

*Full Length Research Paper*

# Spectrum of $\alpha$ -thalassemia mutations in Qazvin Province, Iran

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$\alpha$ -Thalassemia is a widespread inherited disease particularly prevalent in the middle East Asia population, including Iran. The aim of this study was to define the molecular spectrum and frequency of  $\alpha$ -thalassemia mutations in prospective couples of Qazvin province. A total of 120,000 subjects were studied during 10 years (1998-2008). Individuals present with hypochromic and microcytic parameters with normal haemoglobin  $\alpha$ -2 (HbA2), without iron deficiency were included in the study. Molecular detection of  $\alpha$ -globin mutations were performed by gap-PCR, reverse dot blot hybridization (RDB) and sequencing. Results show that six different kinds of mutations are present in this region. In 22 subjects, most prevalent  $\alpha$ -thalassemia mutations were  $\alpha^{3.7}$ , followed by  $\alpha^{20.5}$  and  $\alpha^{5nt}$ . Most  $\alpha$ -thalassemia couples had consanguineous relationships and Kordish ethnicity. In conclusion, in spite of relatively low incidence of  $\alpha$ -thalassemia mutations in Qazvin province, the spectrum and frequency of mutations are different from other parts of Iran. It might be due to migration of several ethnic groups to Qazvin.

**Key words:**  $\alpha$ -Thalassemia, mutations, prospective marriage couples, Qazvin.

## INTRODUCTION

The  $\alpha$ -thalassemias are classified broadly to  $\alpha^0$  thalassemias, in which no  $\alpha$  chains are produced from the affected chromosome (16P13.3), and the  $\alpha^+$  thalassemias in which there is a reduced output of  $\alpha$  chains from the particular chromosome. The normal  $\alpha$  genotype can be represented as  $\alpha\alpha/\alpha\alpha$ . The homozygous and heterozygous states for  $\alpha^0$  thalassemia, which most commonly result from deletions of both of the linked  $\alpha$ -globin genes are presented as  $--/--$  (Hydrops fetalis) and  $-/\alpha\alpha$  (minor state), respectively. The  $\alpha^+$  thalassemias may result from the deletion of one of the linked pairs of  $\alpha$ -globin genes or from a mutation (T) that inactivates only one of the pair (mild form). The homozygous and heterozygous states for the deletion forms are represent as  $-/\alpha$  and  $-/\alpha\alpha$ , respectively (Higgs, 2009). The thalassemia field is very extensive. The distribution and popular genetics as well as molecular pathology of the

condition are heterogeneous. Overall, the  $\alpha$ -thalassemias follow a similar distribution to the  $\beta$ -thalassemia, extending from sub-saharan Africa through the Mediterranean region and Middle East, to Indian sub-continent and East and South East Asia. The  $\alpha^0$  thalassemias reach their highest frequency in South East Asia, where in some regions more than 10% of the population are carrier (Bowden et al., 1992; Weatherall, 2001).

Iran with a population of about 75 million is situated in thalassemia belt. Over recent 2 decades, due to the implementation of Iranian national  $\beta$ -thalassemia screening program, the spectrum of  $\beta$ -thalassemia mutations have been defined but the epidemiologic condition and spectrum of  $\alpha$ -thalassemia is required to be elucidated.

In the  $\beta$ -thalassemia screening program, the most important problem encountered is the interpretation of persistent microcytic- hypochromic indices of red blood cells with no other abnormality which is usually due to mild forms of  $\alpha$ -thalassemias but the presence of silent form of  $\beta$ -thalassemia must also be considered.

In recent years, a couple of investigators have performed researches in these individuals to analyze and define the spectrum of their  $\alpha$ -globin gene mutation in different parts or provinces of Iran (Zarbakhsh et al., 2010;

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**Table 1.** Spectrum of  $\alpha$ -thalassemia mutations in prospective couples of Qazvin province, Iran.

Mutation	Frequency (%)	Number
Alpha -3.7	27.27	6
Alpha- 20.5	13.64	3
Alpha 5'nt	13.64	3
Alpha med	9.09	2
Codone 59	4.54	1
Mild form ( $\alpha$ - / $\alpha$ $\alpha$ ) thalassemia	31.81	7
Total	100	22

Zandian et al., 2008; Tamadoni, 2009; Hadavi et al., 2009). Some authors divided Iran into 8 regions according to geographic boundaries and population distributions and the frequencies of  $\alpha$ -thalassemia mutation have been elucidated in these regions (Hadavi, 2007). It is because the Iranian population is a mixture of different ethnic groups and the frequency of  $\alpha$ -thalassemia mutation in various provinces of the country need to be clarified.

In our past studies conducted on  $\beta$ -thalassemia, major patients of Qazvin province (located in central to North West of Iran), the spectrum as well as rare or unexpected  $\beta$ -globin gene mutations were shown (Sarookhani, 2009, 2010).

Here, we report  $\alpha$ -globin gene mutations in suspected  $\alpha$ -thalassemia trait prospective couples of the screening program in Qazvin province. These data can help for diagnosis, prevention and migration effects of population on  $\alpha$ -thalassemia in this region.

## MATERIALS AND METHODS

This is an analytical-descriptive study conducted on prospective couples with microcytic hypochromic abnormality of thalassemia screening program referred to genetic laboratory during 10 years (1998-2008). In the process of screening program, marriage registrars refer prospective couples to a designated local laboratory for premarital screening to obtain hematological parameters from an automated cell counter (using Sysmex<sup>®</sup> instrument).

In the laboratory, the man's red cell indices are checked first. If he has MCV<80 fl and MCH<27 pg, the woman is tested. When both have microcytic-hypochromic conditions, their HbA2 concentrations are measured (using Helena<sup>®</sup> HbA2 kits). If both have a concentration above 3.5% (hallmark for  $\beta$ -thalassemia trait), they are referred to the designated health post for genetic counseling.

Microcytic individuals with a HbA2 concentration in the normal range (1.5-3.5%) are treated with iron (at least one month) and their indices rechecked. If the indices are not corrected and HbA2 remain unchanged, the individual may have two possibilities, Beta silent or normal HbA2  $\beta$ -thalassemia minor, and  $\alpha$ -thalassemia allele (Samavat and Modell, 2004; Mosca et al., 2009).

During 10 years of screening program, we examined the results of over 120,000 prospective couples and individuals having  $\alpha$ -thalassemia mutations criteria were included in the study. Subjects with iron deficiency,  $\beta$ -thalassemia trait and silent forms of  $\beta$ -thalassemia were excluded.

The age, sex, consanguinity between couples, ethnicity and other

demographic data were recorded by reviewing their files. The suspected individuals were referred to genetic centre. Blood samples were collected on EDTA (anti coagulant). DNA was isolated from white blood cells, using salting out method (Miller et al., 1998). The DNA extract was then kept at -70°C until analysis.

Detection of  $\alpha$ -thalassemia mutations in genetic centre was achieved by gap PCR, reverse dot blot hybridization (RDB) and sequencing techniques. For gap PCR, the method of Zohu and Liu et al., (2002), which is a modification of Baysal and Huisman method, was used.

Strip assay<sup>®</sup> kits of  $\alpha$ -globin (Vienna lab. diagnostics, Vienna, Austria) were used for RDB hybridization. DNA sequencing was used for samples in which no mutations were detected at PCR or RDB stages (for samples after the year of 2004). The purified samples were analyzed on an automated sequencer analyzer (ABI-3730XL Capillary system, USA) according to the manufacturer's instructions. The data were statistically analyzed using SPSS version 16 software.

## RESULTS

From over 120,000 prospective couples (individuals) studied, only 28 suspected individual were referred to genetic centre for investigation of  $\alpha$ -thalassemia during ten years (1998 to 2008) of study (Carriers of  $\beta$ -thalassemia minor (typic or silent forms) or one-side normal of prospective couples were excluded).

All referred (suspected) individuals had lowered MCV and MCH (microcytosis and hypochromia) and normal HbA2 levels (<3.5%) without iron deficiency anemia.

22 individuals had a kind of  $\alpha$ -thalassemia mutation. The spectrums of various kinds of mutations are shown in Table 1. A total of 6 different mutations were indentified in 22 individuals. Some mild forms of  $\alpha$ -thalassemia were detected in the remaining 7 individuals. Most of these mild  $\alpha$ -thalassemia mutations were belonged to individuals referred before 2004. Some one-side  $\beta$ -thalassemia and one-side  $\alpha$ -thalassemia couples were also detected. Most prevalent of  $\alpha$ -thalassemia mutations were  $\alpha$ <sup>-3.7</sup>, followed by  $\alpha$ <sup>-20.5</sup> and  $\alpha$ <sup>5nt</sup>.

Different kinds of consanguineous marriages in the  $\alpha$ -thalassemia individuals are shown in Table 2. Total rate of consanguineous marriages in prospective couples was about 64%, with first cousins marriage being the most common (18.18%). Ethnicities of the  $\alpha$ -thalassemia study group are presented in Table 3. The great majority of  $\alpha$ -

**Table 2.** Different kinds of consanguineous marriages in  $\alpha$ -thalassemic prospective couples of Qazvin province, Iran.

Consanguinity/relationship	Number	Frequency (%)
Cousin's son or girl (first cousin marriage)	4	18.18
Cousin's son or girl/ Aunt's son or girl	2	9.1
Aunts son or girl	4	18.18
No relation	8	36.36
Far relationship	4	18.18

**Table 3.** Ethnicities of the  $\alpha$  thalassemia prospective couples of Qazvin province, Iran.

Ethnic	Number	Frequency (%)
Kordish	9	40.9
Turkish	7	31.8
Fars (Persian)	2	9.1
Gilak	1	4.54
Undetermined	3	13.63

**Table 4.** The mean of different hematological parameters in  $\alpha$ -thalassemia trait subjects.

Parameter	Mean	Normal
RBC count	6.71	5-6.5
Hb	13.34	12-17.5
Hct	43.32	38-54
MCV	72.2	80-96
MCH	22.12	27-31
MCHC	30.73	30-36
PLT	237.16	150-400X1000
HbA2	2.82%	1.5-3.5

RBC, Red blood cell; Hb, haemoglobin; Hct, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelet; HbA2, haemoglobin  $\alpha$ -2.

thalassemia carriers belong to Kordish ethnic group, while the least ethnic group is Gilak. Table 4 shows hematological data of  $\alpha$ -thalassemia trait subjects. Although, most parameters especially Hb and Hct values are within normal ranges, RBC indices (MCV, MCH) are slightly decreased.

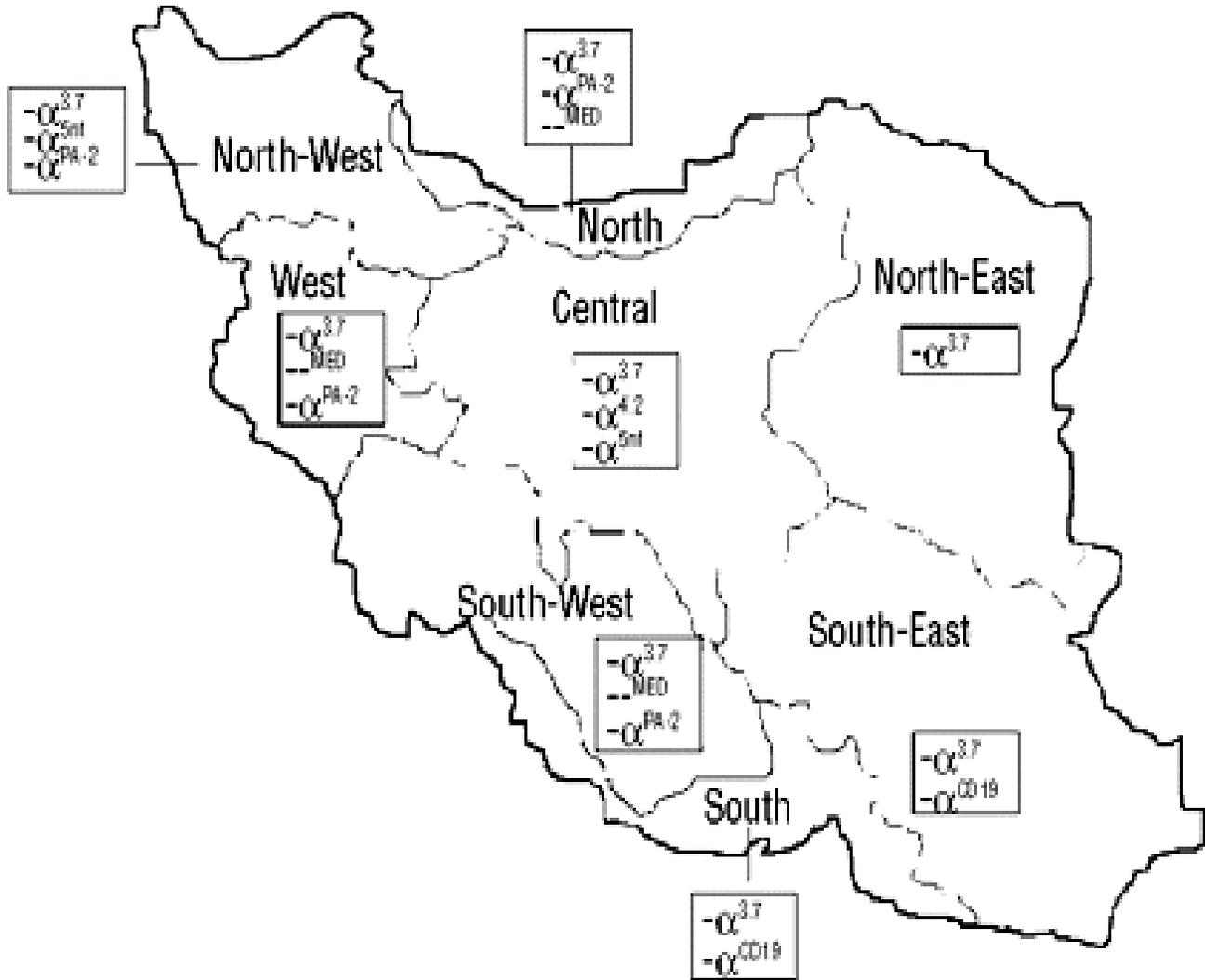
## DISCUSSION

Qazvin with a population of 1.3 million lies between Tehran, Gilan, Mazandaran, Zanjan and Hamadan provinces. Over 10 years of study, only 22 known  $\alpha$ -thalassemia mutations were identified implying that the rate of such mutations is low in this region compared to  $\beta$ -thalassemia mutations. It must be emphasized that one-side normal-prospective couples were excluded for molecular detection in this study. So, the percentages of

$\alpha$ -thalassemia frequency in this region might be much higher than the figures that evaluated in the present study. Zarbakhsh study in Pasteur institute (2010) of Iran revealed the rate of  $\alpha$ -thalassemia in Iran to be very high.

Also, the heterogeneity of  $\alpha$ -thalassemia mutations in this study is lower in comparison to  $\beta$ -globin mutations of the province (sarookhani, 2009, 2010). Among the 6 different  $\alpha$ -globin mutations found,  $\alpha$ <sup>-3.7</sup> is the most frequent. It is also known that this mutation occurs in high frequencies in other provinces of Iran (Tamaddon, 2009; Hadavi et al., 2007) (Figure 1) and neighboring and Mediterranean countries (Abu, 2008; Clark and Higgins, 2000). Mutations of  $\alpha$ <sup>-4.2</sup> and poly A2 which are the next two most frequency mutations in neighboring provinces of Qazvin (Hadavi et al., 2009; Tammadoni et al., 2009) were not found in present study.

A<sup>-20.5</sup> mutation, the second frequent mutation in Qazvin province is not prevalent two neighboring Gilan and



**Figure 1.** Frequencies of  $\alpha$ -thalassemia mutations in different parts of Iran (Hadavi et al., 2007).  $-\alpha^{3.7}$  thalassemia mutation is the predominant mutation in various parts of Iran.

Mazandaran provinces (Hadavi et al., 2009; Tammadoni et al., 2009) and was not detected in Khuzestan (South-west of Iran) province (Zandian, 2008).

Kordish ethnic group had the most  $\alpha$ -thalassemic mutations in Qazvin province ( $\approx 41\%$ ). Interestingly, kordistan province in west part of Iran has similar  $\alpha$ -globin mutations with those of Qazvin (Zarbakhsh et al., 2010; Hadavi et al., 2007). The rate of consanguinity and homogeneity of alleles in between the couples occur in high frequency in the current study. It is implied that  $\alpha$ -thalassemia mutations migrate through families in Qazvin. This condition alarms dangers of consanguineous marriage in producing such harmful diseases as hydrops fetalis and HbH diseases (Abu Ghoush, 2008).

Hematologic data of the  $\alpha$ -thalassemia population of the present study resembles that of  $\beta$ -thalassemia minor

subjects, although, the average levels of MCV and MCH are slightly higher than that of  $\beta$ -thalassemia traits. In Aba ghoush (2008), the mean of MCV and MCH are lower than those of this study. Regarding to normal HbA2 concentrations of  $\alpha$ -thalassemia individual, it is possible to misdiagnose these conditions with Iron deficiency anemia, normal HbA2  $\beta$ -thalassemia minor (silent form),  $\delta\beta$  or  $\gamma\delta\beta$  thalassemia and combined  $\beta/\alpha$  thalassemias. Some laboratory and instrumental pitfalls must be taken in mind as well.

In conclusion, spectrum and frequencies of  $\alpha$ -thalassemia mutations are unique for Qazvin. This condition is similar to  $\beta$ -thalassemia mutations in this region (Sarookhani, 2009). It might be due to the migration of several ethnic groups to Qazvin during the past five decades. Other factors contributing to the pattern of  $\alpha$ -thalassemia mutations must also be taken

into account.

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