

Original research paper

# Alpha thalassemia gene mutations in neonates from Mazandaran, Iran, 2012

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**Aim:** Alpha thalassemia is one of the most prevalent disorders worldwide and carrier frequency of the disease is varied in different parts of the world. Although different studies in Iran and Mazandaran province have been carried out to identify different mutations of alpha globin gene among people with low hematological indices, frequencies of these mutations were unknown in general population, and thus the aim of this study was to evaluate the carrier frequencies of alpha globin gene mutations among neonates in Mazandaran.

**Material and methods:** Four hundred and twelve neonates were collected from a delivery ward of a hospital in Sari. DNA was extracted from their cord blood samples using phenol-chloroform-based method. For the detection of five common alpha thalassemia gene mutations, multiplex-GAP-PCR and PCR-RFLP methods were applied.

**Results:** Sixty three (15.29%, confidence interval, CI 95%: 11.81–18.77) of investigated neonates had at least one of the five evaluated mutations. The  $-\alpha^{3.7}$  deletion had the highest frequency (9.7%, CI 95%: 6.84–12.56) and none of the neonates had  $-\text{Med}$  double gene deletion. The  $-\alpha^{4.2}$  deletion,  $\alpha\alpha^{\text{anti}3.7}$  triplication, and  $\alpha^{-5\text{nt}}$  mutations had frequencies of 4.1% (CI 95%: 2.19–36.01), 2.2% (CI 95%: 0.78–3.62), and 0.49% (CI 95%: –0.18–1.16), respectively.

**Discussion:** Our study showed that in most of the alpha thalassemia carriers just one copy of alpha globin gene was absent and they are not at risk of having children with Hb H disease or hydrops fetalis; however, up to 2.2% of neonates were carriers for  $\alpha\alpha^{\text{anti}3.7}$  triplication and they will be at risk for having a child with thalassemia intermediate if they marry a person which is a carrier of beta thalassemia.

**Keywords:** Alpha thalassemia, Allelic frequency  $-\alpha^{3.7}$  deletion,  $\alpha\alpha^{\text{anti}3.7}$  triplication

## Introduction

Alpha thalassemia is one of the most common inherited disorders in the globin gene throughout the world.<sup>1</sup> Regarding hematologic symptoms a wide spectrum from an asymptomatic carrier to a very severe anemia incompatible with life may be anticipated.<sup>2</sup> The disorder has high incidence rate in Southeast Asia, Mediterranean countries, Middle East, India, and sub-Saharan Africa. Carrier frequency of the disease is varied in different parts of the world, ranging from 1 to 90%.<sup>3</sup> The frequency and distribution of alpha globin gene mutations has diversity in different regions of Iran, an issue that has to be addressed precisely.<sup>1–5</sup>

A typical individual has two copies of alpha globin gene on chromosome 16 at 16p13.3 position. The deletion of one of the two copies of alpha globin gene in a chromosome is known as alpha-thalassemia-2 ( $-\alpha/$

$\alpha\alpha$ ) and loss of two copies in a chromosome is called alpha-thalassemia-1 ( $-/\alpha\alpha$ ). Hb H disease occurs when three  $-$  copies of alpha globin gene are missing ( $-/-\alpha$ ) and in Hb Barts hydrops fetalis syndrome all four copies of the alpha globin gene are absent ( $-/-$ ). Alpha-thalassemia-2 is the most frequent type of a thalassemia throughout the world. Individuals with three active alpha globin gene are clinically and hematologically silent. Carriers with thalassemia trait (cis  $-/\alpha\alpha$  or trans  $-\alpha/-\alpha$ ) show very mild hypochromic microcytic anemia. These individuals are at risk of having children with hydrops fetalis or Hb H disease.<sup>6–8</sup> Point mutations in the alpha-globin genes are less frequent; however, Hb H disease caused by non-deletional mutations ( $\alpha^T \alpha/-$ ) are more severe and the patient is more dependent on blood transfusion compared with deletion-related Hb H disease ( $-/-\alpha$ ). There are a few reports of incidence of Hb H disease caused by homozygosity or compound heterozygosity for nondeletional mutations in  $\alpha 2$ -globin gene.<sup>9,10</sup>

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Pre-marital counseling for identification of beta thalassemia carriers is compulsory in Iran since 1997<sup>11</sup>; however, in many individuals who have reduced hematological indices, no beta globin gene mutations can be found, furthermore, some beta globin gene mutations change the indices very slightly,<sup>12,13</sup> and characterization of alpha globin gene mutations can be of great help in identification of hemoglobinopathies. This information is useful in pre-marital counseling.

### Materials and methods

Four hundred and twelve samples of umbilical cord blood were collected from a delivery unit in Sari, Iran, after receiving consent from infants' parents. Red blood cell (RBC) count, hemoglobin concentration (Hb), mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration, and other hematological indices were evaluated. The samples were then frozen and stored at -80°C up to the time of molecular investigation.

Genomic DNA was extracted from leukocytes using phenol-chloroform-based method and Qiagen DNA extraction kit. Multiplex Gap PCR method described by Oron-Karni *et al.* was applied to detect three common gene deletions in the region, namely -α<sup>3.7</sup>, -α<sup>4.2</sup>, and -Med deletions and alpha triplication mutation (ααα<sup>anti3.7</sup>).<sup>14</sup> PCR-RFLP method using HphI restriction enzyme was also applied to detect α<sup>-5nt</sup> mutation.

### Results

Among 412 investigated neonates with Mazandarani ethnicity, consisting of 198 male (48%) and 214 female cases (52%), 63 (15.29%, CI 95%: 11.81-18.77) cases had at least one mutation on alpha globin gene, including 54 alpha globin deletions, 9 cases of ααα<sup>anti3.7</sup> triplication, and 2 α<sup>-5nt</sup> mutations. Among five investigated mutations -α<sup>3.7</sup> had the highest frequency (9.7%, CI 95% = 6.84 to 12.56) and none of the neonates had -Med double gene deletion. Five neonates had two mutations simultaneously: two cases with -α<sup>3.7</sup> deletion in homozygote state, and three compound heterozygote

samples consisting of -α<sup>3.7</sup> and -α<sup>4.2</sup> deletions, -α<sup>3.7</sup> deletion and ααα<sup>anti3.7</sup> triplication, and -α<sup>4.2</sup> deletion and ααα<sup>anti3.7</sup> triplication (Table 1). The hematological indices relating to each mutation among neonates are shown in Table 2.

Among 824 investigated chromosomes, 68 were affected with one of the studied mutations and overall allelic frequency of alpha globin gene mutations was 0.0825. 37 out of 824 chromosomes had -α<sup>3.7</sup> deletion and this mutation had the highest allelic frequency (0.0485) among five studied mutations. The -α<sup>4.2</sup>, ααα<sup>anti3.7</sup> triplication, and α<sup>-5nt</sup> mutations had allelic frequencies of 0.0206, 0.0109, and 0.0024, respectively. The -α<sup>3.7</sup> and -α<sup>4.2</sup> deletions had the highest and second highest frequencies among mutated alleles (58.8 and 25%, respectively) (Table 3).

### Discussion

The majority of the alpha thalassemia in the Mediterranean countries caused by few common deletions including -α<sup>3.7</sup>, -α<sup>4.2</sup>, -Med, and ααα<sup>anti3.7</sup> triplication.<sup>14</sup> Several studies in Iran have reported the frequencies of alpha thalassemia mutations among patients with reduced hematological indices and the results are varied among different regions of the country.<sup>4</sup> In North of Iran although the frequencies of alpha globin gene mutations was studied among patients with reduced hematological indices, the exact frequencies of common mutations was not studied in the whole population, including those with normal hematological indexes.<sup>15</sup> The aim of this study was to evaluate the allelic frequencies of alpha thalassemia common gene mutations among neonates in Mazandaran.

In comparison with beta thalassemia cases, alpha thalassemia carriers have hematological indices closer to normal individuals,<sup>16</sup> and therefore it is not possible to identify all of them in screening program, which is merely based on evaluation of hematological indices. In Mazandaran province screening program of thalassemia carriers has effectively been in use for several years; however, this study is the first one to report exact frequencies of alpha thalassemia common mutations among neonates in Mazandaran, using molecular methods.<sup>11</sup>

In our previous study we studied hemoglobin alpha chain gene deletion in 680 neonates from Mazandaran using high-performance liquid chromatography (HPLC) for the detection of hemoglobin Bart's and the results showed that 12% (CI 95%: 9.56-14.44) of neonates had one or two alpha globin gene deletions.<sup>17</sup> Although in previous study we reported the frequency of neonates with alpha globin gene deletions, because of the applied technique (HPLC) we could not report the types of deletions, frequencies of point mutations and alpha globin gene triplication. In this study the

**Table 1** Distribution of alpha globin genotypes in 412 neonates, Mazandaran, Iran, 2012

Genotype	Number of cases	Frequency (%)
αα/αα (wild type)	349	84.7
-α <sup>3.7</sup> /αα	34	8.3
-α <sup>4.2</sup> /αα	15	3.6
ααα <sup>anti3.7</sup> /αα	7	1.7
-α <sup>3.7</sup> /-α <sup>3.7</sup>	2	0.48
α <sup>-5nt</sup> /αα	2	0.48
-α <sup>3.7</sup> /-α <sup>4.2</sup>	1	0.24
-α <sup>3.7</sup> /ααα <sup>anti3.7</sup>	1	0.24
-α <sup>4.2</sup> /ααα <sup>anti3.7</sup>	1	0.24

**Table 2** Hematological indices of neonates with one the alpha globin gene mutations

Hematological indices	RBC ( $\times 10^6/\mu\text{l}$ )	Hb (g/dl)	Hct (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
$-\alpha^{3.7}$	$4.4 \pm 0.6$	$12.8 \pm 0.2$	$39.3 \pm 5.0$	$90.1 \pm 6.1$	$29.6 \pm 2.1$	$32.0 \pm 2.9$
$-\alpha^{4.2}$	$4.3 \pm 1.0$	$13.2 \pm 3.3$	$39.6 \pm 8.5$	$92.6 \pm 4.9$	$29.6 \pm 1.2$	$32.0 \pm 1.9$
$\alpha\alpha\alpha^{\text{anti}3.7}$	$4.9 \pm 1.1$	$14.8 \pm 2.9$	$46.7 \pm 9.3$	$95.2 \pm 1.6$	$29.8 \pm 1.1$	$31.7 \pm 0.1$
$\alpha^{-5\text{nt}}$	2.4	7.3	21.9	92.9	31.4	33.9
Neonate with two mutations	$4.62 \pm 0.97$	$12.6 \pm 4.0$	$39.5 \pm 12.5$	$84.4 \pm 8.8$	$26.5 \pm 2.7$	$31.73 \pm 1.1$
Neonates with one of the mutations	$4.3 \pm 0.8$	$12.7 \pm 2.6$	$38.8 \pm 7.5$	$91.2 \pm 5.1$	$29.7 \pm 1.9$	$32.1 \pm 2.6$
Neonates with none of the 5 evaluated mutations	$4.1 \pm 1.0$	$13.1 \pm 1.7$	$40.1 \pm 6.8$	$98.3 \pm 6.0$	$32.3 \pm 2.0$	$32.9 \pm 2.6$

MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume.

frequency of alpha globin gene deletions was 13.1% (CI 95%: 9.84–16.36) which is close to the result of our previous study. Moreover, in our study we reported the frequencies of one point mutation and  $\alpha\alpha\alpha^{\text{anti}3.7}$  triplication.

Our study showed that although up to 15.3% (CI 95%: 11.82–18.78) of Mazandarani neonates are carriers of alpha globin gene mutations, a majority of them had single gene mutations and will be asymptomatic and unidentifiable by complete blood count test at any time in their lives. 2.2% (CI 95% = 0.78 to 3.62) of neonates carry  $\alpha\alpha\alpha^{\text{anti}3.7}$  triplication that causes no significant hematological changes. Different studies have shown that interaction of this mutation with heterozygous beta thalassemia leads to intermediate form of thalassemia which is nowadays called non-transfusion-dependent beta thalassemia major.<sup>16,17</sup> Since around 10% of Mazandarani population are carriers for different beta thalassemia mutations,<sup>11</sup> simultaneous presence of  $\alpha\alpha\alpha^{\text{anti}3.7}$  triplication and beta globin gene mutation in a couple should be considered in genetic counseling.<sup>18,19</sup>

Molecular analysis of alpha globin gene mutations in 255 patients with hypochromic and microcytic anemia by Tamaddoni *et al.* in Mazandaran province showed 21 different mutations in 89% of the patients. In that study most frequent mutations were  $-\alpha^{3.7}$  deletion (44.9%), polyadenylation signal 2 ( $\alpha^{\text{Polya}2}$ ) (AATAAA > AATGAA) (18.2%),  $-\alpha^{4.2}$  deletion (9.1%),  $\alpha^{-5\text{nt}}$  (6.5%), and  $-\text{Med}$  (4.3%).<sup>15</sup> In our study all of these mutations were studied among randomly collected neonates except polyadenylation signal 2 ( $\alpha^{\text{Polya}2}$ ) (AATAAA > AATGAA) which was excluded

and  $\alpha\alpha\alpha^{\text{anti}3.7}$  triplication included in our study. The results of our study on  $-\alpha^{3.7}$  deletion was similar to their work, confirming it to be the most common mutation in North of Iran, but the frequency of  $-\alpha^{4.2}$  deletion in our work was much higher than theirs (25% versus 9.1% among mutated alleles) and frequencies of  $\alpha^{-5\text{nt}}$  (6.5%), and  $-\text{Med}$  (4.3%) mutations were less than previous report.

In order to detect different hemoglobin disorders, a study on 8500 individuals, participating in thalassemia counseling program, was carried out in another city in Mazandaran province. Attendants were categorized as alpha or beta thalassemia carriers, according to their hematological indices and these cases underwent molecular examination for the detection of alpha globin gene mutations. Among molecularly investigated cases 47.5% had  $-\alpha^{3.7}$  mutation.<sup>19</sup> Our study shows higher, almost two folds, frequency of alpha thalassemia carriers in Mazandaran, compared with the above mentioned study. Since our study covers the cases randomly, regardless of their hematological indices, therefore the results are more reliable to reflect exact carrier frequencies of alpha thalassemia mutations in the whole population.

Recently, Zarbakhsh *et al.* reported the frequency of alpha thalassemia carriers detected in Tehran premarital screening program using molecular techniques.<sup>4</sup> Among 479 patients with low hematological indices, 95 cases had at least one deletion on alpha globin genes, and a frequency of 19.83% (CI 95% = 11.81–25.85) was observed. Tehran has a population of 14 million with diverse ethnicity. In both studies  $-\alpha^{3.7}$  has the highest frequency. In Tehran just 2 out of 958 investigated chromosomes had the  $-\alpha^{4.2}$  deletion; however, this deletion has the second highest frequency in Mazandaran.

Unlike Tehran, where the  $-\text{Med}$  double gene deletion was found in four cases, in our study no  $-\text{Med}$  double gene mutation was observed. In our study nine cases were diagnosed to have  $\alpha\alpha\alpha^{\text{anti}3.7}$  triplication while this mutation was not reported in their study.<sup>4</sup>

Nevertheless, in another study on Tehran population, the frequency of this mutation was reported to be close to Mazandaran (2.14%).<sup>20</sup> Comparing all the available studies in different provinces of Iran,

**Table 3** Allelic frequencies of different mutations among 824 investigated alleles

Mutation	Number of affected alleles	Allelic frequency
$-\alpha^{3.7}$	40	0.0485
$-\alpha^{4.2}$	17	0.0206
$\alpha\alpha\alpha^{\text{anti}3.7}$	9	0.0109
$\alpha^{-5\text{nt}}$	2	0.0024
$-\text{Med}$	0	0.0000
Mutations (total)	68	0.0825

one may conclude that the total carrier frequency of alpha globin gene mutations is similar in different regions of Iran; however, the spectra of mutations and their frequency rate are dissimilar in different provinces, reflecting various ethnicities living in Iran. It needs to be clarified here, on the case of  $-\alpha^{3.7}$  deletion, which has the highest frequency among alpha globin gene deletions worldwide, the same result was obtained in all reports from various provinces of Iran.

Neonatal screening of the 418 cord blood samples in United Arab Emirates (UAE) demonstrates that 49% of the cases had an alpha globin gene defect. Comparing to Mazandaran province, frequency of alpha thalassemia carriers is higher in UAE, but it has to be noted this high frequency rate is mostly due to high allelic frequency of  $-\alpha^{3.7}$  deletion in that population (0.2847 in UAE versus 0.0485 in Mazandaran), but if we compare the next common mutations in the two populations, we realize that other mutations do not have very high frequencies in UAE and for instance,  $-\alpha^{4.2}$  deletion which is the second most common mutation in both societies, has a higher allelic frequency rate in Mazandaran (0.0072 versus 0.0206). The results of similar studies from Southeast Asia, Brazil, and Georgia have shown that carriers with  $-\alpha^{3.7}$  deletion are more common than other alpha thalassemia gene mutations, and this mutation has to be considered the most common alpha globin gene mutation worldwide.<sup>21</sup>

## Conclusion

Hereby we conclude that, in comparison with previous studies on beta thalassemia mutations in Mazandaran, mutations on alpha globin gene are more prevalent, and because of the role  $\alpha\alpha^{\text{anti}3.7}$  triplication in thalassemia intermedia, we strongly recommend that molecular evaluation of  $\alpha\alpha^{\text{anti}3.7}$  triplication and beta thalassemia gene mutations should be included in pre-marital and prenatal screening programs.

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