

Original Article

Molecular and Hematologic Analysis of Hemoglobin Q-Iran and Hemoglobin Setif in Iranian Families

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Background: Single nucleotide mutations in $\alpha 1$ or $\alpha 2$ genes produce abnormal α -chain hemoglobins. Hemoglobin Q disorders including hemoglobin Q-Iran, hemoglobin Q-Thailand, and hemoglobin Q-India are important hemoglobin variants. Herein, we report on the presence and hematologic and molecular features of hemoglobin Q-Iran [$\alpha 75$ (EF4) Asp→His] in 20 members of 11 families including nine children and hemoglobin Setif [$\alpha 94$ (G1) Asp→Tyr] in 10 individuals from five families consisting of five children and their affected parents living in western Iran.

Methods: A polymerase chain reaction-RFLP procedure using Ava II restriction enzyme was designed to confirm the presence of two α -chain variants. To find the coinheritance with α -thalassemia, the presence of deletions of $-\alpha 3.7$, $-\alpha 20.5$, $--MED$, $--SEA$, and nondeletion defects of IVSI (-5 bp) and hemoglobin CS was examined using polymerase chain reaction-based approaches.

Results: The mean \pm SD level of hemoglobin Q-Iran was 20.4 \pm 4.4%. Three out of 18 individuals with hemoglobin Q-Iran were heterozygous for $-\alpha 3.7$ deletion ($-\alpha 3.7/\alpha$). The coinheritance of hemoglobin Q-Iran and $-\alpha 3.7$ deletion resulted in significantly ($P=0.002$) higher levels of hemoglobin Q-Iran (26.7 \pm 3.8 %). In those heterozygous for hemoglobin Setif, the level of this hemoglobin was 17.8 \pm 5.6 %.

Conclusion: The polymerase chain reaction-RFLP method described here is a simple, rapid, and inexpensive procedure for the diagnosis of abnormal α -chains in developing countries.

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Keywords: α -Globin variants • α -thalassemia • hemoglobin Q-Iran • hemoglobin Setif • Iran

Introduction

Single nucleotide mutations in $\alpha 1$ or $\alpha 2$ genes produce abnormal α -chain hemoglobins (Hbs). Hb Q disorders including Hb Q-Iran [$\alpha 75$ (EF4) Asp→His], Hb Q-Thailand [$\alpha 74$ (EF3) Asp→His], and Hb Q-India [$\alpha 64$ (E13) Asp→His] are important Hb variants.^{1,2}

All three of these hemoglobins are slow-moving variants with an Asp→His substitution that migrates at the electrophoretic position of Hb S at alkaline pH.² Heterozygous individuals for Hb Q-Thailand generally present with moderate red cell microcytosis due to the association of the mutation with the deletion $-\alpha 4.2$ kb, but those carrying Hb Q-Iran or Hb Q-India are hematologically normal and no association with an α -thalassemic phenotype has been reported.^{1,3}

Hb Setif [$\alpha 94$ (G1) Asp→Tyr] is an α -chain Hb variant with electrophoretic mobility similar to Hb S at alkaline pH. Hb Setif is much less soluble than Hb A and induces pseudosickling of the red cells *in vitro*.⁴ Substitution of Asp by Tyr produces an unstable Hb with decreased O₂ affinity and cooperativity present with a low-percent (12 – 17%) expression.^{5–7} Hb Setif has been found in Algeria, Iran, Lebanon, Saudi Arabia, Turkey,

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Italy, Malta, and Cyprus.⁵⁻⁹

The spectrum of α -thalassemia alleles includes deletional and nondeletional forms. Most of these deletions are α -thalassemia-1 and α -thalassemia-2. Heterozygotes for α -thalassemia-2, in whom only one of the α -globin genes is lost ($-\alpha/\alpha\alpha$), have minimal changes in red blood cell (RBC) indices.¹⁰ A deletion of about 3.7 kb is found in Asians, Mediterraneans, and Africans, consistent with a deletion created by nonhomologous crossing over between two α -globin loci. This rightward deletion is designated $-\alpha 3.7$.¹¹⁻¹³ α^+ -Thalassemia due to nondeletional defects, in most cases, results from single or oligonucleotide mutations in the region of the α -gene sequence that are critical for normal expression. One of these mutations, found in the Mediterraneans, is a splicing mutation of the IVS-1 donor site (-5 bp). Hb Constant Spring (Hb CS) is a chain termination mutation (TAA \rightarrow CAA) that has been detected in South Asia.^{13,14}

The objective of the present study was to find the molecular and hematologic features of α -chain variants in western Iran, to provide adequate hematologic information about abnormal Hbs for physicians and genetic counselors, and also to establish a molecular approach in the detection of these abnormal Hbs in Iran.

Materials and Methods

During a survey for finding abnormal Hbs, 16 individuals consisting of 14 children who were referred to the Clinic of Kermanshah University of Medical Sciences had abnormal Hbs. For each case, both the parents were also studied for abnormal Hbs. In one affected individual, the family study was impossible. Since in 14 families the abnormal Hb was present in one of the parents, we were able to study 30 individuals (17 females and 13 males) including 14 children aged two and a half to 16 years and 16 adults aged 24–56 years, from 16 unrelated families which their abnormal Hbs had electrophoretic mobility similar to Hb S on cellulose acetate at alkaline pH. Hb electrophoresis was carried out on cellulose acetate at pH 8.6¹⁵ and the percent of the abnormal Hb was detected using a densitometer.

Hb A₂ was determined by microcolumn chromatography using the anion exchange resin diethylaminoethyl (DEAE) cellulose with glycine-KCN developer.¹⁵ Hb F was determined by the alkaline denaturation method.¹⁶ DNA was

extracted from whole blood by the phenol-chloroform extraction method.¹⁷

DNA analysis

Direct sequencing of DNA revealed the presence of the Hb Setif mutation (GAC \rightarrow TAC) at codon 94 or the Hb Q-Iran mutation (GAC \rightarrow CAC) at codon 75. To confirm the presence of both Hb variants, a polymerase chain reaction (PCR)-RFLP system was designed with the enzyme *Ava II*. The presence of Hb Q-Iran creates a restriction site and Hb Setif eliminates a restriction site as shown in Figure 1. A 406-bp fragment of the gene $\alpha 2$ was amplified using primers 5' AGG CCC TGG AGA GGT GAG 3' and 5' GTG ATC CTC TGC CCT GAG AG 3'. Amplification was carried for 30 cycles at 94°C for one min, 55°C for one min, 72°C for one minute, with a final extension period of five min at 72°C. Fifteen microliter of amplified PCR product was digested with 10 units of *Ava II* restriction enzyme and electrophoresed on 2% agarose gel. In normal individuals, the 406-bp fragment is digested by *Ava II* restriction enzyme to four fragments (225, 89, 58, and 34 bp). The presence of Hb Q-Iran creates a restriction site within the 225-bp fragment (165 and 60 bp), while the presence of Hb Setif abolishes a restriction site, and produces only three fragments (314, 58, and 34 bp).

PCR-based strategies were used for the detection of the deletions $-\alpha 3.7$, $-\alpha 20.5$, $--MED$, $--SEA$, and point mutations IVSI (-5 bp) and Hb CS as described previously.^{11,18}

Statistics

Quantitative variables were expressed as mean \pm SD. Levene's test was used to compare the variances. Independent-sample Student's *t*-test was used to compare the mean percentage of Hb Q-Iran between children and adults and also between individuals with $-\alpha 3.7$ and those without this deletion. Using these two tests, the mean percentage of Hb Setif was also compared between children and adults. $P < 0.05$ was considered statistically significant. SPSS (version 11.5) was used for statistical analysis.

Results

We found Hb Q-Iran in 20 members of 11 unrelated families and Hb Setif in 10 members of five unrelated families. All studied individuals

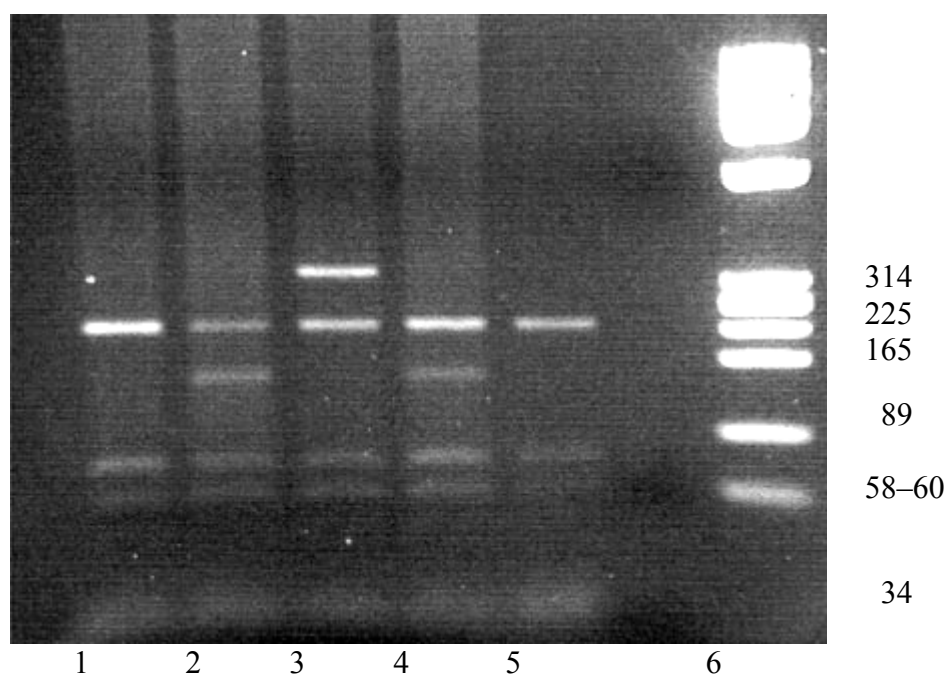


Figure 1. Agarose gel electrophoresis pattern of *Ava II* restriction map of Hb Q-Iran and Hb Setif. Lanes 1 and 5 show digested PCR products from normal subjects. Lanes 2 and 4 depict digested PCR products with *Ava II* from two carriers of Hb Q-Iran. Lane 3 indicates digested PCR product from a carrier of Hb Setif. Lane 6 shows the DNA molecular weight marker (ϕ 174 *Hae III*).

came from Kermanshah, western Iran. Table 1 indicates the levels of Hb Q-Iran, Hb F, and Hb A₂ in carriers of Hb Q-Iran. The levels of Hb Setif, Hb F, and Hb A₂ in heterozygous individuals with Hb Setif are shown in Table 2. The mean \pm SD level of Hb Q-Iran was 20.4 \pm 4.4%. The mean \pm SD level of Hb Q-Iran was 19.7 \pm 4.6% in children and

20.9 \pm 4.4% in adults ($P=0.57$). In those heterozygous for Hb Setif, the level of Hb Setif was 17.8 \pm 5.6%; it was 17.2 \pm 6% in children and 18.4 \pm 5.8% in adults ($P=0.75$). All patients were analyzed for deletional ($-\alpha 3.7$, $-\alpha 20.5$, $--MED$, $--SEA$) and IVSI (-5 bp) and the nondeletional α -thalassemia Hb CS, and all except three had a

Table 1. Hemoglobin data in subjects carrying Hb Q-Iran.

Family	Sex/Age	Hb Q-Iran (%)	Hb F (%)	Hb A ₂ (%)	α -Globin gene status	
1	Proband	F/16	22.8	0.9	1.8	$\alpha\alpha/\alpha\alpha$
	Father	M/45	21.0	0.6	1.2	$\alpha\alpha/\alpha\alpha$
2	Proband	F/24	21.0	0.7	0.7	$\alpha\alpha/\alpha\alpha$
3	Proband	F/25	19.7	1.1	1.7	$\alpha\alpha/\alpha\alpha$
	Mother	F/56	19.5	1.6	1.5	$\alpha\alpha/\alpha\alpha$
4	Proband	F/5	15.1	0.5	2.8	$\alpha\alpha/\alpha\alpha$
	Mother	F/26	22.4	0.2	2.0	$-\alpha 3.7/\alpha\alpha$
5	Proband	M/2.5	14.7	0.9	2.2	$\alpha\alpha/\alpha\alpha$
	Mother	F/28	14.3	0.6	2.0	$\alpha\alpha/\alpha\alpha$
6	Proband	M/12	15.4	0.3	2.2	$\alpha\alpha/\alpha\alpha$
	Mother	F/35	14.2	0.2	2.2	$\alpha\alpha/\alpha\alpha$
7	Proband	M/12	19.1	0.9	1.6	$\alpha\alpha/\alpha\alpha$
	Mother	F/37	25.2	0.6	1.4	$\alpha\alpha/\alpha\alpha$
8	Proband	M/10	18.8	0.5	2.1	$\alpha\alpha/\alpha\alpha$
	Mother	F/42	18.4	0.8	1.6	$\alpha\alpha/\alpha\alpha$
9	Proband	F/10	29.3	1.6	1.0	$-\alpha 3.7/\alpha\alpha$
10	Proband	F/12	20.3	2.3	2.7	*ND
	Mother	F/36	28.4	2.0	1.2	$-\alpha 3.7/\alpha\alpha$
11	Proband	M/8	21.9	1.3	2.3	$\alpha\alpha/\alpha\alpha$
	Mother	F/34	25.5	1.6	2.0	*ND
	Mean \pm SD	—	20.4 \pm 4.4	0.96 \pm 0.59	1.8 \pm 0.54	—

*ND=not determined.

Table 2. Hemoglobin data in individuals heterozygous for Hb Setif.

Family	Sex/Age	Hb Setif (%)	Hb F (%)	Hb A ₂ (%)	α -Globin gene status	
1	Proband	M/11	18.5	0.4	1.4	$\alpha\alpha/\alpha\alpha$
	Mother	F/37	19.0	0.5	1.3	$\alpha\alpha/\alpha\alpha$
2	Proband	M/4	12.7	1.1	1.2	$\alpha\alpha/\alpha\alpha$
	Father	M/37	10.8	1.0	1.1	$\alpha\alpha/\alpha\alpha$
3	Proband	M/3	12.5	0.3	1.9	$\alpha\alpha/\alpha\alpha$
	Mother	F/25	25.0	0.4	1.1	*ND
4	Proband	M/3.5	15.4	1.3	2.4	$\alpha\alpha/\alpha\alpha$
	Mother	F/29	14.7	1.5	0.7	$\alpha\alpha/\alpha\alpha$
5	Proband	M/14	27.1	0.9	1.7	$\alpha\alpha/\alpha\alpha$
	Father	M/40	22.7	1.0	1.8	$\alpha\alpha/\alpha\alpha$
	Mean \pm SD	—	17.8% \pm 5.6	0.84 \pm 0.41	1.46 \pm 0.49	—

*ND=not determined.

normal α -genotype. Three (17%) out of 18 individuals with Hb Q-Iran also were heterozygous for the $-\alpha^{3.7}$ deletion ($-\alpha^{3.7}/\alpha\alpha$).

A significantly ($P=0.002$) higher percentage of Hb Q-Iran (26.7 \pm 3.8%) was found in the presence of $-\alpha^{3.7}$ deletion compared to those with normal α chains (18.7 \pm 3.4%).

Discussion

Iran has been known as a country with high prevalence of hemoglobinopathies.¹⁹ However, application of molecular procedures for identification of abnormal Hbs in clinical laboratories in Iran is not common and many cases with abnormal Hb variants still remain undiagnosed.

Lorkin et al.¹ have reported three cases of Hb Q with substitution of aspartic acid to histidine at position $\alpha 75$ (EF4), the so-called Hb Q-Iran. The latter, in the heterozygous state has normal hematology and has not been reported in association with a thalassemic phenotype. The quantity of this Hb variant in the heterozygous state has been reported to be 17 – 19%.^{1,2} We found a level of 14.2 – 29.3% for Hb Q-Iran in heterozygous individuals.

Hb Setif was originally described in an Algerian family.⁸ Transversion of G to T at codon 94 (GAC \rightarrow TAC) of α_2 -globin gene results in the replacement of Asp with Tyr. It has been found that Hb Setif produces pseudosickling of RBC *in vitro* by intracellular crystallization of insoluble Hb.⁴ It has been suggested that, a disturbance in the tertiary structure in the region G1 of the α -chain, involves in the development of a hydrogen bond between the $\alpha 1$ and $\beta 2$ interface, which may produce contacts with other molecules in the vicinity and could explain the self-association into crystals.⁴ A range of 12 – 17% has been reported

for Hb Setif.⁵⁻⁷ In the present study, a range of 10.8 – 27.1% was established for Hb Setif.

Finding Hb Q-Iran in 20 cases from 11 unrelated families and Hb Setif in 10 members of five unrelated families during one year study on structural Hb variants suggests a high incidence of these α -chain variants among the population of western Iran. Furthermore, we found a family with four members in whom the father was a carrier of Hb Setif and the mother was heterozygous for Hb Q-Iran, with two affected children with Hb Setif and Hb Q-Iran, respectively.

α -Thalassemia was first intensively studied in Asian populations, where it occurs at a very high incidence. The most common molecular defects underlying α -thalassemia ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) involve the deletion of one or the other of the duplicated α -globin genes.^{13,14} In these populations, association between Hb Q-Thailand and the α -thalassemic determinant $-\alpha^{4.2}$ kb was also found.¹ However, the association between Hb Q-Iran and α -thalassemia has not been described.

We found the coinheritance of Hb Q-Iran and the $-\alpha^{3.7}$ deletion in 17% of carriers of Hb Q-Iran (three out of 18) that resulted in increased expression of Hb Q-Iran (26.7%) compared to those with normal α -chains (18.7%). In agreement with our results that an elevation of Hb Q level occurs in the presence of α^+ -thalassemia, Abraham et al.³ reported that the coinheritance of Hb Q-India and α^+ -thalassemia causes increased expression of Hb Q-India.

The high prevalence of Hb Q-Iran and Hb Setif encountered in Kermanshah Province, western Iran, suggests that this region could be a hotspot for these mutations, differing from other regions of Iran, such as Hormozgan and Tehran where other large deletions and point mutations responsible for α -thalassemia have been found.^{20,21}

The information provided by this study of the presence and molecular and hematologic characteristics of Hb Q-Iran and Hb Setif in western Iran is important for clinical management and precise diagnosis of cases with these α -chain variants. Furthermore, the PCR-RFLP method described here is a simple, rapid, and inexpensive procedure that could be applied for the diagnosis of abnormal α -chains in developing countries.

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