5}$, $\alpha 2^{-5\text{nt}}$, $-\alpha^{3.7}$, and $-\alpha^{4.2}$, mutations including $\alpha 2^{-\text{Poly A}1}$, $\alpha 2^{-\text{Poly A}2}$ and $\alpha 1$ cd 59 G>A point mutation and $\alpha\alpha\alpha^{\text{anti-3.7}}$ gene triplication have been reported in several studies from our country and the frequency of $-\alpha^{3.7}$ deletion has been reported to be rather high as in other populations of Mediterranean basin [6–8]. In our study, it was aimed to investigate the frequency of α -thal gene mutations in individuals with anemia, microcytosis and normal iron values referred to our laboratory for either detection of α -thal gene mutations or diagnosis of Hb H before marriage at Hatay province located at the south of Turkey.

Materials and methods

Among the 330 normal individuals with moderate anemia, microcytosis and normal iron level who referred to Medical Biology and Genetic Department of Mustafa Kemal University, Medicine School for either detection of α -thal gene mutations or diagnosis of

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Hb H before marriage between September, 2008 and May, 2010, 97 individuals (thirty nine men with a mean age of 27.79 ± 9.02 and fifty eight women with a mean age of 25.38 ± 8.04) with α -thal gene mutations were included to the study. DNA was isolated from EDTA whole-blood samples by salting out method described by Miller et al. [9], whereas reverse dot blot method (α -globin StripAssay, ViennaLab Diagnostics, Vienna, Austria) was used to analyze α -thal gene mutations. By using this kit, 22 mutations frequently seen in Mediterranean region were screened. Erythrocyte, Hb, MCV, MCH, and RDW values were measured by using an automatic cell counter.

Ethics

The study was approved by the institutional review board and written informed consent was obtained from the subjects. The study was in compliance with the Helsinki declaration.

Results

Table 1 presents α -globin genotypes and hematological parameters of 97 individuals and Table 2 presents allele frequencies. Of the patients, deletional mutations were detected in 81.8% of the patients, whereas non-deletional mutations in 18.2%. Of the 330 individuals tested for α -thal gene mutations, 9 distinct mutations including $-\alpha^{3.7}$, $-\alpha^{4.2}$, $-\alpha_{-MED}$, $-\alpha_{-FIL}$, and $-\alpha^{20.5}$ deletions and $-\alpha 2^{5nt}$, $\alpha 2^{-Poly A1}$, $\alpha 2^{-Poly A2}$ and $\alpha\alpha\alpha^{anti-3.7}$ non-deletional mutations were detected in 97 patients (29.9%). As presented in Table 2, the frequency of these 9 distinct mutations were as follows: $-\alpha^{3.7}$ in 43.81% (n = 85); $-\alpha 2^{5nt}$ in 6.70% (n = 13); $-\alpha_{-MED}$ in 5.67% (n = 11); $\alpha 2^{Poly A2}$ in 2.57% (n = 5); $\alpha 2^{Poly A1}$ in 0.51% (n = 1); $-\alpha^{4.2}$ in 0.51% (n = 1); $-\alpha^{20.5}$ in 1.03% (n = 1), $-\alpha_{-FIL}$ in 1.03% (n = 1) and $\alpha\alpha\alpha^{anti-3.7}$ gene triplication in 1.54% (n = 3). $-\alpha_{-FIL}$ mutation was detected in one patient, which was detected for the first time in Turkey.

Discussion

α -Thal is commonly seen in tropical and sub-tropical regions of the world and carrier frequency can reach up to 80–90% in some regions [10–14]. In our study, 9 distinct gene mutations were detected including two single gene deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$), three double-gene deletions ($-\alpha_{-MED}$, $-\alpha^{20.5}$, $-\alpha_{-FIL}$) and four non-deletional mutations ($-\alpha 2^{5nt}$, $\alpha 2^{Poly A1}$, $\alpha 2^{Poly A2}$, $\alpha\alpha\alpha^{anti-3.7}$). As shown in Table 3, some variations are seen in the mutations detected regarding frequency, although mutation types are similar to those seen in other regions of our country. In the present study, 15 different genotypes and 9 different allele distributions were observed in 97 patients. The most commonly observed mutation was $-\alpha^{3.7}$ gene deletion in $\alpha 1$ gene which was detected in 74 (76.3%) of 97 patients. This mutation was

Table 2

Allele frequency of α -thal mutations in individuals from Hatay, Turkey.

Mutation	Allele (n = 194)	%
$-\alpha^{3.7}$	85	43.81
$-\alpha 2^{5nt}$	13	6.70
$-\alpha_{-MED}$	11	5.67
$\alpha 2^{Poly A2}$	5	2.57
$\alpha 2^{Poly A1}$	1	0.51
$-\alpha^{4.2}$	1	0.51
$-\alpha_{-FIL}$	1	0.51
$-\alpha^{20.5}$	1	0.51
$\alpha\alpha\alpha^{anti-3.7}/\alpha\alpha$	3	1.54
Total	121	62.37

seen in 85 (43.81%) of 194 alleles. Other studies from Turkey have also reported that $-\alpha^{3.7}$ mutation is frequently observed [6–8]. In a study on patients with Hb H from Cukurova region by Cürük et al., it was shown that there was $-\alpha^{3.7}$ mutation in 19 (59.3%) of 32 patients and allele frequency was 29.6% [7]. In a study on 25 patients with Hb H, Oner et al. found the allele frequency of $-\alpha^{3.7}$ deletion as 28% [6]. In a large series by Guvenc et al., $-\alpha^{3.7}$ deletion was detected in 181 of 450 alleles from 225 patients with α -thal and allele frequency was reported as 40.66% [8]. In a study by Sütçü et al., in which distribution of α -thal mutations was investigated at Isparta province, allele frequency of $-\alpha^{3.7}$ was reported as 5.55% [15]. $-\alpha^{3.7}$ gene deletion has worldwide distribution and it is the most frequent mutation in many populations. It is the most frequently seen mutations in West Asia countries including Iran (40–93%), Saudi Arabia (64%), Jordan (43%), United Arab Emirates (28.4%), South Cyprus (72.8%), Oman (58.3%), Tunisia (22.5%) and Israel (51%) [16–26]. Also, it is the most frequently seen mutation in Europe with the allele frequencies of 58.2% in Holland, 46.94% in Sicily and 52.41% in Spain [27–29]. This is also true for Malaysia (45.9%), Brazil (10.7%) and North Thailand (58.3%) [30–32]. The results of the above-mentioned studies support that $-\alpha^{3.7}$ single gene deletion is prevalent worldwide rather than being limited to East Asia including our country and indicate that our results are in agreement with literature.

In our study, the second most commonly seen mutation is $-\alpha 2^{5nt}$ non-deletional mutation at IVS-1 region of $\alpha 2$ gene, which was detected in 6.70% of carrier alleles. $\alpha 2^{5nt}$ mutation was detected in homozygote genotype ($\alpha 2^{5nt} \alpha / \alpha 2^{5nt} \alpha$) in 4 patients; in heterozygote genotype ($\alpha \alpha / - \alpha 2^{5nt} \alpha$) in 2 patients; and compound heterozygote genotype ($-\alpha^{3.7} / - \alpha 2^{5nt} \alpha$) in 3 patients. In the study by Oner et al., it was the sixth most frequently detected mutation with a frequency of 8% [6]. In the study by Cürük et al., it was at fifth order among 6 different mutations in terms of frequency (4.68%), while it was detected in only one (5.55%) of 18 chromosomes in 9 patients with heterozygote α -thal mutation in the study by Sütçü et al. [7,15].

Table 1

α -Thal genotypes and mean hematological values in patients with α -thal.

Genotype	n	%	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RBC
$-\alpha^{3.7}/\alpha\alpha$	56	57.73	12.89 \pm 1.96	76.88 \pm 8.80	22.05 \pm 3.32	33.76 \pm 1.33	4.98 \pm 0.79
$-\alpha^{3.7}/-\alpha^{3.7}$	11	11.34	9.68 \pm 1.12	58.14 \pm 6.52	19.48 \pm 2.75	32.92 \pm 1.38	5.20 \pm 0.66
$\alpha\alpha/-\alpha_{-MED}$	10	10.30	11.25 \pm 0.21	64.70 \pm 3.39	22.80 \pm 3.39	35.10 \pm 3.39	5.01 \pm 0.84
$\alpha 2^{5nt} \alpha / \alpha 2^{5nt} \alpha$	4	4.12	9.42 \pm 0.99	62.80 \pm 2.50	19.50 \pm 0.63	31.10 \pm 1.08	4.83 \pm 0.39
$-\alpha^{3.7} / - \alpha 2^{5nt} \alpha$	3	2.06	11.10 \pm 0.14	64.20 \pm 0.27	20.90 \pm 0.28	31.97 \pm 1.02	5.21 \pm 0.08
$-\alpha^{3.7} / - \alpha 2^{Poly A2}$	2	2.06	13.15 \pm 0.35	67.90 \pm 0.56	21.40 \pm 0.14	31.50 \pm 1.03	6.13 \pm 0.23
$\alpha\alpha / - \alpha 2^{5nt} \alpha$	2	2.06	11.40 \pm 0.70	71.90 \pm 2.96	25.00 \pm 1.69	34.65 \pm 0.91	4.57 \pm 0.14
$\alpha\alpha\alpha^{anti-3.7}$	3	3.09	14.96 \pm 0.95	78.16 \pm 0.35	27.63 \pm 0.70	35.86 \pm 0.32	5.40 \pm 0.20
$-\alpha^{3.7} / - \alpha^{20.5}$	1	1.03	11.20	65.60	21.70	33.00	5.18
$-\alpha^{3.7} / - \alpha_{-MED}$	1	1.03	10.60	62.60	20.60	32.80	5.17
$\alpha\alpha / \alpha 2^{Poly A2} \alpha$	1	1.03	9.60	77.80	24.70	31.70	3.88
$\alpha\alpha / - \alpha^{4.2}$	1	1.03	12.30	76.20	25.90	32.10	4.86
$\alpha\alpha / - \alpha_{-FIL}$	1	1.03	9.80	73.60	24.3	31.80	4.89
$\alpha\alpha / \alpha 2^{Poly A1}$	1	1.03	7.80	65.60	20.70	31.50	3.77
Total	97.0	100.00	-	-	-	-	-

Table 3
Allele frequency of α -thal mutations in Turkish population.

Mutation	Present study	Oner et al.	Cukurova region	Adana	Isparta	Cyprus Turks
$-\alpha^{3.7}$	43.81	28.00	29.6	40.66	5.55	30.00
$-\alpha^{2.5nt}$	6.70	8.00	4.68	0.00	5.55	10.00
$-\alpha^{MED}$	5.67	20.00	14.06	9.55	27.77	40.00
$\alpha^{2^{Poly A2}}$	2.57	10.00	7.81	2.00	0.00	4.00
$\alpha\alpha\alpha^{anti-3.7}/\alpha\alpha$	1.54	0.00	0.00	1.11	0.00	0.00
$\alpha^{2^{Poly A1}}$	0.51	0.00	4.68	0.66	0.00	12.00
$-\alpha^{4.2}$	0.51	12.00	1.56	0.66	0.00	0.00
$-\alpha^{FIL}$	0.51	0.00	0.00	0	0.00	0.00
$-\alpha^{20.5}$	0.51	22.00	18.75	3.33	11.10	4.00
Reference no.		[6]	[7]	[8]	[15]	[33]

Guvenc et al. failed to detect this mutation in their study [8]. It was the fourth most common mutation in Cyprus Turks with a frequency of 10% [33]. This mutation was the fourth most common mutation in Iran with an allele frequency ranging from 2.5% to 6.5, whereas it has a frequency of 11.1% in Israel [18–20,26,34].

$-\alpha^{MED}$ double-gene deletion, the third most frequently detected mutation, was found in 11 of 194 chromosomes with a frequency of 5.67% in our study. This mutation was the second most commonly seen mutation in the studies by Çürük et al. and Guvenc et al., whereas the third most commonly seen mutation in the study by Oner et al. with allele frequencies of 9.55%, 14.06% and 20%, respectively. In the study by Sütçü et al., it was found in 5 of 18 chromosomes in 9 patients and frequency was reported as 27.77% [6–8,15]. It was the most frequently seen deletional mutation in Cyprus Turks with a frequency of 40% [33].

In our study, the third and fourth most frequently seen mutations were $\alpha^{2^{Poly A2}}$ point-mutation and $\alpha\alpha\alpha^{anti-3.7}$ triplication, respectively. $\alpha^{2^{Poly A2}}$ mutation was detected in two patients with Hb H as a compound heterozygote genotype in association with $-\alpha^{3.7}$ gene deletion with an allele frequency of 2.57%. $\alpha^{2^{Poly A2}}$ mutation was discovered during analyses of patients with Hb H belonging to a large Turkish family living in Adana province and it was demonstrated in association with MED-II deletion [35]. Çürük et al. reported that it was the fourth most frequently seen mutation with a frequency of 7.81% and demonstrated that it was present in 3 patients with Hb H in association with MED-II deletion [7]. Guvenc et al. and Oner et al. reported this as the fifth most frequently seen mutation with an allele frequency of 2% and 10%, respectively [6,8].

$\alpha\alpha\alpha^{anti-3.7}$ triplication is a sporadic mutation which doesn't cause any clinical or hematological sign in carriers, but association of this triplication with β -globin mutation may lead the phenotype of dominant β -thalassemia intermedia [36]. In our study, heterozygote $\alpha\alpha\alpha^{anti-3.7}$ triplication was detected in 3 patients, with an allele frequency of 1.54%. Guvenc et al. detected this mutation at a frequency of 1.11% [8]. In an Iranian population, it was reported that the carrier frequency of this gene triplication was 2.14% in 280 patients with normal hematological findings and 1.7% in 117 patients with borderline hematological findings [37].

In our study, $-\alpha^{FIL}$, $-\alpha^{20.5}$, and $-\alpha^{4.2}$ deletions and $\alpha^{2^{Poly A1}}$ non-deletional mutation were detected in only one patient with an allele frequency of 0.51% for each. $-\alpha^{FIL}$ double-gene deletion hasn't been encountered in Turkey so far and it was shown for the first time in Turkey by this study. This mutation was first characterized in 1988 by Fischel-Ghodsian et al. [38]. It is most prevalent in countries at Southwest Asia, particularly Philippines [39,40]. In the previous studies from Turkey, the frequency of $\alpha^{20.5}$ double-gene deletion was found to be between 3.3% and 18.75% [6–8,15,35]. The frequency is 1.8–4.8% in Iran [34], 4.58% in Sicily [28], 12.1% in Greece, 0.9% in Netherlands [27] and 7.8% in South Cyprus [33]. The allele frequency of $-\alpha^{4.2}$ deletion was found as 1.5% and 0.66% in two distinct studies from Turkey, although it is prevalent worldwide. As similar the frequency of $\alpha^{2^{Poly A1}}$ non-deletional mutation was found as 4.68% and 0.66 [7,8]. Deletional or non-deletional mutations were detected

in 121 chromosomes of 97 patients included in our study. When rates of deletional and non-deletional mutations were considered, it was seen that, of the patients, 81.8% carry either a single- or double-gene mutations, while 18.2% carry a non-deletional mutation. In other studies from Turkey, it has been reported that the rates of deletional mutations are markedly higher than non-deletional mutations in Turkish patients [6–8,15]. In our study, we observed 12 different genotypes and 9 different mutations in 97 patients; in addition, $-\alpha^{FIL}$ double-gene deletion was detected in one patient, which hasn't been shown in Turkey so far. Our results show that α -thal mutations are highly heterogeneous as well as deletional and $-\alpha^{3.7}$ single gene deletion is particularly prevalent at Hatay province in agreement to other studies from Turkey. Present results may be helpful for genetic counseling and prenatal diagnosis. The present study is also important in terms of the potential contribution to development of national database of hemoglobinopathy gene mutations.

Conflict of interest

The authors of this paper have no conflicts of interest, including any financial interests, personal or other relationships and/or affiliations relevant to the subject matter or materials included.

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