

## The spectrum of Alpha and Beta Thalassemia Mutations: A 10-year Population-based Study of the Premarital Health Screening Program in West of Iran

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### Abstract

**Background:** In various cancers, Ganoderic Acid A (GAA), an active triterpenoid derived from Ganoderma Background: Thalassemia refers to a category of inherited disorders resulting from defects in synthesizing one or several chains of hemoglobin (Hb). The present study aimed to determine the frequency of alpha and beta-thalassemia mutations in Kurdistan province, Iran.

**Materials and Methods:** In this retrospective cross-sectional study, the laboratory data of 340 patients with thalassemia (170 females and 170 males), who were candidates for genetic testing in Kurdistan province, were examined over ten years (2006-2016). The participants were Kurd couples selected from the premarital health screening program.

**Results:** In this demographic study, 20 beta mutations and nine alpha mutations were identified. Among the beta-thalassemia mutations, intervening sequence or intron No. 2- first nucleotide change as splice site mutation (IVS-II-1) (26.1%), codons 8/9 (14.8%), and intervening sequence or intron No. 1- first nucleotide (IVS-I-1) (12.2%) change as splice site mutation, had the highest frequency rates, respectively, constituting 53% of the beta mutations. In addition,  $\alpha 3.7$ (82.7%),  $-\alpha 4.2$ (8.3%), and  $--MED$  (Mediterranean deletion) (3.75%) were the most frequent alpha mutations, which constituted more than 90% of the alpha mutations.

**Conclusion:** According to the results, the most frequent mutations in the HBB gene are IVS-II-1, Codons 8/9, and IVS-I-1, and in the HBA gene are  $\alpha 3.7$ ,  $-\alpha 4.2$ , and  $--MED$  in Kurdistan province. In addition, the role of race and ethnicity as significant, influential factors in thalassemia was observable in the findings. The obtained results also indicated the communication pattern between the studied region's populations. Identifying common thalassemia mutations in an area could greatly benefit the early detection of thalassemia carriers in genetic laboratories and enhance thalassemia prevention programs.

**Keywords:** Alpha Thalassemia, Beta Thalassemia, Iran, Mutation

### Introduction

Thalassemia refers to a category of autosomal recessive disorders with different phenotypes resulting from defects in human globin gene synthesis (1) and alpha-thalassemia and beta-thalassemia. Beta thalassemia is the most common hereditary genetic blood disorder characterized by the abnormal or low production of hemoglobin (Hb), which

could lead to life-threatening anemia (2). More than 300 different b-globin gene mutations have been identified as the cause of beta-thalassemia worldwide (3). Bone marrow transplantation is the only effective treatment of severe thalassemia, which imposes a substantial burden on the patients' families and the community. Therefore, using thalassemia carrier

screening and prenatal diagnosis is essential to prevent the birth of children with severe thalassemia (4). Beta-thalassemia mutations are highly prevalent in Iran since it is a country located in the 'thalassemia belt' region. Given that Iran is ethnically diverse, beta-thalassemia mutations have different prevalence rates in the various areas of Iran (5). Alpha thalassemia is another common form of classic thalassemia, which reduces hemoglobin production and leads to anemia, thereby causing pallor, weakness, fatigue, and other severe complications. A more severe type is known as the hemoglobin (Hb) Bart's hydrops fetalis syndrome, also referred to as Hb Bart's syndrome or alpha thalassemia major. The milder form is known as the hemoglobin H (HbH) disease. In hydrops fetalis, excess fluid builds up in the body before birth, and severe anemia and hepatosplenomegaly are the main characterizations of the Hb Bart's syndrome. As a result of these severe health issues, most infants may be stillborn or die soon after birth. The Hb Bart's syndrome could also cause severe complications in women during pregnancy, including preeclampsia, preterm labor, and abnormal bleeding during pregnancy. Furthermore, the HbH disease is associated with mild-to-moderate anemia, hepatosplenomegaly, jaundice, and deformities such as a prominent forehead and the overgrowth of the upper jaw, which commonly appear in early childhood and affect individuals' life into adulthood. In Iran's health program, moderate and severe forms of alpha and beta-thalassemia are part of premarital screening (6). The national thalassemia prevention program started two decades ago in Iran, significantly reducing the number of these patients (7, 8). According to this program and the Ministry of Health, Treatment, and Medical Education guidelines, all couples in Iran must be screened before marriage. Couples with anemia who are suspected of thalassemia

minor are referred for genetic counseling, followed by genetic testing so that mutations could be identified in thalassemia carriers as candidates for prenatal diagnosis (PND) and interventions aimed at reducing the incidence of the disease. Kurdistan province is located in the west of Iran. In the latest Iranian population and housing census in 2016, the people of this province was estimated at 1,603,011 (<https://www.amar.org.ir>). Iraqi Kurdistan borders Kurdistan province on the west, West Azerbaijan and Zanzan provinces on the north, Kermanshah province on the south, and Zanzan and Hamedan provinces on the east. According to Iran's National Database of Administrative Divisions (<http://irdv.ncc.org.ir>), Kurdistan province consists of 10 counties, including Dehgolan, Saqqez, Marivan, Kamyaran, Baneh, Divandarreh, Qorveh, Bijar, Sanandaj, and Sarvabad. Thalassemia mutations have been previously studied in Kurdistan province; however, most of these studies were focused on patients with beta-thalassemia and thalassemia major. This comprehensive research was performed in 10 years to examine  $\alpha$  and  $\beta$  globin gene mutations in Kurd thalassemia carriers living in different regions of Kurdistan province, Iran.

## Materials and Methods

This retrospective cross-sectional study was conducted based on the standard guidelines of Iran's national thalassemia screening program. All the couples identified during the ten years of the screening program (2006-2016), who/ had become candidates for genetic testing, were recruited for the study. Notably, only Kurdish participants were selected for the study. The participants had been referred to the health centers in Sanandaj, Saqqez, Marivan, Kamyaran, Baneh, Divandarreh, Qorveh, Dehgolan, Bijar, and Sarvabad.

### Hematological Analysis

The initial diagnosis was based on abnormal blood indices, such as the mean

corpuscular volume (Mean corpuscular volume [MCV]; <80.0 fl) and/or the mean corpuscular Hb (Mean corpuscular hemoglobin [MCH]; <27.0 pg) and Hb A2 (>3.5%) and the absence of iron deficiency anemia.

### **Genetic Analysis**

To perform genetic testing, five milliliters of peripheral blood were collected from each participant, and their genomic DNA (Deoxyribonucleic Acid) was extracted using the salting-out method, agarose gel, and DNA electrophoresis. The quality and quantity of the extracted genomes were controlled using the NanoDrop spectrophotometer. Sanger sequencing (9) and detection of mutations in the beta-globin gene (HBB) were also used to assess beta-thalassemia. Table I shows the primers used for target sequence amplification (PCR protocol is in 35 Cycles as the following program; 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 60 seconds). Sanger sequencing is a 'gold standard' method for detecting short genetic variants, as described by F. Sanger in 1975. The method is based on DNA sequencing with chain-terminating inhibitors by fluorescent-labeled ddNTPs (dideoxynucleotides) and DNA-polymerase on unique PCR (Polymerase chain reaction) product fragments. In this method, after enzymatic purification (Removes excess PCR primers and unincorporated dNTPs) by Exosap-IT, the PCR products of the target fragment is used in a secondary PCR reaction with only one primer (forward or reverse) rather than two primers. The multiplex ligation-dependent probe amplification (MLPA) method (MRC-Holland/SALSA®MLPA®Probmix P140-C1 HBA) was used to assess the alpha thalassemia gene for frequent point mutations. The large mutations in the HBA1 (Hemoglobin Alpha Locus 1) and HBA2 (Hemoglobin Alpha Locus 2) genes since large genomic deletions/duplications represent a significant proportion of causative

mutations in alpha thalassemia. MLPA is one of the few techniques that can quickly and accurately identify large deletions and insertions along with target point mutations (10). Moreover, this technique could quantify the dosage and distinguish heterozygotic changes from homozygotic changes in alpha thalassemia. MLPA is designated for distinguishing cis from trans mutational changes in HBA1 and HBA2 in two alleles. This PCR-based technique amplifies the specific probes with universal primers rather than the target DNA through a multiplexing PCR reaction. The probe mixes (used for detecting more than 40 target sequences) are designed for specific DNA sequences. Each pair of probes is synthesized as two half probes: the 5' MLPA probe and the 3' MLPA probe. In addition to the target-specific site, they contain universal primer sites and stuffer sequences. In the first step of the MLPA procedure, the target DNA is denatured, and the MLPA probes are hybridized onto the target sequence (16 hours of incubation at 60°C). The third step is the ligation step, in which the probe pairs could be ligated into a larger oligonucleotide by the ligase enzyme. This step is only possible when both primers are hybridized correctly and have no gap with their adjacent targets. After ligation, the resulting complete probes (only bound and ligated probes) were amplified by PCR with only one pair of fluorescently labeled primers, which bind to the universal sites of the complete probe. The amplification products were separated by capillary electrophoresis with fluorescence detection. In this step, the stuffer sequences with various known lengths could characterize the presence of the nucleotide or correct the wild-type nucleotide in each targeted sequence. The MLPA results were analyzed in the COFFALYSER or Gene Marker software.

### **Statistical Analysis**

Data analysis was performed in SPSS version 24 (IBM Inc, Chicago, IL, USA).

This was a descriptive and strategic study aimed at defining more frequent mutations for clinical usage in a specific geographical, ethnic group (Kurdish people). Descriptive statistics were also applied, including the mean and standard deviation of blood indices for each genotype.

### Ethical Consideration

The study protocol was approved by the Ethics Committee of Kurdistan University of Medical Sciences (No. IR.MUK.REC.1399). Written informed consent was obtained from all the participants in the thalassemia screening program.

### Results

In total, 356 couples from the 10-year health screening program were identified as candidates for genetic testing in the initial examination. After excluding non-Kurd (from other provinces) or non-Iranian couples, 340 Iranian-Kurd couples (170 females and 170 males) were recruited. Over half of the cases (54.4%)

were from Sanandaj and Saqqez. One-third of the participants (47% or 27.6%) had a consanguineous marriage, and the remaining had a non-consanguineous marriage. The highest number of couples was in Sanandaj (39.1%), Saqqez (15.3%), and Daivandarreh (10.3%). Table II shows the mean age and the mean blood indices based on gender and type of thalassemia.

Overall, 20 different beta-thalassemia mutations were identified, and the first eight mutations constituted 81% of all the mutations (Table III). Table IV shows the blood indices of the more common mutations (frequency>5) by the type of mutation. Nine different alpha thalassemia mutations were identified, and the first three mutations constituted about 95% of all the mutations (Table V). Table VI shows the blood indices of the more common mutations (frequency>5) by the type of mutation. Table VII displays the results of the present study, as well as the similar studies conducted in Iran.

Table I: Primer Sequences Used for HBB Gene

Fragment	Primer Sequence	Target Size
1	5'- CTGAGGGTTTGAAGTCCAACCTCC - 3' : Forward	766
	5'- CTGTACCCTGTTACTTCTCCCCTTC - 3' : Reverse	
2	5'- ATGTATCATGCCTCTTTGCACC - 3' : Forward	578
	5'- GCACTGACCTCCCACATTCC - 3' : Reverse	

Note: fragment 1 is for exon 1, intron 1, and exon 2 sequencing; fragment 2 is for intron 2 and exon 3 sequencing.

Table II: Mean Age and Blood Indices by Type of Thalassemia

Patients	N	Age (year)	MCV (fl)	MCH (pg)	Hb A <sub>2</sub> (%)
Beta thalassemia	115	23.3±4.5	68.2±5.6	20.7±2.3	3.9±1.2
Alpha thalassemia	118*	24.4±5.3	76.8±5.8	23.9±2.1	1.95±0.8
Both alpha and beta thalassemia carriers	4	24±7.1	71.3±5.7	21.5±2.2	2.5±0.9
Total carriers	233	23.8±4.9	72.7±7.1	22.4±2.7	2.9±1.4
Total	340	23.9±4.7	73.8±6.9	22.7±2.8	2.5±1.3

\*Note: 14 carriers of alpha thalassemia had two mutations; MCV: mean corpuscular volume (femtoliters [fl]=10<sup>-5</sup> liter), normal adult range: 80-100 fl, a cutoff in Iran's program is lower than 80 fl; MCH: mean corpuscular hemoglobin (picograms), normal adult range: 27-33 pg, a cutoff in Iran's national program is lower than 27 pg; HbA<sub>2</sub>: hemoglobin A<sub>2</sub>, HbA<sub>2</sub>% was normally distributed (mean: 2.73±0.27; n=2,340), a cutoff in Iran's national program is higher than 3.5% (up to 7%; >7% is suspected of HgS (Hemoglobin S), HbG (Hemoglobin G), HbE (Hemoglobin E) or HbC (Hemoglobin C).

Table III: Mutations and Their Frequencies Identified in Beta Thalassemia Carriers

Name	HGVS Nomenclature	Mutated Alleles (n)	Frequency (%)
IVS-II-1 (G>A)	HBB:c.315+1G>A	30	26.1
Codons 8/9 (+G)	HBB:c.27_28insG	17	14.8
IVS-I-1 (G>A)	HBB:c.92+1G>A	14	12.2
IVS-I-6 (T>C)	HBB:c.92+6T>C	11	9.6
Codon 44 (-C)	HBB:c.135delC	7	6.1
Codon 39 (CAG>TAG)	HBB:c.118C>T	5	4.3
IVS-I-110 (G>A)	HBB:c.93-21G>A	5	4.3
Codons 36/37 (-T)	HBB:c.112delT	4	3.5
IVS-I-5 (G>C)	HBB:c.92+5G>C	3	2.6
Codons 22-24 (-7 bp): (-AAGTTGG)	HBB:c.68_74delAAGTTGG	3	2.6
Codon 15 (-T)	HBB:c.46delT	3	2.6
Hb Lepore	NG_000007.3:g.63290-70702del	3	2.6
Codon 5 (-CT)	HBB:c.17_18delCT	2	1.7
Codon 22 (GAA>TAA)	HBB:c.67G>T	2	1.7
Codon 121 (GAA>TAA)	HBB:c.364G>T	1	0.9
CAP +22 (G>A)	HBB:c.-29G>A	1	0.9
HbS or CD 6 (GAG>GTG)	HBB:c.20A>T	1	0.9
CAP +20 (C>T)	HBB:c.-31C>T	1	0.9
-88 (C>T)	HBB:c.-138C>T	1	0.9
Codon 25/26 (+T)	HBB:c.-78dupT	1	0.9
		115	100

\*Note: The first column of the table is the conventional nomenclature of the pathologic mutations of beta thalassemia; IVS: intronic variants standing for intervening sequence; the second portion is the intron number, and the third portion is the nucleotide number; CD: codon number, CAP: cap sequence of the m-RNA

Table IV. Association of Observed Mutations with Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, and Hb A<sub>2</sub>

Mutation	MCV (fl)		MCH (pg)		Hb A <sub>2</sub> (%)	
	M±SD	Min.; Max	M±SD	Min.; Max	M±SD	Min.; Max
IVS-II-1 (G>A)	70.25±6.25	62-79	21.66±2.34	19-26	5.29±1.47	<b>4.1-6.4</b>
Codons 8/9 (+G)	64.8±3.11	61-68	19.2±0.84	18-20	4.50±0.57	<b>4-5.2</b>
IVS-I-1 (G>A)	68.04±9.89	61-75	21.08±5.65	17-25	5.14±0.93	<b>48-6.7</b>
IVS-I-6 (T>C)	69.25±4.50	63-73	21.08±5.65	19-23	5.14±0.93	<b>3.7-5.8</b>
Codon 44 (-C)	67.78±2.76	70-72	20.08±1.45	18-21	4.68±0.73	<b>3.7-5.6</b>
Codon 39 (CAG>TAG)	63.77±2.61	63-68	18.93±1.25	18-21	4.99±0.53	<b>4.4-5.5</b>
IVS-I-110 (G>A)	65±4.49	56-67	19.95±1.06	17-20	4.5±0.42	<b>3.9-4.8</b>

\*All genotypes are in the heterozygote state; Hb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular Hb; Min.: minimum; Max.: maximum.

Table V. Mutations and Their Frequencies Identified in Alpha Thalassemia Carriers

Name	HGVS Nomenclature	Mutated Alleles (n)	Frequency (%)
-α <sup>3.7</sup> (3.7 Kb deletion)	NG_000006.1:g.34164-37967del3804	110	<b>82.7</b>
-α <sup>4.2</sup> (4.2 Kb deletion)	N/A	11	<b>8.3</b>
--MED	N/A	5	<b>3.75</b>
PolyA2 (AATAAA>AATGAA)	HBA2:c.*92A>G	2	<b>1.5</b>
CD 19 (-G) or CD 19 GCG>GC-	HBA2:c.60delG	1	<b>0.75</b>
-24C>G or CAP+14C>G	HBA1/HBA2:c.-24C>G	1	<b>0.75</b>
CD 90 (AAG>TAG)	HBA2:c.271A>T	1	<b>0.75</b>
Hb Q- Iran or CD 75 GAC>CAC	HBA1/HBA2:c.226G>C	1	<b>0.75</b>
Hb Constant Spring or CD 142 (TAA>CAA) >172aa	HBA2:c.427T>C	1	<b>0.75</b>
Total		133	<b>100</b>

\*Note: the first column of the table is the conventional nomenclature of the pathologic mutations of alpha thalassemia; MED: Mediterranean; -α<sup>3.7</sup>=3.7 Kb deletion in one of the alpha chain HBA1s or HBA2s; CD: codon number; PolyA2: polyadenylation tail sequence in mRNA; CAP: cap sequence in 5' side of HBA2 gene

Table VI. Association of Mutations with Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, and HbA<sub>2</sub>

Genotype*	MCV (fl)		MCH (pg)		Hb A <sub>2</sub> (%)	
	M±SD	Min.; Max	M±SD	Min.; Max	M±SD	Min.; Max
-α <sup>3.7</sup> (3.7 Kb deletion)	76.72±5.96	59-85	23.95±2.19	19-26	1.97±0.84	<b>1.1-3.2</b>
-α <sup>4.2</sup> (4.2 Kb deletion)	74.81±3.11	71-78	24.2±0.84	20-28	2.05±0.57	<b>1.5-2.9</b>
--MED	72.39±7.07	58-84	22.14±2.91	14-30	2.83±1.43	<b>1.8-4.7</b>

\*Note: 14 carriers of alpha thalassemia were compound heterozygote and α-trait, except for two carriers, which were Cis (i.e. -/aa); the remaining carriers were trans (i.e. -a/-a).

Table VII. Comparison of Frequency Rates of Most Frequent Mutations in Identified HBB Gene and Reported Values of Similar Studies in Iran in Adjacent Geographical Areas of Kurdistan Province or Populations with Ancestral Relationships

Geographical Region	Region	IVS-II-1 (G>A)	Codons 8/9 (+G)	IVS-I-1 (G>A)	IVS-I-6 (T>C)	Codon 44 (-C)	Codon 39 (CAG>TAG)	IVS-I-110 (G>A)	Codons 36/37 (-T)	IVS-I-5 (G>C)
West of Iran	Present study	26.1	14.8	12.2	9.6	6.1	4.3	4.3	3.5	2.6
	Kurdistan (south of province) (5)	23.71	19.59	6.19	8.25	1.03	12.37	7.22	6.19	6.19
	Kurdistan (Sanandaj) (11)	31.8	13.6	12.1	1.52	1.52	4.55	1.52	1.52	1.52
	Kurdistan (center and north of province) (12)	22.5	15.94	9.42	2.9	1.45	1.45	3.7	7.97	1.45
	Kurdistan and West Azerbaijan (Kurds) (13)	35	15.7	8	3.4	1.7	1.7	6	4.2	3.4
	Kermanshah (14)	45.8	15.9	3.5	5.5	N/A	2	8	1.5	1
	Lorestan (15)	27.7	10.8	N/A	N/A	N/A	N/A	11.6	23.8	4.7
Northeast of Iran	North Khorasan (16)	11.23	4.79	N/A	1.81	4.56	1.73	2.51	N/A	42.03

\*All mutation frequencies reported in percentages; rare: <1%; N/A: no report of the respective mutation in the study; North Khorasan Kurds peoples have ancestral relationship with Turkey's Kurds and north of Kurdistan province peoples; Kermanshah and Lorestan provinces have ancestral relationships with Kurdistan province.

## Discussion

In the historical background of the thalassemia in the middle east (including Iran) as the most frequent region of thalassemia worldwide, some effects have a central role in this phenomenon; Heterozygote advantage for acquired infections like malaria (as the main reason for high frequency), the regional founder effect (as the main reason of specific variants changes in various regions) and high rate of consanguine marriages (as recent cultural effects of maintaining of the disease frequency). The present study aimed to determine the frequency of mutations in patients with classic

thalassemia (alpha and beta) in the Kurdistan province of Iran due to specific founder effects. Overall, nine types of alpha mutations and 20 types of beta mutations were identified in the 10 years in Kurdistan province during the national premarital thalassemia prevention program. Among the beta-thalassemia mutations, IVS-II-1, codons 8/9, and IVS-I-1 had the highest frequency, constituting 53% of all the mutations. In a previous study on the Kurds of Ilam province (west of Iran), IVS-II-1, codons 8/9, 36/37, and IVS-I-110 were reported to be the most frequent mutations, and IVS-II-1 alone constituted 59% of all the mutations (7).

Consistent with this finding, IVS-II-1 has been reported to have the highest frequency rate in Iran (23%) (8) and the north of Iraq (28.7%) (17). A study conducted in Siirt province in the southeast of Turkey (mostly inhabited by Kurds) aimed to determine the types and frequency of mutations in patients with beta-thalassemia, and 13 mutations were identified, of which IVI-I-110 (G>A; HBB: c.93-21G>A) had the highest frequency (38.9%). Other frequent mutations included IVS-II-1 (G>A; HBB: c.315\_1G>A; 11.1%), -30 (T>A; HBB: c.-80T>A; 9.25%), and IVS-I-1 (G>A; HBB: c.92p1G>A; 9.25%). These mutations were observed in 68.5% of the samples (3). Among these 13 mutations, one was identified in the Kurdish origin, and another was detected of the Kurdish and Iranian origin. Other studies performed in the Kurdish regions of Turkey have reported the most frequent mutations to be IVS-I-110, IVS-I-1, codons 8/9, IVS-II-745, and IVS-II-I (18-20). These mutations were also widespread in the present study. In another study conducted in the Isfahan and Chaharmahal, and Bakhtiari provinces of Iran, Fr36/37 (-T) and IVS-II-1 (G>A) were reported to be the most frequent beta mutations (21).

Compare to our results, in a review paper results of the De Sanctis V. & et al. in 2017, the neighbor countries of Iran relatively have a similar variants of beta thalassemia frequency. In this review the top 5 frequent variants in Iran are; IVS-2-1 (G>A) (28.7%); IVS-1-1(G>A) (17.7%); codon 8 (-AA) (9.1%); codon 8/9 (9.1%); codon 39 (C>T) (9.1%)- in Eastern Province of Saudi Arabia are; IVS-2-1 (G>A) (27.5%); IVS-1-5 (G>C) (23.2%); codon 39 (C>T) (20.3%); IVS-1-1 (G>A) (5.8%); IVS-1-25 bp (4.4%)- in Kuwait IVS-2-1 G>A and IVS-1-6 T>C accounted for 63.9% of all mutations - in Syria are; IVS-1-110 (G>A) (17.0%), IVS-1-1 (G>A) (14.7%), codon 39 (C>T) (14.4%), IVS-2-1 (G>A) (9.8%), codon 8 (-AA) (6.2%), and in Azerbaijan Three mutations

(codon 8-AA, IVS-2-1(G>A) and IVS-1-110 (G>A) account for over 80% of thalassemia genes (22). These variants frequency are significantly different compare with the European countries.

In the present study, - $\alpha$ 3.7, - $\alpha$ 4.2, and -MED were the most frequent alpha mutations, constituting more than 90% of all the alpha mutations. In a study performed in Erbil (Iraq), six alpha mutations were identified, and - $\alpha$ 3.7 was the most frequent alpha mutation (62.86%); this finding is in line with the present study (20). The result was expected as Erbil is mainly populated by Kurds. In another research, Al-Allawi et al. (2009) also stated that - $\alpha$ 3.7 and -MED were the most frequent alpha thalassemia gene mutations in the residents of Dohuk (a Kurdish province of Iraq) (21). In another study performed in Khuzestan province (Iran), - $\alpha$ 3.7 and -MED were reported to be the most frequent alpha mutations (23).

Compared to HBA, HBB gene variations have a wider spectrum, making it a better index of genetic similarities for ethnic group variations in a shorter period in different geographical regions. Contrary to HBB, alpha thalassemia gene variations need a more extended period, making it a better index for far geographical populations. A comparison of our findings with similar studies conducted in Iran shows that the mutation spectrum of the  $\beta$ -globin gene in Kurdistan province is identical to the other regions of Iran. In addition to the overall status of common mutations in Iran, common mutations in Kurdistan province are mainly similar to those in other Kurdish regions inside and outside Iran. Race and ethnicity are significant, influential factors in thalassemia, according to the results of the present study. On the other hand, the obtained results reflected the pattern of communication between different populations in the region.

## Conclusion

Given the high frequency of carriers and beta-thalassemia patients in Kurdistan province, our findings could be used in prenatal diagnosis programs to identify high-risk carriers and fetuses. Identifying common mutations in a region could contribute to the rapid and early detection of thalassemia carriers in genetic laboratories while improving thalassemia prevention programs throughout the country. To summarize and compare the frequency of genetic variants, it could be stated that both ethnicity and geographical proximity could be considered the determinants of the similarity of genetic variants in different populations.

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## Conflict of interest

The authors declare no conflict of interest.

## References

- Hemminki K, Li X, Försti A, Sundquist J, Sundquist K. Thalassemia and sickle cell anemia in Swedish immigrants: genetic diseases have become global. *SAGE open medicine* 2015; 3:1-8.
- Kattamis A, Forni GL, Aydinok Y, Viprakasit V. Changing patterns in the epidemiology of  $\beta$ -thalassemia. *Eur J Haematol* 2020;105(6):692-703.
- Yılmaz S. The spectrum of  $\beta$ -Thalassemia mutations in Siirt province, southeastern Turkey. *Hemoglobin* 2019;43(3):174-181.
- Zhu Y, Shen N, Wang X, Xiao J, Lu Y. Alpha and beta-Thalassemia mutations in Hubei area of China. *BMC Med. Genet* 2020; 21(1):6-11.
- Alibakhshi R, Moradi K, Aznab M, Dastafkan Z, Tahmasebi S, Ahmadi M, et al. The Spectrum of  $\alpha$ -Thalassemia Mutations in Kurdistan Province, West Iran. *Hemoglobin* 2020;44(3):156-161.
- Colosimo A, Gatta V, Guida V, Leodori E, Foglietta E, Rinaldi S, et al. Application of MLPA assay to characterize unsolved  $\alpha$ -globin gene rearrangements. *Blood Cells Mol Dis* 2011;46(2):139-144.
- Moradi K, Aznab M, Tahmasebi S, Omidniakan L, Bijari N, Alibakhshi R. Distribution of HBB Gene Mutations in the Kurdish Population of Ilam Province, West Iran. *Hemoglobin* 2020;44(4):244-248.
- Najmabadi H, Karimi-Nejad R, Sahebjam S, Pourfarzad F, Teimourian S, Sahebjam F, et al. The  $\beta$ -thalassemia mutation spectrum in the Iranian population. *Hemoglobin* 2001;25(3):285-296.
- Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci* 1977;74(12):5463-5467.
- Os PG, Schouten JP. Multiplex Ligation-dependent Probe Amplification (MLPA®) for the detection of copy number variation in genomic sequences. *PCR Mutation detection protocols: Springer; 2011: 97-126.*
- Fathollah-Pour A, Naghshi-Zadeian R. Common  $\beta$ -Thalassemia mutation in the city of Sanandaj. *Sci J Kurd Univ Med Sci* 2003; 3:21-26.
- Darabi A, Keshavarzi F, Sedaghatikhayat B, Salehifar P, Masoudifar M, Rad AF, et al. Beta globine gene mutations in blood transfusion-dependent beta-thalassemia major patients in Kurdistan Province hospitals. *Sci J Kurd Univ Med Sci* 2015;20(4): 1-6.
- Haghi M, Khorshidi S, Hosseinpour Feizi MA, Pouladi N, Hosseinpour Feizi AA.  $\beta$ -Thalassemia mutations in the Iranian Kurdish population of Kurdistan

and West Azerbaijan provinces. *Hemoglobin* 2009;33(2):109-114.

14. Mehrabi M, Alibakhshi R, Fathollahi S, Farshchi MR. The spectrum of  $\beta$ -thalassemia mutations in Kermanshah Province in West Iran and its association with hematological parameters. *Hemoglobin* 2013; 37(6):544-552.

15. Kiani AA, Mortazavi Y, Zeinali S, Shirkhani Y. The molecular analysis of  $\beta$ -thalassemia mutations in Lorestan Province, Iran. *Hemoglobin* 2007;31(3):343-349.

16. Jaripour ME, Hayatigolkhatmi K, Iranmanesh V, Zand FK, Badiei Z, Farhangi H, et al. Prevalence of  $\beta$ -thalassemia mutations among northeastern Iranian population and their impacts on hematological indices and application of prenatal diagnosis, a seven-years study. *Mediterr J Hematol Infect Dis* 2018; 10(1): 1-13.

17. Al-Allawi NA, Hassan K, Sheikha AK, Nerweiy FF, Dawood RS, Jubrael J.  $\beta$ -Thalassemia mutations among transfusion-dependent thalassemia major patients in Northern Iraq. *Mol Biol Int* 2010(1): 1-4.

18. Genc A, Tastemir Korkmaz D, Buyukleyla M, Celiker M. Prevalence and molecular analysis of  $\beta$ -thalassemia in Adiyaman, Turkey. *Hemoglobin* 2012; 36(2):131-138.

19. Hakan Ince H, Ayyildiz O, Kalkanli S, Batun S, Muftuoglu E. Molecular Basis of  $\beta$ -Thalassemia Mutations in Diyarbakir in the Southeastern Region of Turkey. *Hemoglobin* 2003; 27(4):275-278.

20. Oner AF, Özer R, Uner A, et al.  $\beta$ -Thalassemia mutations in the east of Turkey. *Turk J Haematol* 2001;18(4):239-241.

21. Heidari Soureshjani E, Vallian S, Mirahmadi Babaheidari S, Abasian F. Prevalence of various mutations in Beta thalassaemia in Province of Chahar Mahal Bakhtiari and Isfahan and its association with haematological parameters. *J Iran Blood Transfus Organ* 2017;14(2):109-117.

22. De Sanctis V, Kattamis C, Canatan D, Soliman AT, Elsedfy H, Karimi M, et al.  $\beta$ -thalassemia distribution in the old world: an ancient disease seen from a historical standpoint. *Mediterr J Hematol Infect Dis* 2017; 9(1): 1-8.

23. Keikhaei B, Slehi-Fard P, Shariati G, Khosravi A. Genetics of Iranian alpha-thalassemia patients: a comprehensive original study. *Biochem Genet* 2018;56(5):506-521.