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Original Article

Molecular Spectrum of Beta-Globin Mutations in Transfusion-Dependent Patients with Thalassemia in Qazvin Province, Iran

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Abstract

Background: Beta thalassemia is a common inherited disease, resulting from one or more of 200 different mutations in the beta-globin gene. Qazvin province has attracted migrations of several different populations due to industrialization during the past five decades. The aim of this study was to define the molecular spectrum of beta-thalassemia mutations in Qazvin province.

Methods: Ethylen diamin acetic acid-containing venous blood samples were collected from 100 patients with transfusion-dependent beta-thalassemia from the department of Pediatrics in Qods hospital. Age, sex, history, and consanguinity between the parents were recorded by reviewing the patients' files. DNA was isolated from leukocytes using the standard procedure. Amplification refractory mutation system (ARMS) technique was used for molecular detection of mutations. Direct sequencing analysis was applied for DNA samples when no mutation was detected with ARMS.

Results: Of the 200 chromosomes investigated, 11 types of mutations were identified by ARMS technique while direct sequencing revealed the remaining alleles (9 types of mutations). Total 20 different mutations discovered by this two-step approach. Abundant alleles (IVS II-1, IVS I-10, FSC 8/9) accounted for 59.3% of the mutations. IVS II-1 with a frequency of 31.3 % was the most common while HbS, Cd 74/75 and Cd 15, each with a frequency of 0.55%, had the least frequencies.

Conclusion: Beta thalassemia mutations are very heterogeneous in Qazvin province. Extensive ethnic and genetic admixture has resulted in unexpectedly high number of different mutations, most of them similar to that of north and north-western provinces of Iran. Different mutations in this region suggest migration of chromosomes from distant places and genetic admixture.
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Keywords ● Beta thalassemia ● mutations ● gene frequency

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Introduction

Beta thalassemia is considered the most common autosomal single gene disorder worldwide. This disorder can be found in more than 60 countries with a carrier (*heterozygotes*) population of up to 150-200 million or 4.5%

of the world population. At least 300,000 lethally affected homozygotes are born annually.[1,2]

Iran, with more than 18000 affected individuals represents one of the areas in the world with an unusually high prevalence of beta thalassemia. Provinces around the Persian Gulf and the Caspian Sea with a gene frequency of more than 10% constitute the thalassemia major zones in Iran.[3,4]

The disease is resulting from one or more of a total of about 200 different mutations in the beta globin gene. Therefore, there are numerous gene mutations responsible for beta thalassemia. With regard to regional differences in the frequency of these mutations, defining the spectrum of these mutations in different countries or areas is necessary.[3]

Information provided on the distribution and the frequency of beta thalassemia alleles is useful to establish a program for carrier screening, genetic counseling, prenatal diagnosis, and for physicians to establish specific therapeutic approaches for patients with beta thalassemia major.[5]

The present study was undertaken to define the spectrum of beta thalassemia mutations in Qazvin province.

Materials and Methods

Specimens

This is a descriptive-analytical study performed during 2005-2006. Venous blood samples were collected prior to transfusion in Ethylen diamin acetic acid-containing tubes from 100 patients with transfusion-dependent beta thalassemia major who referred to the department of Pediatrics in Qods hospital, Qazvin province.

The age, sex, duration, start of transfusion, and consanguinity between the parents were recorded by reviewing their files after obtaining permission from patients or their parents.

DNA was isolated from white blood cells, using salting out method.[6] The DNA extract was then kept at -70 °C until analysis.

Methods

Amplification refractory mutation system (ARMS) technique was used for molecular detection of mutations. Sequencing analysis was applied for DNA samples when no mutation was detected with ARMS.[7,8] In all the above characteristics, mean central statistical data were estimated.

Polymerase chain reaction (PCR)-ARMS primers were synthesized in TAG Corporation (Copenhagen, Denmark), for 13 most common mutations in the Mediterranean populations.[8,9] Sequences of allele-specific oligonucleotide primers (normal and mutant) used are listed in table1.

PCR reaction mixture contained 2 µl of genomic DNA, 0.5 µl 10 mM dNTP mix, 1 µl common (B or C or D) primer, 1 µl of either normal (N) or mutant (M) primers, 2.5 µl 10X PCR buffer, 1.25 µl 50 mM MgCl2, 0.5 µl Taq DNA polymerase and 16.25 distilled water (total volume 25 µl). All reaction mixture constituents were purchased from Fermentase Corporation (Russia).

The reaction tubes were then subjected to thermal cycles on a DNA thermal cycler (MWG-BIOTECH, Germany). The thermal cycling regimen consisted of 30 cycles: preheating at 94 °C for 2 min, denaturing at 94 °C for 1 min, variable annealing temperatures (depending on each mutations), and extension at 72 °C for 1 min.[5,7-9]

All samples were analyzed simultaneously alongside positive and negative controls (known genomes) for particular mutation. An 861 bp internal control band was also amplified in all reactions indicating successful PCR. The PCR products were finally analyzed by electrophoresis.

Direct Sequencing

To check the efficiency of PCR-ARMS technique as well as to identify the rare beta thalassemia alleles in the study, the beta globins of 30 samples were amplified by two sets of primers (sense/anti sense and forward / reverse).[10] The resulting segments and nucleotides respectively, contain most of the known mutant sites specific for the Mediterranean population.

Following amplification, the PCR products were purified, using an Applied Biosystem purification kit. The purified samples were analyzed on an automated sequencer analyzer (ABI-3730XL Capillary system, USA) according to the manufacture's instructions. Preparation of sequencing primers (table 1) and sequencing process were performed in TAG Corporation (Copenhagen, Denmark).

Results

The mean age of the patients at the time of blood collection was 16.5 (+7.7) years. Of the 100 studied patients with thalassemia major, 47 (47%) were male and 53 (53%) were female. In 43% of the patients' families, the parents were first or second cousin relatives and in 53% of families, the parents had no relation to each other (no consanguineous marriage). The ethnicities of the parents of the study population are provided in figure 1. In addition, 30 patients (30%) with the mean age of

Table 1: Oligonucleotide primers used for mutation detection by ARMS and direct sequencing.

Common primer which used	Primer sequences (normal or mutant)	Primer name	
Common D	5´- CAAACAGACACCATGGTGCACCTGACTCCT-3	Codon 5	N
	5´- TCAAACAGACACCATGGTGCACCTGAGTCG-3´		M
	5´- ACACCATGGTGCACCTGACTCCTGAGCAGA-3´	Codon 8	N
	5´- ACACCATGGTGCACCTGACTCCTGAGCACG-3´		M
Common B	5´- GGTTTCATATTGCTAATAGCAGCTACAATCGAGC-3´	IVS II-745	N
	5´- GGTTTCATATTGCTAATAGCAGCTACAATCGAGG-3´		M
Common C	5´- TTAAACCTGTCTTGTAACCTTGATACCCAC-3´	IVS I-1	N
	5´- TTAAACCTGTCTTGTAACCTTGATACCGAT-3´		M
	5´- CTCCTTAAACCTGTCTTGTAACCTTGTTAC-3´	IVS I-5	N
	5´- CTCCTTAAACCTGTCTTGTAACCTTGTTAG-3´		M
	5´- AAGAAAACATCAAGGGTCCCATAGACTGAC-3´	IVS II-1	N
	5´- AAGAAAACATCAAGGGTCCCATAGACTGAT-3´		M
	5´- TCTCCTTAAACCTGTCTTGTAACCTTCATA-3´	IVS I-6	N
	5´- TCTCCTTAAACCTGTCTTGTAACCTTCATG-3´		M
	5´- TAAACCTGTCTTGTAACCTTGATACCAACC-3´	Codon 30	N
	5´- TAAACCTGTCTTGTAACCTTGATACCAACG-3´		M
	5´- CAGATCCCCAAAGGACTCAAAGAACCTGTG-3´	Codon 39	N
	5´- CAGATCCCCAAAGGACTCAAAGAACCTGTA-3´		M
	5´- GGTAAGGACTCAAAGAACCTCTGGGTCCAA-3	FSC 36/37	N
	5´- GGTAAGGACTCAAAGAACCTCTGGGTCCAG-3´		M
	5´- AGCATCAGGAGTGGACAGATCCCCAATGG-3´	Codon 44	N
	5´- CAGCATCAGGAGTGGACAGATCCCCAATGA-3´		M
	5´- ACCAGCAGCCTAAGGGTGGGAAAATACACC-3´	IVS I-110	N
	5´- ACCAGCAGCCTAAGGGTGGGAAAATAGAGT-3´		M
	5´- CCTTGCCCCACAGGGCAGTAACGGCACACT-3´	FSC 8/9	N
	5´- CCTTGCCCCACAGGGCAGTAACGGCACACC-3´		M
	5´- ACCTCACCCTGTGGAGCCAC-3´	Common C	
	5´- CCCCTTCCTATGACATGAACTTAA-3´	Common D	
		Common B	
	5´-TTGAGGATTCGGTCACGGTCTTCT-3´	Sequencing primer Sense	
	5-TTTTCCCTTACACCCTCCAGTCAC -3´	Sequencing primer Anti sense	
	5´- CAATGTATCATGCCTCTTTGCACC-3´	Control A	
	5´- GAGTCAAGGCTGAGAGATGCAGGA-3´	Control B	
	5 - CTTAGGCTGCTGGTGGTCTACC-3´	Sequencing primer F	
	5 - AGCACTTTCTTGCCATGAGCC-3´	Sequencing primer R	

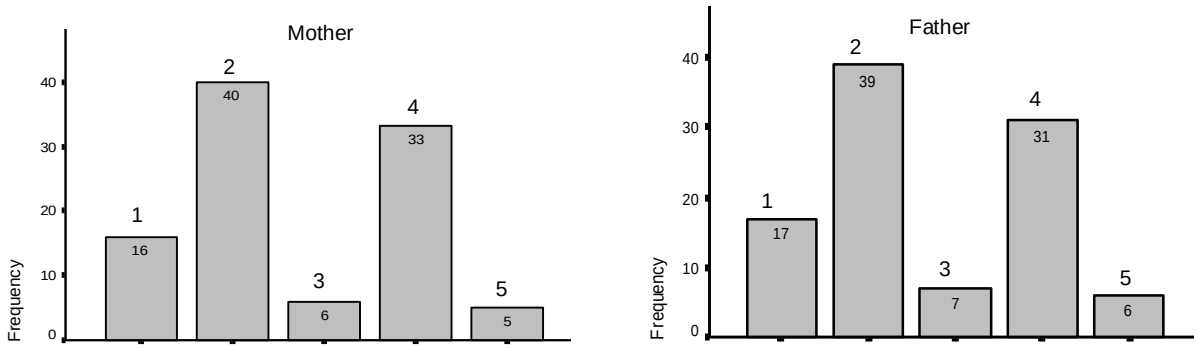

Figure 1: Ethnicities of the parents of the study population: 1: Persian, 2: Turkish, 3: Kurdish, 4: Gilak and 5: Miscellaneous.

10.7±5.6 years had undergone splenectomy. Forty seven patients (47%) were homozygous (having same genotype) and 53 (53%) were double heterozygous of different beta thalassemia mutations.

Of the 200 chromosomes investigated, 140 chromosomes were identified by PCR-ARMS techniques while direct sequencing uncovered the remaining 60 alleles. Totally 20 different mutations were discovered by this two-step approach.

Figures 2 and 3 show examples of mutation detection by ARMS technique as well as direct sequencing in a patient with FSC 36/37 mutation.

In spite of thalassemia major phenotype in one patient, his two alleles remained unknown even after beta globin gene sequence analysis.

The frequencies of various mutations in unrelated transfusion-dependent beta thalassemia patients (n=92) in Qazvin province are given in table 2.

Based on these data, three abundant alleles (IVS II-1, IVS-I-110 and FSC 8/9) accounted for 59.3% of the mutations. IVS II-1 with a frequency of 31.3% was the most common allele.

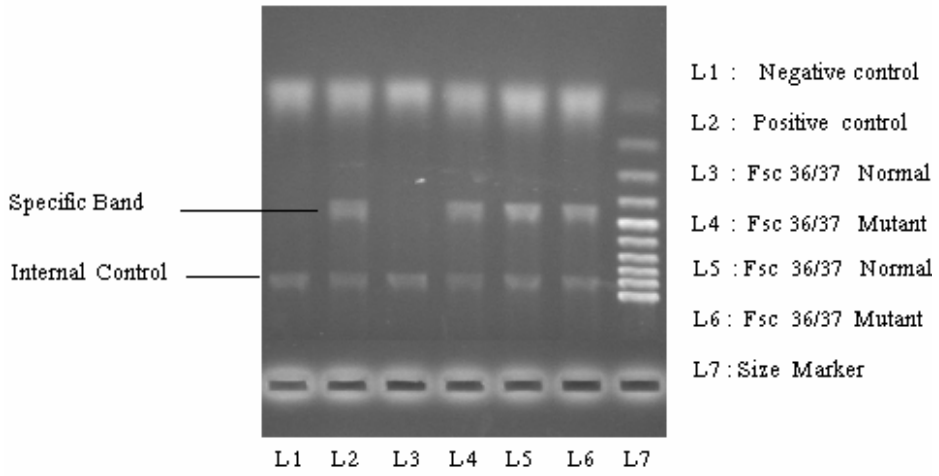

Figure 2: Detection of FSC 36/37 mutation by ARMS technique.

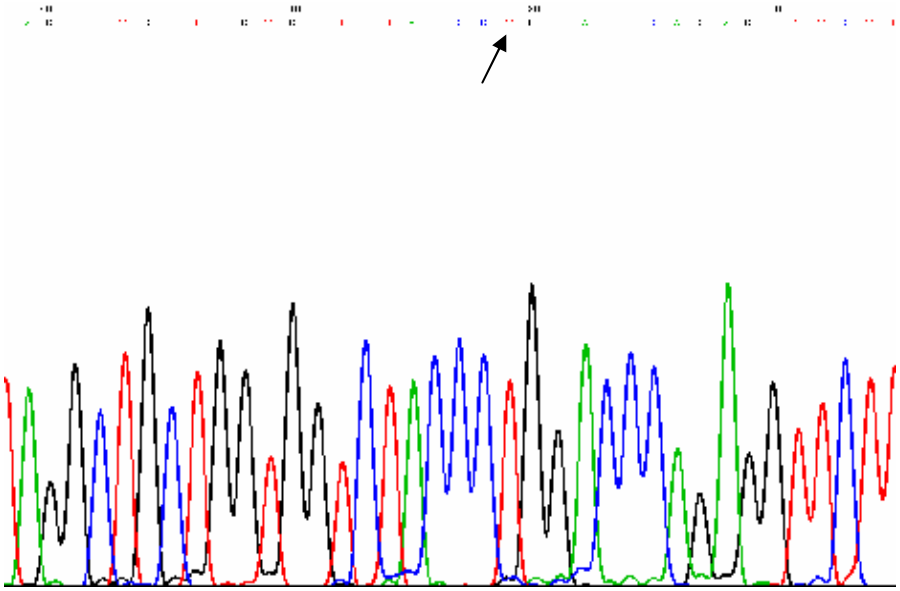

Figure 3: Detection of FSC 36/37 mutation by direct sequencing. Deletion of T nucleotide (arrow) of 419 related to codons CCT and TTG, results in CCT and – GG, hence frame shift change of the following codons.

Table 2: Frequencies of various mutations in patients with beta thalassemia major in Qazvin province.

Allele	Detected	Frequency %
IVS II-1	57	31.3
IVS I-110	35	19.2
FSC 8/9	16	8.8
Codon 30& Hb Monroe	11	6.1
Codon 44	11	6.1
IVS I-5	10	5.5
FSC 36/37	9	4.95
IVS I-1	8	4.4
IVS I-6	3	1.64
Codon 5	3	1.64
IVS II-745	2	1.1
5 ' UTR	2	1.1
Codon 15	2	1.1
Codon 39	2	1.1
IVS I-130	2	1.1
Nt -30	4	2.1
Codon 24	1	0.55
Codon 74/75	1	0.55
Hbs	1	0.55
No mutation	2	1.1
SUM=20	SUM=82	SUM=100

The frequency of different kinds of low or rare prevalent mutations was 40.7% (table 2).

Discussion

Qazvin is a small province with about 1.2 million populations in the north of Iran surrounded by Tehran, Guilan, Mazandaran, Zanjan and Hamedan provinces.

In Qazvin, most of beta globin mutations had been cumulated into IVS II -1, IVS I-110 and frame shift of codon 8 of beta globin gene, which account for nearly 59.3% of all abnormalities.

Each country or area is found to have only 3-4 common mutations accounting for 70% or more of the beta thalassemia alleles.[11] Our results show that in Qazvin this value is 59.3% that is less than those of the other areas.

In Iran, there are numerous gene mutations responsible for beta thalassemia. These mutations are related to Iranian, Mediterranean, Kurdish, Turkish, Egyptian, Tunisian, Indian, Asian-Indian, Chinese, and Afro-American origin.[12]

IVS II nucleotide 1 mutation was found to be the most common mutation in Qazvin. This is in complete accordance with the data known about the provinces neighboring Qazvin (Mazandaran 62%, Guilan 57 %, and Tehran 51.3 %),[12-14] and it is possible to consider this mutation as a common mutation (34% frequency) in Iran.[15]

IVS I nucleotide 110 mutation was found to be the second common mutation. This mutation is also the second common mutation (25.5% frequency) in the north-west provinces of Iran.[9] In these provinces, FSC 8/9 mutation was found to have the highest frequency (29.9%) but it has the third position in Qazvin.

An important finding of the present study is that in such a small province like Qazvin, the distribution of low frequent or rare mutations are high (40.7%) in comparison with the other provinces of the country,[9,12-16] or other countries.[17-19] Three of these mutations are unique to Qazvin province (table 2).

Hb Monroe results from a splice site point mutation in the last nucleotide of beta-globin exon1 that is also the penultimate nucleotide of codon 30 of the beta-globin peptide. After the original reports in 1989, Hb Monroe has been reported in low frequency, mostly in compound heterozygosity with other beta globin mutations.[20] The parents of the patient with this mutation in the present study were first cousin relatives.

Codon 74/75 is a rare frame shift mutation in the world with β° phenotype in Turkish ethnic groups.[21] There was no relationship (consanguinity) between the parents of the patient with this mutation in the current study.

In spite of having thalassemia major phenotype, one patient (2 alleles) detected as having "no mutation". This abnormality may result from defects in beta-globin Locus Control Region (LCR). The parents of this patient were first cousin relatives.

To the best of our knowledge, these three mutations have not been reported previously from Iran.[4,15,22-25] An advantage of this study was simultaneous application of PCR-ARMS and DNA sequencing techniques which could detect such uncommon mutations.

A major factor for this genetic diversity and thalassemia gene dissemination is the migration of people. Qazvin province has attracted migrations of several different populations due to industrialization. During the past five decades extensive ethnic (genetic) admixture has resulted in unexpectedly high number of different mutations. Most of these mutations are similar to those of north and north-west provinces of Iran.

In Conclusion, the people in Qazvin province exhibit a genetic heterogenecity that is unparalleled to the other provinces of Iran.

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Conflict of Interest: None declared

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