Elucidating the spectrum of α-thalassemia mutations in Iran

α thalassemia (α-thal) is one of the most common hemoglobin (Hb) disorders in the world.[1] α-globin genes are located on chromosome 16. The majority of α-thal mutations are deletions but point mutations are found as well.[2] Since the Iranian population is a mixture of different ethnic groups, frequency and distribution of α-globin mutations in various regions of the country need to be clarified. These findings can contribute to a wider understanding of this disorder.

Haematologica 2007; 92:992-993

This study included 653 individuals of different ethnic origins selected from patients referred to our center between January 1998 and February 2006. Subjects presented with low MCV (<85 fL), low MCH (<27dg), normal or slightly reduced hemoglobin (Hb) levels (<85g/dL), and normal HbA_2 (<85dL).

A polymerase chain reaction (PCR) method introduced by Baysal and Huisman[3] was used to screen all samples for the two α-thal single gene deletions $-\alpha^{3.7}$ and $-\alpha^{4.2}$, and the --MED double gene deletion. Before 2004, analyses were performed by KK Women's and Children Hospital in Singapore for nine mutations including: --SEA, --THAI, --FIL, -20.5 kb double gene deletions, and five point mutations (Hb Constant Spring, Hb Quong Sze, Hb Pakse, Hb Adana, Cd 30 del GAG) on a total of 264 patients.

After 2004, 389 patient samples were comprehensively analyzed using the α-globin StripAssay (ViennaLab Diagnostics, Vienna, Austria). This test included the following α-globin mutations: --SEA, --THAI, --FIL, -20.5 kb double gene deletions, anti-3.7 gene triplication, two point mutations in the α1 gene (cd 14: TGG-TAG; cd 59: GGC-GAC Hb Adana) and eleven mutations in the α2 gene (initiation cd: ATG-ACG; cd 19 del G: GCG-GC, IVS1 -5nt: TGAGG del; cd 59: GGC-GAC; cd 125: CTG-CCG Hb Quong Sze; cd 142: TAA-CAA Hb Constant Spring; cd 142: TAA-AAA Hb Icaria; cd 142: TAA-TAT Hb Pakse; cd 142: TAA-TCA Hb Koya Dora; poly A-1: AATAAA-AATAAG; poly A-2 AATAAA-AATGAA). Samples that showed no mutation with this method were further analyzed by DNA sequencing of the α-1

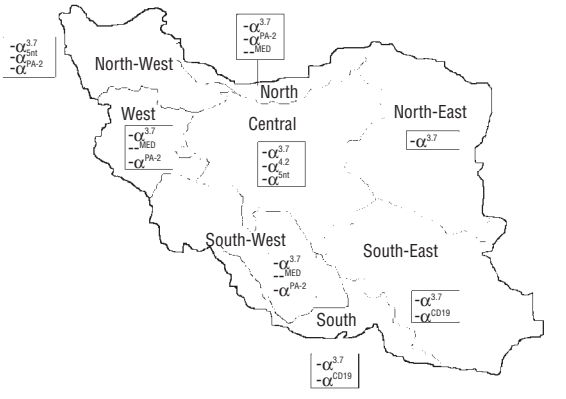

Figure 1. Iran is divided into eight regions according to geographic boundaries and population distributions, and the frequent genotypes are shown in each region.

Table 1. Geographic distribution and spectrum of α-thalassemia mutations in Iran (number and frequencies). A total of 650 (49.8%) wild-type genotypes were identified.

Mutation (%)	*Central (%)*	*North (%)*	*South (%)*	*South-West (%)*	*West (%)*	*South-East (%)*	*North-East (%)*	*North-West (%)*	*All (% of mutation)*	*% of total*
$-\alpha^{3.7}$	69 (53.9)	111 (40.9)	40 (93.0)	85 (69.6)	26 (63.4)	20 (71.4)	9 (75.0)	35 (83.3)	395 (60.2)	30.2
α^{polyA2}	4 (3.1)	47 (16.9)	–	9 (7.3)	3 (7.3)	1 (3.6)	1 (8.3)	2 (4.8)	67 (10.2)	5.1
$-\alpha^{4.2}$	12 (9.3)	26 (9.6)	1 (2.3)	5 (4)	–	–	1 (8.3)	1 (2.4)	46 (7.0)	3.5
α^{-5nt}	12 (9.3)	13 (4.8)	–	2 (1.6)	2 (4.9)	2 (7.1)	–	2 (4.8)	33 (5.0)	2.5
--MED	4 (7.0)	16 (12.1)	–	6 (9.8)	3 (14.6)	1 (7.1)	–	–	30 (4.6)	3.3
α^{CS} Hb Constant Spring	2 (1.5)	17 (6.2)	–	1 (0.8)	–	–	1 (8.3)	1 (2.4)	22 (3.4)	1.7
α^{cd19}	3 (2.4)	3 (1.1)	2 (4.7)	3 (2.4)	1 (2.4)	3 (10.7)	–	–	15 (2.3)	1.1
$-\alpha^{20.5}$	6 (4.8)	6 (2.2)	–	-	–	–	–	–	12 (1.8)	0.9
α^{polyA1}	3 (2.4)	5 (1.8)	–	2 (1.6)	–	–	–	1 (2.4)	11 (1.7)	0.8
α^{cd59} Hb Adana	2 (1.5)	3 (1.1)	–	1 (0.8)	2 (4.9)	–	–	–	8 (1.2)	0.6
α^{IC} Hb Icaria	–	3 (1.1)	–	–	1 (2.4)	–	–	–	4 (0.6)	0.3
α^{cd26}	1 (0.8)	2 (0.7)	–	–	–	–	–	–	3 (0.5)	0.2
α^{cd99}	2 (1.5)	–	–	–	–	–	–	–	2 (0.3)	0.2
$-\alpha^{St}$	–	–	–	2 (1.6)	–	–	–	–	2 (0.3)	0.2
anti-3.7 αααα triplication	1 (0.8)	1 (0.3)	–	–	–	–	–	–	2 (0.3)	0.2
α^{cd103}	1 (0.8)	–	–	–	–	–	–	–	1 (0.2)	0.1
Total number of mutant alleles	*123*	*255*	*43*	*116*	*38*	*27*	*12*	*42*	*656 (100)*	*50.2*
Tested Genotypes	*270*	*522*	*66*	*214*	*74*	*42*	*24*	*94*	*1306*	*100*

and α-2 genes. We divided Iran into eight different regions (Figure 1). Geographic distribution and frequency of the identified genotypes are presented in Table 1 and Figure 1. The -$\alpha^{3.7}$ mutation was the most frequent α-thalassemia mutation, contributing to 60.2% of α-thal alleles. Sixteen other α-globin mutations were found, nine of which (--MED, -$\alpha^{4.2}$, $\alpha^{PolyA2(AATGAA)}$, α^{CS}, α-^{5nt}, -$(\alpha)^{20.5}$, $\alpha^{PolyA1\ (AATAAG)}$, α^{cd19}, α^{cd59}) were present in frequencies above one percent, while seven mutations were found less frequently (Table 1). No mutation was found using either PCR methods in 85 individuals studied after 2004. Further DNA sequencing of the α-1 and α-2 genes identified mutations in 47 patients. Since no DNA sequencing was performed before 2004, 67 of those patients remain unidentified. In total, 105 individuals (16.1%) had no identified α-globin mutation. We identified two novel mutations by sequence analysis: a single nucleotide mutation at the initiation codon of α gene (ATG-to-AGG) and a codon 99 mutation on α-1 gene (AAG-to-TAG), which is a stop codon. Iran, located in the Middle East between Iraq and Pakistan, has a population of 70 million. It is in the middle of the so-called *Thalassemia Belt* with a high thalassemia carrier rate.[4,5] Our data agrees with the records of the HbVar globin-specific database by Patrinos *et al.* which shows the most frequent mutations and deletions in Arab countries located along the Persian Gulf as -$\alpha^{3.7}$, -$\alpha^{4.2}$, $\alpha^{PA2(GAA)}$, α^{cs}, and α^{-5nt}.[6,7]

The 75% detection rate before 2004 increased to 90% after 2004 by means of Globin StripAssay and sequencing. The StripAssay is based on gap-PCR and covers the deletions analyzed in a recent review.[8] Gap-PCR detects large α-globin deletions and gene triplication, but cannot detect point mutations or small deletions/insertions. Although the 10% undetected patients is acceptable compared to other studies, sequencing both α genes could increase coverage. α-thalassemia disorder is characterized by a wide clinical and hematologic phenotypic heterogeneity.[8] Undetected cases could also be due to situations like antisense RNA transcription which was clarified in long-range analysis of chromosome structures.[9]

In a country like Iran, with a remarkable prevalence of αβ-globin mutations, the increased likelihood of co-inheritance of α- and β-thal may result in a large variety of phenotypes.[10] Our findings on the prevalence and distribution of α-globin mutations will provide a valuable basis for carrier screening, genetic counseling and prenatal diagnosis, helping the prevention of damaging forms of α-thal mutations.

Valeh Hadavi, Amir Hossein Taromchi,* Mahdi Malekpour,* Behjat Gholami,* Hai-Yang Law,° Navid Almadani,* Fariba Afroozan,* Farhad Sahebjam,* Parisa Pajouh,* Roxana Kariminejad,* Mohammad Hassan Kariminejad,* Azita Azarkeivan,*# Maryam Jafroodi,* Ahmad Tamaddoni,@ Helene Puehringer^ Christian Oberkanins,^ Hossein Najmabadi**

**Kariminejad-Najmabadi Pathology & Genetics Center, Tehran, Iran; °DNA Diagnostic & Research Lab, KK Women's and Children Hospital, Singapore; #Iranian Blood Transfusion Organization (IBTO), Research Center, Iran; @Amirkola Thalassemia Center, Babol University of Medical Science, Babol, Iran; ^ViennaLab Diagnostics GmbH, Vienna, Austria*

Key words: α thalassemia, mutation, Iran.

Correspondence: Hossein Najmabadi, Professor of Genetics, Kariminejad-Najmabadi Pathology & Genetics Center, 14665/154, Tehran, Iran. Phone: international +98.21.88363952. E-mail: hnajm@mavara.com

References

1. Kan YW. Molecular pathology of α-thalassemia. Ann NY Acad Sci 1985;445:28.
2. Higgs DR, Vickers MA, Wilkie AO, Pretorius IM, Jarman AP, Weatherall DJ. A review of the molecular genetics of the human α-globin gene cluster. Blood 1989;73:1081-4.
3. Baysal E, Huisman TH. Detection of common deletional α-thalassemia-2 determinants by PCR. Am J Hematol 1994; 46:208-13.
4. Najmabadi H, Karimi-Nejad R, Sahebjam S, Pourfarzad F, Teimourian S, Sahebjam F, et al. The β-thalassemia mutation spectrum in the Iranian population. Hemoglobin 2001; 25:285-96.
5. Najmabadi H, Pourfathollah AA, Neishabury M, Sahebjam F, Krugluger W, Oberkanins C. Rare and unexpected mutations among Iranian β-thalassemia patients and prenatal samples discovered by reverse-hybridization and DNA sequencing. Haematologica 2002;87:1113-4.
6. Patrinos GP, Giardine B, Riemer C, Miller W, Chui DH, Anagnou NP, Wajcman H, Hardison RC. Improvements in the HbVar database of human hemoglobin variants and thalassemia mutations for population and sequence variation studies. Nucleic Acids Res 2004;32:D537-41.
7. Garshasbi M, Oberkanins C, Law HY, Neishabury M, Kariminejad R, Najmabadi H. α-globin gene deletion and point mutation analysis among Iranian patients with microcytic hypochromic anemia. Haematologica 2003; 88: 1196-7.
8. Patrinos GP, Kollia P, Papadakis MN. Molecular diagnosis of inherited disorders: lessons from hemoglobinopathies. Hum Mutat 2005;26:399-412.
9. Tufarelli C, Stanley JA, Garrick D, Sharpe JA, Ayyub H, Wood WG, et al. Transcription of antisense RNA leading to gene silencing and methylation as a novel cause of human genetic disease. Nat Genet 2003;34:157-65.
10. Najmabadi H, Ghamari A, Sahebjam F, Kariminejad R, Hadavi V, Khatibi T, et al. Fourteen-year experience of prenatal diagnosis of thalassemia in Iran. Comm Gen 2006; 9:93-7.