

Original Article

Identification of α -globin Chain Variants: A Report from Iran

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Abstract

Background: This study was carried out to identify molecular and hematological features of α -globin chain variants and to evaluate their effects on the clinical and hematological characteristics in Iranian individuals suspected of having thalassemia trait.

Methods: Analysis of red blood cell indices, hemoglobin (Hb) analysis and genomic DNA isolation were carried out according to standard methods. For identifying the α -thalassemia (α -thal) genotype, investigation of common Mediterranean α -globin gene deletions ($-\alpha^{3,7}$, $-\alpha^{4,2}$, $-\alpha^{20,5}$ and $--_{MED}$) was performed by Gap-PCR. To characterize chain variants the entire α_1 and α_2 genes that spanned from the promoter region to the poly A tail were amplified and directly sequenced.

Results: In this study, 19 members of 17 unrelated families showed α -chain variants. Among these cases ten α -chain variants that included Hb Setif, Hb Constant Spring (Hb CS), Hb Handsworth, Hb Icaria, Hb Evanston, Hb Val de Marne, Hb Utrecht, Hb Savaria, Hb Adana, and Hb Dartmouth were identified. The hematological profile and molecular basis of these ten α -chain variants and the phenotypic consequences of their interactions were discussed.

Conclusion: The knowledge of the spectrum of α -globin variants present in the Iranian population is essential for the molecular diagnosis and prevention of hemoglobinopathies.

Keywords: α -globin chain variants, α -thalassemia, Iran

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Introduction

Iran is located in the middle of the so-called Thalassemia Belt that has a high thalassemia carrier rate. Thalassemia is more prevalent in the northern (Caspian Sea) and southern (Persian Gulf and Oman Sea) areas of the country (10%). The overall prevalence ranges approximately from 3 to 100 patients per 100,000 in different provinces.^{1,2} There is also a high prevalence of sickle cell trait in Southern Iran (around 1.43%) and Central Iran (around 8%).^{3,4}

In Iran, the initial phases of the Thalassemia Prevention Program began in 1992 under the supervision of the Genetics Department of the Center for Disease Control in the Health Ministry. Because of the successful results of several pilot studies in different regions of high thalassemia prevalence, in 1997 the National Thalassemia Prevention Program was established to diagnose thalassemia carriers at the premarital stage. This program enlists the coordination of all 39 medical universities throughout the country.¹

α -thalassemia (α -thal) is one of the most common hemoglobin (Hb) disorders in the world due to deletion or point mutations in α -globin genes.⁵ The majority of α -thal mutations are deletions however point mutations account for a small proportion of cases.^{5,6} In general, α -globin variants result from point mutations in α_1 or α_2 globin genes leading to abnormal α -chain hemoglobins.⁷ α -thal is common in Iran and the most frequent lesion reported so far is the $-\alpha^{3,7}$ mutation.⁸ The other α -thal mutations ($--_{MED}$, $-\alpha^{4,2}$,

$\alpha^{PolyA2(AATGAA)}$, α^{CS} , α^{5nt} , $-(\alpha)^{20,5}$, $\alpha^{PolyA1(AATAAG)}$, α^{cd19} , and α^{cd59}) are present in frequencies above 1%.⁸

The purpose of this study was to characterize α -globin chain variants at the molecular level. We evaluated their effects on the clinical and hematological outcome in Iranian individuals suspected of thalassemia trait.

Materials and Methods

This study included individuals referred to the Tehran Medical Genetics Laboratory (TMGL) during the past ten years for carrier detection as part of a national program for the prevention of thalassemia. The majority were heterozygous carriers of β -thalassemia, and around 2000 individuals showed an α -thal hematologic profile.

Analysis of red blood cell indices and Hb analysis were carried out according to standard methods. Following obtaining written informed consent, molecular studies were conducted on genomic DNA isolated from peripheral blood cells by a salting-out procedure.⁹ For identifying α -thal genotype, investigation of common Mediterranean α -globin gene deletions ($-\alpha^{3,7}$, $-\alpha^{4,2}$, $-(\alpha)^{20,5}$ and $--_{MED}$) was performed by Gap-PCR as described previously.¹⁰

To characterize chain variants the entire α_1 and α_2 genes that spanned from the promoter region to the poly A tail were amplified and directly sequenced by the chain termination method on an ABI 3730 XL sequencer (Primm, Milan, Italy), as described elsewhere.¹¹

Results

Among 2000 cases screened for their α -thal status, ten α -chain variants were identified in 19 members of 17 unrelated families.

These variants included Hb Setif, Hb CS, Hb Handsworth, Hb Icaria, Hb Evanston, Hb Val de Marne, Hb Utrecht, Hb Savaria, Hb Adana, and Hb Dartmouth. Hematological and molecular anal-

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Table 1. Hematological and molecular features in Iranian individuals with α -globin variants.

No	α -genotype	Age (years)/sex	Origin	Place of birth	RBC ($10^{12}/L$)	Hb (g/dl)	MCV (fL)	MCH (pg)	HbA (%)	HbF (%)	HbA2 (%)	Hb variants(%)
1	$\alpha\alpha/\alpha^{Hb\text{Setif}}\alpha$	20/F	Nahavand	Northwest	3.95	11.6	87	29.3	94.2	0.2	1.6	4.2
2	$\alpha\alpha/\alpha^{Hb\text{Setif}}\alpha$	27/M	Nahavand	Northwest	5.42	14.8	84	27.3	90.7	0.1	1.4	7.8
3	$\alpha\alpha/\alpha^{Hb\text{Setif}}\alpha$	24/F	Kurd	Southwest	4.33	11.4	81.6	26.4	75	1.7	1.5	21.8
4	$\alpha\alpha/\alpha^{Hb\text{Setif}}\alpha$	17/M	Kurd	Southwest	6.22	14.4	71.6	23.1	83.9	0.5	2.4	13.2
5	$\alpha\alpha/\alpha^{Hb\text{Constant Spring}}\alpha$	18/F	Rodsar	North	4.78	13.1	82	27	95.9	2.3	1.8	N
6	$\alpha\alpha/\alpha^{Hb\text{Constant Spring}}\alpha$	25/M	Ahvaz	Southwest	6.06	15.3	73.4	25.2	98	0.1	1.9	N
7	$\alpha\alpha/\alpha^{Hb\text{Constant Spring}}\alpha$	26/M	Lor	West	6.16	15.6	78	25	96.3	1.1	2.6	N
8	$\alpha\alpha/\alpha^{Hb\text{Constant Spring}}\alpha$	28/F	Rodehan	North	5.15	12.7	74.4	24.6	95.3	1.6	3.1	N
9	$\alpha\alpha/\alpha^{Hb\text{Constant Spring}}\alpha$	26/M	Tehran	North	5.09	13.1	75.8	25.7	97.3	0.2	2.5	N
10	$\alpha\alpha/\alpha^{Hb\text{Handsworth}}\alpha$	22/F	Arab	Southwest	4.5	12.3	85.6	27.3	84.6	0.1	2.3	13
11	$\alpha\alpha/\alpha^{Hb\text{Handsworth}}\alpha$	24/M	Arab	Southwest	5.68	16.8	87	29	84.8	0.6	1.9	12.7
12	$\alpha\alpha/\alpha^{Hb\text{Icaria}}\alpha$	23/F	Arab	Southwest	4.3	10.9	77.6	25.3	97.1	0.5	2.4	N
13	$\alpha\alpha/\alpha^{Hb\text{Icaria}}\alpha$	2/F	Arab	Southwest	5	8.4	64.6	16.9	79.9	1.2	0.8	18.1
14	$\alpha\alpha/\alpha^{Hb\text{Evanston}}\alpha$	21/M	Lor	Southwest	6.25	14.8	72	24	95.7	1.3	3	N
15	$\alpha\alpha/\alpha^{Hb\text{Val de Marne}}\alpha$	26/M	NA	NA	6.35	15.8	73	25	56.6	3.1	4.4	35.9
16	$\alpha\alpha/\alpha^{Hb\text{Utrecht}}\alpha$	22/F	Esfahan	Center	4.52	11.5	80	25	95.5	1.6	2.9	N
17	$\alpha\alpha/\alpha^{Hb\text{Savaria}}\alpha$	26/M	Dezful	Southwest	5.63	15.5	81.6	27.5	74.3	1.1	2.6	22
18	$\alpha\alpha/\alpha^{Hb\text{Adana}}\alpha$	28/F	Tehran	North	5.41	12.6	71.3	23.3	NA	0.8	3	N
19	$\alpha\alpha/\alpha^{Hb\text{Dartmouth}}\alpha$	23/F	Naein	Center	5.67	13	75.5	22.9	NA	NA	3.8	N

N:Not detected; NA: Not available.

yses of these variants are summarized in Table 1.

Hemoglobin Constant Spring (HbCS)

Five unrelated individuals with slightly hypochromic and anisocytosis anemia showed HbCS in the heterozygous form. Cellulose acetate electrophoresis was normal. In α -globin gene sequencing, a point mutation at the stop codon of the α_2 -globin gene (TAA>CAA) was identified.

Hb Setif

Hb Setif variant was detected in four members of two families in heterozygous status from Western Iran. The level of this hemoglobin was $11.75\pm 7.6\%$ (range: 4.2% – 21.8%) in adults, as detected by cellulose acetate electrophoresis. Direct sequencing of DNA revealed the presence of the Hb Setif mutation (GAC>TAC) at codon 94. None of the carriers had any significant hematological complaints however one case had hypochromic and microcytic anemia with an MCV of 71.6 fL and MCH of 23.1 pg.

Hb Handsworth

Two unrelated individuals with mild anemia showed this Hb variant. These cases were from Khuzestan, south Iran and were of ethnic Arab origin. The level of Hb Handsworth was 12.7% and 13% in these individuals as detected by cellulose acetate electrophoresis. α_2 -globin gene sequencing revealed a point mutation substitution of Glycine by Arginine at codon 18.

Hb Icaria

This variant was detected in a mother and her daughter from Khuzestan Province, who were of ethnic Arab origin. The mother had heterozygous Hb Icaria with mild anemia, whereas her daughter showed an unusual case of Hb H disease. She had inherited the Hb Icaria (X142K) from her mother, while the second α -globin gene inherited paternally had the α -Med deletion. Hb H was 18.1% as detected by cellulose acetate electrophoresis. The patient had clinical

manifestations for Hb H disease with occasional anemia that necessitated blood transfusions and splenomegaly. Direct α -globin gene sequencing identified a base substitution of TAA>AAA at the termination codon of the α_2 -globin gene.

Hb Evanston

This Hb variant was detected in a person who had slightly hypochromic and anisocytosis anemia in the heterozygous form. He was from Lor, in southwest Iran. This Hb variant was not identified by cellulose acetate electrophoresis. α -globin gene sequencing revealed a point mutation at codon 14 of the α_2 -globin gene (TGG>AGG).

Hb Val de Marne

A 26-year-old male with slightly hypochromic, anisocytosis anemia inherited this variant in the heterozygous state. Hb Val de Marne was 35.9% as detected by cellulose acetate electrophoresis. Automated DNA sequencing revealed an AGC > AGA mutation at codon 133 of the α_2 -globin gene.

Hb Utrecht

This variant was found in a 22-year-old female with mild heterozygous thalassemia phenotype, not detected at the protein level by cellulose acetate electrophoresis. Her mutation was found to be an α_2 codon 129 (CTG > CCG) transition.

Hb Savaria

A 26-year-old female who had no significant clinical features showed this variant in the heterozygous form. The protein was 22% as detected by cellulose acetate electrophoresis. The mutation related to this variant (codon 49 Ser>Arg) was identified by DNA sequencing of the α_2 -globin gene.

Hb Adana

This variant was detected in a 28-year-old female from Tehran in its heterozygous form. This person had hypochromic, anisocytosis

and poikilocytosis anemia with no detectable protein by cellulose acetate electrophoresis. Automated DNA sequencing showed a GGC>GAC mutation at codon 59 of the α_2 -globin gene.

Hb Dartmouth

A 23-year-old female with mild thalassemia phenotype showed this variant in the heterozygous form. This Hb variant was not detected by cellulose acetate electrophoresis, however DNA sequencing revealed a point mutation in the α_2 gene (CTG>CCG at codon 66).

Discussion

Structural hemoglobin variants are mainly caused by a point mutation in the globin gene which changes an amino acid. This substitution in the globin chain may have different outcomes on the function of the protein. While some positions on the globin chains tolerate substitutions with no physiological abnormality or clinical problem, other positions are very sensitive to amino acid substitutions leading to alteration of oxygen affinity, altering binding stability between globin chains, structural defect with thalassaemic effect and altered physical behavior. To date, approximately 1000 hemoglobin variants have been identified worldwide.¹²

We identified hematological and molecular features of ten α -chain variants that included Hb Setif, Hb CS, Hb Handsworth, Hb Icaria, Hb Evanston, Hb Val de Marne, Hb Utrecht, Hb Savaria, Hb Adana, and Hb Dartmouth.

In this study Hb CS was the most frequent α -globin variant, which agreed with a previous report.⁸ All carriers showed slightly hypochromic and anisocytosis anemia. This variant is the most common non-deletional α -thal mutation in Southeast Asia and Southern China.^{13,14}

The second most frequent variant seen in this study was Hb Setif. Hb Setif is caused by the substitution of Asp by Tyr at codon 94 that produces an unstable α_2 chain Hb with decreased O_2 affinity.¹⁵ Nozari et al. were the first to report this variant in the Iranian population.¹⁶ Hb Setif has also been found in Algeria, Lebanon, Saudi Arabia, Turkey, Italy, Malta, and Cyprus.^{15,17-19} This variant has a migration pattern similar to HbS on cellulose acetate electrophoresis and it can be confused with HbS. Earlier reports have shown that patients did not have significant hematological complaints. Our patients also did not have significant hematological variations, with the exception of one case that showed hypochromic and microcytic anemia with MCV of 71.6 fL and MCH of 23.1 pg. The range of 12% – 17% has been reported for Hb Setif.¹⁷ In the present study, a range of 4.2% – 21.8% was established for this variant. Hb Setif was found in four members of two unrelated families in this study, whereas ten members of five unrelated families and twelve subjects from six families have been previously reported.^{15, 20} All were from the west of Iran, which has suggested a high prevalence of this variant in this region of Iran, particularly amongst the Kurdish population.

For the first time in individuals of Iran, Hb Handsworth was shown in two unrelated individuals of Arab ethnic origin. This variant has also been reported in Saudi Arabian patients.²¹ Therefore, this may reflect a shared origin of the Handsworth variant in this part of the world.

Hb Icaria, a rare structural Hb and thalassaemic variant reported from Greek, Yugoslavian, Macedonian, and Iranian families^{8, 22-24} has been identified in this study. This variant is difficult to detect

in peripheral blood samples by the more commonly used techniques due to its very low concentration and electrophoretic mobility, which is slower than that of Hb A₂ at an alkaline pH.²² In two members of a family affected by Hb Icaria, we have described an unusual case of HbH disease caused by the combination of α^0 -thalassaemia allele ($-\text{Med}$) with Hb Icaria, only the second report thus far worldwide.²⁴ The patient had severe clinical manifestations of HbH disease, with anemia necessitating blood transfusions and the presence of splenomegaly with 18.1% Hb H, which was similar to those reported in Greek individuals who were $-\text{Med}/\alpha$ Hb Icaria.²⁴

Hb Evanston, another rare α chain variant was originally observed in black families.^{25,26} This variant has also been reported in Indian and Afghani families^{27,28} and recently in an Iraqi individual.²⁹ This is the first report of the very rare heterozygous Hb Evanston in Iran.

There have been two previous reports on Hb Val de Marne (Hb Footscray) detected in French and Chinese families.^{30,31} This is the third report of this variant which was found in a 26-year-old male with 35.9% Hb Val de Marne. The individual was slightly hypochromic and had anisocytosis anemia. This case was diagnosed with heterozygous Hb Val de Marne. This variant displays a slightly higher auto-oxidation rate than Hb A.³¹

Hb Utrecht was first reported in 1996 in six individuals from a three-generation Dutch family.³² This variant found in a female from Esfahan Province in Central Iran, with the mild thalassemia phenotype, is the second reported worldwide.

Hb Savaria has been detected in individuals from Hungary, Yugoslavia, Kenya, and the United States.³³⁻³⁶ This is the first report of this variant (22%) in the Iranian population.

As for Hb Adana, due to a mutation in the α_2 gene, it was detected in a 28-year-old female. This variant has been reported in eight individuals in a previous study in the Iranian population,⁸ which has indicated a notable frequency. One has to be cautious about the clinical consequences of this variant, because the compound heterozygous combination of Hb Adana on the α_2 -globin gene and α^0 -thalassaemia deletion allele results in a severe phenotype and prenatal diagnosis should be offered to at-risk couples.³⁷ However, co-inheritance of Hb Adana on the α_1 gene with α^0 -thalassaemia deletion allele results in less severe clinical symptoms.³⁸

Hb Dartmouth is the last one reported in this study. This variant was first reported in monozygotic twins and their father, who was of Scottish-Irish ancestry.³⁹ In the present study, as the second reported case in the world, we found this variant in a 23-year-old female with mild thalassemia phenotype from Naein, Central Iran. Hb Dartmouth in combination with α^0 -thalassaemia deletion mutation causes severe anemia that necessitates transfusions.³⁹

In conclusion, Iran is recognized as a country with a high prevalence of hemoglobinopathies. The knowledge of the spectrum of α -globin variants present in the Iranian population are essential for the molecular diagnosis and prevention of hemoglobinopathies. In addition, non-deletional α -thal mutations can result in severe forms of HbH disease. Therefore, more work is needed to identify the frequency of these mutations in order to determine potential risk to public health. However, patients who co-inherit α^0 -thalassaemia deletion mutations along with α -chain variants are rare and there is little information reported on their clinical manifestation.

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