Indian J Hematol Blood Transfus (Apr-June 2015) 31(2):223–228
DOI 10.1007/s12288-014-0406-0

ORIGINAL ARTICLE

Alpha-Thalassemia Mutations in Adana Province, Southern Turkey: Genotype-Phenotype Correlation

Sevcan Tug Bozdogan · Ozge Ozalp Yuregir · Nurhilal Buyukkurt · Huseyin Aslan · Zeynep Canan Ozdemir · Tomasz Gambin

Received: 10 January 2014 / Accepted: 11 May 2014 / Published online: 13 June 2014

Abstract To look over the distribution of the mutations in a large series from Adana province, Southern Turkey, and determine the genotype-phenotype correlation of the frequent mutations. Among the 2500 individuals with mild or moderate anemia, microcytosis, and normal iron levels that were referred to our Genetic Diagnosis Center, a population consisting of 539 individuals were included in the study and tested for alpha-thalassemia mutations by using reverse dot blot hybridization technique. Twelve different mutations were detected in 539 patients. Among the 12 different mutations found, the most frequent mutations were the $-\alpha^{3.7}$ (63.3 %), $-^{MED}$ (11.7 %), $-^{20.5}$ (10.7 %), $\alpha 2^{IVS1(-5nt)}$ (3.9 %), and $\alpha 2^{polyA-2}$ (3.5 %). The most frequent genotypes were $-\alpha^{3.7}/\alpha\alpha$ (35.8 %), $-\alpha^{3.7}/-\alpha^{3.7}$(18.9 %), $-^{20.5}/\alpha\alpha$ (11.5 %), and $-^{MED}/\alpha\alpha$ (10.4 %), respectively. There were statistically significant differences in hematological findings between $-\alpha^{3.7}/-\alpha^{3.7}$ and $-^{MED}/\alpha\alpha$, even though both have two mutated genes in the genotype. Our results show that alpha-thalassemia mutations are highly heterogeneous as well as deletional and $-\alpha^{3.7}$ single gene deletion is particularly prevalent at Adana province in agreement to other studies from Turkey.

Keywords Alpha-thalassemia · Mutation · Phenotype-genotype correlation

S. T. Bozdogan (✉)
Department of Medical Genetics, Mersin University, Faculty of Medicine, Mersin, Turkey
e-mail: sevcantb@gmail.com

O. O. Yuregir · H. Aslan
Genetics Diagnosis Center, Seyhan Practice Center, Adana Numune Training and Research Hospital, Adana 01170, Cukurova, Turkey

N. Buyukkurt
Department of Hematology and Bone Marrow Transplantation Unit, Baskent University Adana Training and Research Hospital, Adana, Turkey

Z. C. Ozdemir
Department of Pediatrics, Division of Pediatric Hematology, Adana Numune Training and Research Hospital, Adana, Turkey

T. Gambin
Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

Introduction

Microcytic hypochromic anemia is a common condition of alpha-thalassemia (OMIM 141800), which is the most common single-gene disorder in Turkey as it is worldwide [1]. Adana is located in the southern part of Turkey and takes place in the eastern Mediterranean coast of the thalassemic belt. Alpha-thalassemia is a genetic defect extremely frequent worldwide, especially in Southeast Asian, Mediterranean, and Middle Eastern populations, and is characterized by the decrease or complete suppression of α-globin polypeptide chains, resulting from over 128 different deletions or point mutations of the alpha globin genes [2, 3].

Alpha-globin genes (α1 and α2) are located at the short arm of chromosome 16 (16p13.3). There are four functional α genes, termed as αα/αα, in normal individuals [4]. The clinical signs are caused mostly by the deletion of one (–α/) or both (–/) cis-linked α-globin genes and less frequently by non-deletional mutations ($\alpha^T\alpha$ or $\alpha\alpha^T$) [4, 5].

Alpha-thalassemia is an autosomal recessive disorder characterized by microcytic hypochromic anemia and a

clinical phenotype varying from almost-asymptomatic to lethal hemolytic anemia [6]. Alpha-thalassemia is often initially suspected on the basis of a routine full blood count. The key components of the full blood count include hemoglobin, red blood cell (RBC) number, mean corpuscular volume (MCV/fl), mean corpuscular hemoglobin (MCH/pg), and red cell distribution width. All affected individuals have a variable degree of anemia, reduced MCH, reduced MCV, and a normal or slightly reduced level of the minor HbA_2 at electrophoresis. A wide range of clinical phenotypes is produced depending on the number of alpha-thalassemia alleles [7]. Carriers of a single mutant α-globin gene are clinically silent and show little to no hemoglobin (Hb) abnormalities. Those with two affected genes have mild microcytic hypochromic anemia with normal Hb A_2 levels. Mutations in three α-globin genes lead to Hemoglobin H (HbH) disease due to a marked imbalance in the globin chain synthesis ratio. Homozygosity for alpha-thalassemia is the cause of HbH disease, characterized by the development of hemolytic anemia of a variable degree, and the lethal Hb Barts hydrops fetalis syndrome [8].

Although it is accepted that the clinical phenotypes vary according to the number of mutated genes, this study was planned for the prediction of phenotypes that can vary not only according to the number of mutated genes, but also to the character of the mutation.

Materials and Methods

Study Design

The study was approved by the Ethical committee and written informed consent was obtained from the subjects. The study was in compliance with the Helsinki declaration. Among the 2500 individuals with mild or moderate anemia, microcytosis, and normal iron levels that were referred to Adana Numune Training and Research Hospital Genetic Diagnosis Center for detection of thalassemia mutations, a population consisting of 539 individuals were included in the study. We investigated patients who were referred to our center for probable iron deficiency anemia and beta thalassemia, at first step. Hence we evaluated patients' blood serum iron, total iron binding capacity, ferritin, transferring saturation index, peripheral blood smear and hemoglobin electrophoresis. Patients with beta thalassemia and iron deficiency anemia were excluded from the study. We detected small group patients who are hematologically compatible with iron deficiency anemia as well as thalassemia. These criteria were low hemoglobin, low MCV and MCHC, but normal or higher RBC level according to the age.

Hematological parameters were analyzed using (on) the CD 3700 automatic cell counter (Abbott, Santa Clara, CA). The high performance liquid chromatography was performed to detect the carriers of hemoglobin variants. The Primus Ultra2 Resolution method was used according to manufacturers' instructions (Primus Corporation, Kansas City, USA). The age, sex, spleen status, transfusion history, and hematological parameters, including total hemoglobin, RBC, HbA_2, HbF, MCV, and MCH, were recorded for each patient. Skeletal deformity was absent in all subjects. After obtaining written informed consent, 2 ml of blood was collected in EDTA from each patient.

Molecular Analysis

Blood samples were collected and DNA was extracted by the salting out procedure [9]. Alpha globin mutations were analyzed using multiplex-PCR and reverse-hybridization assay according to the instructions provided by the manufacturer (Alpha-globin StripAssay; ViennaLab Diagnostics, Vienna, Austria), covering the following 21 mutations: two single gene deletions (−3.7, −4.2), five double gene deletions [–MED, –SEA, –THAI, –FIL, –20.5 kb], anti-α-3.7-kb α triplication, two point mutations on the α1 gene [codon 14 (T*G*G > T*A*G); codon 59 (G*G*C > G*A*C) (Hb Adana)], and 11 mutations on the α2 gene [initiation codon A*T*G > A*C*G; codon 19 (GC*G* > GC-), IVS-I(−5 nt) (−TGAGG); codon 59 (G*G*C > G*A*C); codon 125 (C*T*G > C*C*G) (Hb Quong Sze); Hb Constant Spring (HbCS) α142, Term → Gln, *T*AA > *C*AA; Hb Icaria, α142, Term → Lys, *T*AA > *A*AA; Hb Paksé, Term → Tyr, TA*A* > TA*T*, Hb Koya Dora, α142, Term → Ser, T*A*A > T*C*A; polyadenylation signal (polyA) AATAA*A* > AATAA*G* (polyA1); polyA2 AAT*A*AA > AAT*G*AA] [5].

Table 1 Number and frequency of mutated α-globin alleles in patients from Adana, Turkey

Mutation	Number of alleles	Frequency (%)
−3.7	450	63.3
–MED	83	11.7
–20.5	76	10.7
IVS1(-5nt)	28	3.9
α2PolyA-2	25	3.5
anti-3.7	19	2.7
α2 Poly A-1	10	1.4
−4.2	7	1
Hb Icaria	5	0.7
α1 cd 59 [G > A](Hb Adana)	4	0.6
α2 cd 142 [T > C](Constant Spring)	2	0.3
–SEA	2	0.3

Table 2 Alpha-thalassemia mutations and mean hematological values for each genotype in patients from Adana

Genotype	Number	Frequency (%)	Mean MCV ± SD (min–max)	Mean MCH ± SD (min–max)	Mean MCHC ± SD (min–max)	Mean Hb ± SD (min–max)	Mean RBC ± SD (min–max)
$-\alpha^{3.7}/\alpha\alpha$	193	35.8	74.9 ± 7.6 (49.4–89.9)	25 ± 3.2 (13.9–33.6)	33.3 ± 1.8 (27.6–44.4)	12.2 ± 2.2 (5.7–18)	5.2 ± 3.4 (2.2–12.8)
$-\alpha^{3.7}/-\alpha^{3.7}$	102	18.9	63.7 ± 7.4 (47.1–83.3)	20.5 ± 2.7 (14.7–29.7)	32.2 ± 1.3 (29.4–36.5)	10.8 ± 1.9 (7.1–15.3)	5.3 ± 0.7 (2.6–7.1)
$-^{20.5}/\alpha\alpha$	62	11.5	65.5 ± 3.5 (57.4–72.2)	20.8 ± 1.3 (18–23.5)	31.7 ± 1.1 (29.6–36.6)	11.9 ± 1.1 (9.9–14.6)	5.8 ± 0.5 (4.7–6.9)
$-^{MED}/\alpha\alpha$	56	10.4	65.5 ± 3.0 (58.7–72.2)	21.1 ± 1.2 (18–23.3)	32.2 ± 1.1 (30.2–35.4)	11.9 ± 1.2 (9–14.7)	5.7 ± 0.6 (4.4–6.9)
$-^{MED}/-\alpha^{3.7}\alpha$	24	4.5	58.1 ± 5.0 (51.3–68.2)	18.1 ± 1.3 (16.2–21)	31.2 ± 1.4 (28.6–34.2)	9.4 ± 1.0 (7.7–11.8)	5.2 ± 0.6 (4.2–6.6)
$\alpha^{anti-3.7}/\alpha\alpha$	19	3.5	67.7 ± 7.7 (50.3–91.7)	23.5 ± 5.2 (15.2–33.4)	32.5 ± 2.0 (28.5–37.4	10.2 ± 2.3 (6.3–15.8)	4.4 ± 0.9 (2.4–6.1)
$-^{20.5}/-\alpha^{3.7}$	12	2.2	58.6 ± 6.5 (49.4–69.8)	18.7 ± 1.6 (16.4–20.7)	31.6 ± 1.7 (28.9–34.9)	9.3 ± 1.2 (7.3–11.7)	5.1 ± 0.9 (3.7–7.1)
$\alpha2^{IVS1(-5nt)}/\alpha\alpha$	11	2	71.9 ± 8.0 (55.4–85.5)	24.2 ± 2.9 (17.3–28.2)	33.4 ± 1.1 (31.1–35.1)	12.5 ± 1.4 (9.9–14.8)	5.3 ± 0.7 (4.1–6.4)
$\alpha2^{PolyA-2}/\alpha\alpha$	9	1.7	74.1 ± 6.7 (59.9–82.2)	25 ± 2.6 (19–27.7)	33.7 ± 1.3 (31.7–35.5)	11.7 ± 1.4 (9.1–13.3)	4.7 ± 0.4 (3.7–4.9)
$-\alpha^{3.7}/\alpha2^{IVS1(-5nt)}$	8	1.5	65.3 ± 3.9 (59.8–71.7)	20.7 ± 1.3 (18.8–22.9)	31.6 ± 0.7 (30.1–32.3)	11.4 ± 0.6 (10.7–12.1)	5.5 ± 0.4 (4.7–6.2)
$\alpha2^{PolyA-2}/\alpha2^{PolyA-2}$	5	0.9	67.1 ± 7.0 (57.3–73.8)	20.6 ± 2.8 (16.9–23.2)	30.6 ± 1.1 (29.4–31.5)	10.6 ± 1.3 (9.2–11.9)	5.2 ± 0.3 (4.7–5.5)
$\alpha2^{PolyA-1}/\alpha\alpha$	5	0.9	70.5 ± 3.2 (68.5–74.2)	23 ± 0.8 (22.1–23.5)	32.6 ± 1.1 (31.7–33.8)	11.7 ± 0.2 (11.6–11.9)	5.2 ± 0.2 (4.7–5.5)
$-\alpha^{3.7}/\alpha\ 2^{PolyA-2}$	5	0.9	67.8 ± 3.7 (62.6–72.4)	21.5 ± 1.1 (20.8–23.5)	31.8 ± 1.1 (30.6–33.2)	12 ± 0.9 (10.7–13.1)	5.7 ± 0.4 (5.3–6.3)
$\alpha2^{IVS1(-5nt)}/\alpha\ 2^{IVS1(-5nt)}$	4	0.7	60.7 ± 5.4 (55.6–68.2)	19.7 ± 1.7 (17.8–22.0)	32.4 ± 0.8 (31.8–33.5)	10.2 ± 0.8 (9.8–11.4)	5.2 ± 0.3 (5.0–5.6)
$\alpha^{Hb\ Adana}/\alpha\alpha$	3	0.6	75.5 ± 2.8 (72.6–78.2)	25.5 ± 1.0 (24.4–26.3)	33.8 ± 0.3 (33.6–34.1)	13.4 ± 1.0 (12.8–14.4)	5.2 ± 0.2 (5.0–5.5)
$-\alpha^{4.2}/\alpha\alpha$	3	0.6	77 ± 1.9 (75.6–78.3)	25.5 ± 1.6 (24.3–26.6)	33.1 ± 2.8 (31.1–35.1)	11.7 ± 0.3 (11.5–11.9)	4.6 ± 0.4 (4.3–4.9)
$\alpha2^{PolyA-1}/\alpha2^{PolyA-1}$	2	0.4	65.5 ± 5.7 (61.4–69.5)	20.5 ± 0.8 (19.9–21.0)	31.2 ± 1.6 (30.1–32.3)	8.6 ± 1.1 (7.8–9.4)	4.2 ± 0.7 (3.7–4.8)
$\alpha^{ConstantSpring}/\alpha\alpha$	2	0.4	84.4 ± 6.3 (79.9–88.8)	27.5 ± 2.3 (25.9–29.1)	32.6 ± 0.3 (32.4–32.8)	11.8 ± 1.6 (10.6–12.9)	4.3 ± 0.2 (4.1–4.4)
$-^{SEA}/\alpha\alpha$	2	0.4	62 ± 7.1 (57–67)	20 ± 2.0 (18.6–21.4)	32.3 ± 0.6 (31.9–32.7)	11.8 ± 0.5 (11.4–12.1)	5.9 ± 0.3 (5.7–6.1)
$\alpha^{HbIcaria}/\alpha\alpha$	2	0.4	77.4 ± 7.0 (72.4–82.3)	25.9 ± 3.2 (23.6–28.1)	33.4 ± 1.1 (32.6–34.1)	11.9 ± 3.1 (9.7–14.1)	4.6 ± 0.6 (4.1–5.0)
$-^{MED}/-\alpha^{4.2}$	2	0.4	60.8 ± 0.1 (60.7–60.8)	18.6 ± 0.1 (18.5–18.6)	30.5 ± 0.1 (30.4–30.6)	8.5 ± 0.7 (8, 9)	4.3 ± 0.01 (4.3–4.3)
$-^{MED}/\alpha\ 2^{IVS1(-5nt)}$	1	0.2	71 (NA)	20.8 (NA)	29.3 (NA)	9.4 (NA)	4.5 (NA)
$\alpha^{HbIcaria}/\alpha^{HbIcaria}$	1	0.2	69.5 (NA)	20.1 (NA)	29 (NA)	10.2 (NA)	5.1 (NA)
$-\alpha^{3.7}/\alpha2^{PolyA-1}$	1	0.2	58.6 (NA)	18.7 (NA)	31.9 (NA)	11.4 (NA)	6.1 (NA)
$-\alpha^{3.7}/\alpha^{Hb\ Adana}$	1	0.2	63.1 (NA)	21 (NA)	33.3 (NA)	11.4 (NA)	5.4 (NA)
$-\alpha^{3.7}/\alpha^{HbIcaria}$	1	0.2	71 (NA)	22.7(NA)	32 (NA)	10.5 (NA)	4.6 (NA)
$-\alpha^{3.7}/-\alpha^{4.2}$	1	0.2	64.4 (NA)	19.9 (NA)	30.9 (NA)	11.6 (NA)	5.8 (NA)
$-^{20.5}/\alpha^{2PolyA-2}$	1	0.2	61.1 (NA)	18 (NA)	29.3 (NA)	9.2 (NA)	5.1 (NA)
$-^{20.5}/-\alpha^{4.2}$	1	0.2	64.1 (NA)	19 (NA)	29.6 (NA)	11.4 (NA)	6 (NA)

SD Standart Deviation. *NA* Not Available

Statistical Analysis

Statistical analysis was carried out by custom scripts implemented in R (http://www.r-project.org) and an independent sample *t* test was used for comparison of hematological parameters. Genotypic and allelic frequencies were also calculated.

Results

A total of 539 patients met the eligibility criteria for the study. Of the 539 patients (37.1 % male and 62.9 % female), the mean age was 27.7 ± 17.6 (range 1–82) years.

The frequencies of α-globin allele and genotypes and the corresponding mean hematological values for all 539 patients were listed in Table 1 and Table 2. A total of 12 different α-globin variants were found, the most common 5 alpha-thalassemia mutations were −3,7, which comprises 63.3 % of the total mutated alleles, followed by –MED (11.7 %), −20.5 (10.7 %), α2 IVS1 (−5nt) (3.9 %), and α2 PolyA-2 (3.5 %). These five mutations make up 93.1 % of total mutations. Hemoglobin Constant Spring (HbCS) was detected in two patients for the first time in Turkey. Furthermore, –SEA deletion, the most frequent alpha-thalassemia mutation in China and Southeast Asia, was detected in only two patients (Table 1).

In this study we report that 29 alpha-thalassemia genotypes have been found in the Adana region of Turkey and that the most common genotypes have prevalence of 35.8 % ($-\alpha^{3.7}/\alpha\alpha$), 18.9 % ($-\alpha^{3.7}/-\alpha^{3.7}$), and 11.5 % ($-^{20.5}/\alpha\alpha$), respectively (Table 2). Hematological parameters were analyzed in relation to the genotype and the MCV value was found to be the lowest in the $-^{MED}/-\alpha^{3.7}$ and the highest in the $-\alpha^{3.7}/\alpha\alpha$ genotype. The MCH and the MCHC levels were also consistent with this finding. In summary, hematological indices were further reduced as the number of functional genes decreased (Table 2).

When the hematological values of the non-deletional mutations were compared to deletional mutations of genotypes that had one gene defect, there was no statistically significant difference (Table 3).

We also compared the hematological findings of patients who had the most common three genotypes with two gene deletions (Group 1: $-\alpha^{3.7}/-\alpha^{3.7}$; Group 2: $-^{MED}/\alpha\alpha$; Group 3: $-^{20.5}/\alpha\alpha$). We identified a significant difference between RBC and MCV indices, although both groups have two deleted genes in their genotype (Table 4).

Discussion

The thalassemias and abnormal hemoglobins constitute a group of the most common hereditary blood disorders in the world caused by quantitative or structural defects in hemoglobin synthesis [2, 3]. In our country, a broad range of mutations have been observed, probably because of Turkey's location amongst three continents and the influence of different cultures over the course of history. The Turkish population is a mixture of different ethnic groups; hence, the frequency and distribution of α-globin mutations in various regions of the country need to be clarified.

Mutations in α-globin genes are characterized by the absence or reduced expression of α-globin chains. Gene deletions including $-\alpha^{17.4}$, $-\alpha^{26.5}$, $-\alpha^{20.5}$, $\alpha2^{-5nt}$, $-\alpha^{3.7}$, and $-\alpha^{4.2}$, mutations including $\alpha2^{-Poly\ A1}$, $\alpha2^{-Poly\ A2}$ and α1 cd 59 G > A point mutation and $\alpha\alpha\alpha^{anti-3.7}$ 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 [4, 5]. The most common genotypes in Iraq were $-\alpha^{3.7}/\alpha\alpha$, $-^{MED}/\alpha\alpha$ and $-\alpha^{3.7}/-\alpha^{3.7}$ which were detected in 65.1 % of the study group [6]. In the present study, 12 different alleles and 29 genotypes were identified. $-\alpha^{3.7}$ mutation was the most common with an allele frequency of 63.3 %. Oner et al. [10] found the allele frequency of $-\alpha^{3.7}$ deletion as 28 % in 25 patients with alpha-thalassemia. In a study on patients with alpha-thalassemia from Cukurova region by Curuk et al., it was shown that there was $-\alpha^{3.7}$ mutation in 59.3 % of patients and allele frequency was 29.6 % [11]. Guvenc et al. detected $-\alpha^{3.7}$ deletion in 181 of 450 alleles from 225 patients with alpha-thalassemia and allele frequency was reported as 40.66 % [12]. In a study by Sutcu et al. [13] in which distribution of alpha-thalassemia mutations was investigated at Isparta province, $-\alpha^{3.7}$ allele frequency was 5.55 %. $-\alpha^{3.7}$ gene deletion is the most frequently seen mutations in West Asia countries including Iran (40–93 %), United Arab Emirates (28.4 %), Saudi Arabia (64 %), Oman (58.3 %), Tunisia (22.5 %), Jordan (43 %), and Israel (51 %) [14–20]. Similarly, $\alpha^{3.7}$ gene deletion is the most frequently seen mutation in Europe with the allele frequencies of 58.2 % in Holland, 46.94 %

Table 3 The comparison of heterogeneous deletions with heterogeneous non-deletional mutations for samples with single affected gene

	p Value	Mean non-deletions ± SD (min–max)[a]	Mean deletions ± SD (min–max)[b]
Hb	0.842	12.2 ± 2.2 (9.1–14.8)	12.2 ± 1.4 (5.7–18)
RBC	0.372	5.2 ± 3.4 (3.7–6.4)	4.9 ± 0.6 (2.2–12.8)
MCV	0.525	74.9 ± 7.5 (55.4–88.8)	73.9 ± 7.1 (49.4–89.9)
MCH	0.744	24.9 ± 3.2 (17.3–29.1)	24.8 ± 2.6 (13.9–33.6)
MCHC	0.647	33.3 ± 1.8 (31.1–35.5)	33.4 ± 1.1 (27.6–44.4)

[a] $\alpha2^{IVS1(-5nt)}/\alpha\alpha$, $\alpha2^{PolyA2}/\alpha\alpha$, $\alpha2^{Poly\alpha\ -1}/\alpha\alpha$; $\alpha^{Hb\ Adana}/\alpha\alpha$; $\alpha^{HbConstantSpring}/\alpha\alpha$; $\alpha^{Hb\ Icaria}/\alpha\alpha$

[b] $-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{4.2}/\alpha\alpha$

Table 4 Comparison of hematologic values of the most common three deletional genotypes

	$-\alpha^{3.7}/-\alpha^{3.7}$ vs $-^{20.5}/\alpha\alpha$			$-\alpha^{3.7}/-\alpha^{3.7}$ vs $-^{MED}/\alpha\alpha$			$-^{MED}/\alpha\alpha$ vs $-^{20.5}/\alpha\alpha$		
	Group1 (mean SD)	Group 3(mean ± SD)	*p* value	Group 1(mean ± SD)	Group 2(mean ± SD)	*p* Value	Group 2(mean ± SD)	Group 3(mean ± SD)	*p* Value
Hb	10.8 ± 1.8	11.9 ± 1.1	0.0000024	10.8 ± 1.9	11.9 ± 1.2	0.00001	11.9 ± 1.2	11.9 ± 1.1	0.880
RBC	5.3 ± 0.7	5.8 ± 0.5	0.0000002	5,3 ± 0.66	5,7 ± 0.58	0.00027	5.7 ± 0.6	5.8 ± 0.508	0.2727
MCV	63.7 ± 7.4	65.5 ± 3.5	0.04322	63.7 ± 7.4	65.5 ± 2.9	0.04437	65.5 ± 2.9	65.5 ± 3.5	0.94
MCH	20.5 ± 2.7	20.8 ± 1.3	0.40154	20.5 ± 2.7	21.1 ± 1.2	0.07639	21.1 ± 1.2	20.8 ± 1.3	0.2
MCHC	32.2 ± 1.3	31.7 ± 1.1	0.02771	32.2 ± 1.3	32.2 ± 1.1	0.8713	32.2 ± 1.1	31.7 ± 1.1	0.0266

in Sicily and 52.41 % in Spain [21]. This is also true for Malaysia (45.9 %), Brazil (10.7 %) and North Thailand (58.3 %) [22]. These results show that $-\alpha^{3.7}$ gene deletion is prevalent worldwide including our country and indicate that our results are in agreement with literature.

The $-\alpha^{3.7}$ deletion is the most frequent α-globin mutation, while frequencies of the $\alpha\alpha\alpha^{anti-3.7}$ triplication are only sporadically known [23]. The $\alpha\alpha\alpha^{anti-3.7}$ triplication was found lower than $-\alpha^{3.7}$ deletion in our study, similar to other studies worldwide.

$-^{MED}$ double-gene deletion, the second most frequently detected mutation, was found with a frequency of 11.7 % in our study. This mutation was the second most commonly seen mutation in the studies by Curuk 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 [10–12]. In the study by Sutcu et al. [13] it was reported as 27.77 %. The most frequently seen deletional mutation in Cyprus Turks with a frequency of 40 % was $-^{MED}$ double-gene deletion [24].

Hemoglobin Constant Spring (HbCS), usually seen in Asian populations, is characterized by an elongated α chain with additional 31 amino acid residues, caused by a point mutation in the termination codon of the α2-globin gene (HBA2 c.427T > C) [25, 26]. HbCS was detected in two patients for the first time in Turkey. –SEA deletion, the most frequent alpha-thalassemia mutation in China and Southeast Asia, was detected in only two patients [27, 28].

The key to successful detection of the thalassemias is the initial hematological parameters. Hematological parameters of patients with alpha-thalassemia were compared with those of patients with iron deficiency anemia and α- thalassemia. We know that RDW level is increased more in iron deficiency than thalassemia while RBC is increased in thalassemia [29, 30]. As it is known that the hematological parameters can manifest differences according to the type of the mutation, we revealed significant alterations for different types of mutations. Although two functional genes were found in each group, there was a statistically significant difference between $-\alpha^{3.7}/-\alpha^{3.7}$, $-^{MED}/\alpha\alpha$, and $-^{20.5}/\alpha\alpha$, while there were no difference between the latter two. The levels of transcription of the two alpha globin genes differ; the α2 gene produces two to three times more α-globin than the α1 gene and our results support this information [29]. We did not determine any statistically significant difference between the hematological values of the non-deletional mutations as compared to deletional mutations of genotypes that had one gene defect. However, the non-deletion alpha-thalassemia mutations should have more severe effects on α-globin gene expression [31].

The limitation of this study is not confirming the results with functional studies. On the other hand, many patients with anemia suspected of alpha thalassemia were excluded from this study because none of these mutations were found in these patients with the reverse dot blot method.

Our results show that alpha-thalassemia mutations are highly heterogeneous as well as deletional and $-\alpha^{3.7}$ single gene deletion is particularly prevalent at Adana province in agreement to other studies from Turkey. These results may be useful for genetic counseling and diagnosis. The present study is also important in terms of the potential contribution to development of national database of hemoglobinopathy gene mutations.

Acknowledgments None.

References

1. Kan YW, Golbus MS, Dozy AM (1976) Prenatal diagnosis of alpha-thalassemia. Clinical application of molecular hybridization. N Engl J Med 295:1165–1167
2. Celik MM, Gunesacar R, Oktay G, Duran GG, Kaya H (2013) Spectrum of α-thalassemia mutations including first observation of—(FIL) deletion in Hatay Province Turkey. Blood Cells Mol Dis 51:27–30
3. Sengchanh S, Sanguansermsri T, Horst D, Horst J, Flatz G (2005) High frequency of alphathalassemia in the So ethnic group of South Laos. Acta Haematol 114:164–166
4. Higgs DR, Vickers MA, Wilkie AOM, Pretorius I-M, Jarman AP, Weatherall DJ (1989) A review of the molecular genetics of the human a-globin gene cluster. Blood 73:1081–1104
5. Puehringer H, Najmabadi H, Law HY, Walter Krugluger W, Viprakasit V, Serge Pissard S, Baysal E, Taher A, Farra C, Al-Ali

A, Al-Ateeq S, Oberkanins C (2007) Validation of a reverse-hybridization StripAssay for the simultaneous analysis of common a-thalassemia point mutations and deletions. Clin Chem Lab Med 45:605–610
6. Al-Allawi NA, Badi AI, Imanian H, Nikzat N, Jubrael JM, Najmabadi H (2009) Molecular characterization of alpha-thalassemia in the Dohuk region of Iraq. Hemoglobin 33:37–44
7. Old JM (2003) Screening and genetic diagnosis of haemoglobin disorders. Blood Rev 17:43–53
8. Zandian K, Nateghi J, Keikhaie B, Pedram M, Hafezi-Nejad N, Hadavi V, Oberkanins C, Azarkeivan A, Law HY, Najmabadi H (2008) a-Thalassemia mutations in Khuzestan Province Southwest Iran. Hemoglobin 32:546–552
9. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 16:1215
10. Oner C, Gürgey A, Oner R, Balkan H, Gümrük F, Baysal E, Altay C (1997) The molecular basis of Hb H disease in Turkey. Hemoglobin 21:41–51
11. Cürük MA (2007) Hb H (beta4) disease in Cukurova Southern Turkey. Hemoglobin 31:265–271
12. Guvenc B, Yildiz SM, Tekinturhan F, Dincer S, Akyuzluer I, Okten S, Erkman H (2010) Molecular characterization of alpha-Thalassemia in Adana, Turkey: a single center study. Acta Haematol 123:197–200
13. Sutcu R, Aylak F, Kocak H, Sipahi T, Vural H, Delibas N (2011) The investigation of distribution of hereditary alpha-thalassemia mutations in Isparta reservoir. Eur J Basic Med Sci 1:28–32
14. Zandian K, Nateghi J, Keikhaie B, Pedram M, Hafezi-Nejad N, Hadavi V, Oberkanins C, Azarkeivan A, Law HY, Najmabadi H (2008) alpha-thalassemia mutations in Khuzestan Province Southwest Iran. Hemoglobin 32:546–552
15. Hellani A, Fadel E, El-Sadadi S, El-Sweilam H, El-Dawood A, Abu-Amero KK (2009) Molecular spectrum of alpha-thalassemia mutations in microcytic hypochromic anemia patients from Saudi Arabia. Genet Test Mol Biomarkers 13:219–221
16. Abu Ghoush MW (2008) Subtypes of alpha thalassemia diagnosed at a Medical Center in Jordan. TAF Prev Med Bull 7:373–376
17. El-Kalla S, Baysal E (1998) alpha-Thalassemia in the United Arab Emirates. Acta Haematol 100:49–53
18. Hassan SM, Hamza N, Jaffer Al-Lawatiya F, Jaffer Mohammed A, Harteveld CL, Rajab A, Giordano PC (2010) Extended molecular spectrum of beta- and alpha-thalassemia in Oman. Hemoglobin 34:127–134
19. Siala H, Ouali F, Messaoud T, Bibi A, Fattoum S (2008) alpha-Thalassaemia in Tunisia: some epidemiological and molecular data. J Genet 87:229–234
20. Oron-Karni V, Filon D, Shifrin Y, Fried E, Pogrebijsky G, Oppenheim A, Rund D (2000) Diversity of alpha-globin mutations and clinical presentation of alpha-thalassemia in Israel. Am J Hematol 65:196–203
21. Di Bella C, Salpietro C, La Rosa M, Cuppari C, Piraino B, Cutri MR, Rigoli L (2006) Identification of alpha-thalassemia mutations in subjects from Eastern Sicily (Italy) with abnormal hematological indices and normal Hb A2. Ann Hematol 85:829–831
22. Souza AE, Cardoso GL, Takanashi SY, Guerreiro JF (2009) Alpha-thalassemia (3.7 kb deletion) in a population from the Brazilian Amazon region: santarém, Pará State. Genet Mol Res 8:477–481
23. Moosavi SF, Amirian A, Zarbakhsh B, Kordafshari A, Mirzahoseini H, Zeinali S, Karimipoor M (2011) The carrıer frequency of α-globin gene triplication in an Iranian populatıon with normal or borderline hematological parameters. Hemoglobin 35(4):323–330
24. Baysal E, Kleanthous M, Bozkurt G, Kyrri A, Kalogirou E, Angastiniotis M, Ioannou P, Huisman TH (1995) alpha-Thalassaemia in the population of Cyprus. Br J Haematol 89:469–496
25. Laig M, Pape M, Hundrieser J, Flatz G, Sanguansermsri T, Das BM, Deka R, Yongvanit P, Mularlee N (1990) The distribution of the Hb constant spring gene in Southeast Asian populations. Hum Genet 84:188–190
26. Clegg JB, Weatherall DJ, Milner PF (1971) Haemoglobin Constant Spring–a chain termination mutant? Nature 234:337–340
27. Xiong F, Sun M, Zhang X, Cai R, Zhou Y, Lou J, Zeng L, Sun Q, Xiao Q, Shanq X, Wei X, Zhanq T, Chen P, Xu X (2010) Molecular epidemiological survey of haemoglobinopathies in the Guangxi Zhuang Autonomous Region of southern China. Clin Genet 78:139–148
28. Xu XM, Zhou YQ, Luo GX, Liao C, Zhou M, Chen PY, Lu JP, Jia SQ, Xiao GF, Shen X, Li J, Chen HP, Xia YY, Wen YX, Mo QH, Li WD, Li YY, Zhuo LW, Wanq ZQ, Chen YJ, Qin CH, Zhonq M (2004) The prevalence and spectrum of alpha and beta thalassaemia in Guangdong Province: implications for the future health burden and population screening. J Clin Pathol 57:517–522
29. Galanello R, Cao A (2011) Gene test review. Alpha-Thalassemia. Genet Med 13:83–88
30. Rahim F (2009) Microcytic hypochromic anemia patients with thalassemia: genotyping approach. Indian J Med Sci 63:101–108
31. Di Marzo R, Lo Gioco P, Giambona A, Acuto S, Sammarco P, Oddo G, Maggio A (1986) Clinical severity of non-deletion form of HbH disease (–Med/alpha alpha thal). Scand J Haematol 36:39–43