Iranian Journal of Pediatric Society
Volume 2, Number 1, January 2010: 4-8

Original Article

Wide Spectrum of Mutations in the Beta-Globin Gene Causing Beta-Thalassemia Major in Southwest Iran

Hamid Galehdari [1], Mohammad Pedram [2], Bahaoddin Salehi [3], Behnaz Andashti [1]
[1] Department of Genetics, Shahid Chamran University of Ahwaz, Ahwaz, Iran, [2] Research Center for Thalassemia and Hemoglobinopathies, Jondishapoor Medical University, Ahwaz, Iran, [3] Genetic Lab and PND Center of Shafa Hospital, Ahwaz, Iran

ABSTRACT

Background and Aim: *Beta-thalassemia (β-thalassemia) is characterized by the reduced synthesis of the hemoglobin beta chain. Nowadays, more than 200 disease-causing mutations in beta-globin (β-globin) gene have been identified. Beta-thalassemia is the most common monogenic disease worldwide and one of the widespread hereditary disorders in Iran. Considering the vast spectrum of beta-thalassemia mutations, it has multi-ethnic population. The gene frequency of beta-thalassemia is high and varies considerably in each region. Therefore, it is necessary to determine the frequency and distribution of beta-thalassemia mutations in different regions.*

Materials and Methods: *In the present study, two hundred and two beta-thalassemia major patients were subjected for genomic DNA extraction from whole blood. Amplification and subsequent sequencing of the beta-globin gene has been made by specific primers.*

Results: *Thirty mutations were found in 404 studied alleles. Our results show that IVSII-1(G>A) with 21.3% (86/404 alleles) represents the most common mutation, followed by the four mutations namely CD36/37 (-T) (16%), IVSI-110(G>A) (17.8%), IVSI-5(G>C) (6.9 %) and CD5 (-CT) (5.2%), respectively.*

Conclusion: *Our findings indicate that the Khuzestan population possesses a wide variety of thalassemia allelic distribution. These results can be used as basis for prenatal diagnosis of beta-thalassemia, especially in the south west of Iran.*

Iranian J Pediatr Soc 2010; 2(1): 4-8

Keywords: *Beta-globin, Beta-thalassemia, DNA sequencing, Gene mutations*

Corresponding author: *Hamid Galehdari, Ph.D.*
Address: *Department of Genetics, Shahid Chamran University of Ahwaz, Ahwaz, Iran*
Tel: *+98-611-3331045****; Fax:*** *+98-611-3331045*
Email: *galehdari@scu.ac.ir*
Received: *June 2009;* ***Accepted:*** *July 2009*

INTRODUCTION

Thalassemia is an inherited hematological disorder with an autosomal recessive pattern that is usually caused by mutations in the β-globin gene. Mutations generally involve the replacement, insertion or deletion of the normal

(βA) gene (1). β-thalassemia is widespread in Mediterranean, Southeast Asian, African and Middle east populations and is due to a heterogeneous collection of mutations that seriously restricts or completely eliminates the production of β-globin protein (2). It is usually clinically manifested in homozygotes as a severe transfusion-dependent hemolytic anemia (Cooley's anemia).

Although about 200 mutations of the β-globin gene which can lead to β-thalassemia major have been characterized worldwide (3), a subset of common mutations is present in each ethnic group in which the disease is prevalent (4-10). For example, four such mutations- the single nucleotide deletion mutation between the codons 36-37 of the gene and three RNA processing (splicing) mutations in the first and second intervening sequence at nucleotide positions 1, 6 and 110 (βIVS I-1, βIVS I-110 and βIVSII-1, respectively)- together account for more than 70% of the β-thalassemia alleles present in Iran's populations (11).

The frequency, broad geographic distribution, and clinical severity of these hemoglobinopathies make testing for carriers and prenatal diagnosis a critical public health issue in these areas. Current hematological tests are adequate for the screening of carriers, but the identification of specific mutations and prenatal diagnosis during the first trimester of pregnancy is the main goal in molecular diagnosis (12).

Meanwhile, molecular diagnosis of β-thalassemia is a more complex task because many different mutations can give rise to defective β-globin-chain synthesis (13).

In order to solve the above mentioned problem and to create a data base for prenatal molecular diagnosis, we aimed to investigate 202 patients affected with beta-thalassemia major. All the patients were registered in the Thalassemia center of the Shafa hospital in Ahwaz, Iran

MATERIALS AND METHODS

***Clinical assessment*:** The project was approved by the Medical University of Jondishapoor's ethics board. In this project, 202 patients with different ethnicities that had referred to Shafa hospital of Ahwaz from September 2007 to June 2008 were studied. After obtaining informed consent, all participants were questioned in regard to their personal medical history and a family tree was drawn.

***Sample collection and DNA extraction*:** Five ml of EDTA-whole blood from 202 beta-thalassemia major affected individuals was collected and the DNA was extracted by the routine salting out method.

Polymerase Chain Reaction (PCR): One hundred nanogram extracted DNA from each sample was amplified by PCR using primers as described in table 1. The 25μL reaction mixture contained 2.5μL PCR buffer, 0.75 μL MgCl 2 (1.5 mM), 0.5 μL of all four deoxynucleoside

Table 1. The primers and their sequences that were used in this study are listed below. All the primers were designed with the special software 'primer3out'

Primer name	Primer sequence	Applied in
BT-NF	5-AACTCCTAAGCCAGTGCCAGAAGA-3	PCR FORWARD
BT-NR	5-CACTGACCTCCCACATTCCCTTTT-3	PCR REVERSE
BT-seq-1F	5-AGGTACGGCTGTCATCAC-3	SEQUENCING FORWARD
BT-seq-501F	5-CATGGCAAGAAAGTGCTC-3	SEQUENCING FORWARD
BT-seq-683R	5-AGGTACGGCTGTCATCAC-3	SEQUENCING REVERSE
BT-seq-2F	5-ATCTCTTTCTTTCAGGGC-3	SEQUENCING FORWARD

triphosphates (each at 0.2 mM), 0.5 µL of each forward and reverse primers (each at 25 pmol/µL), and 2.5 U of *Taq* DNA polymerase. After an incubation time at 94°C for 3 min, 30 cycles including 94°C for 30 sec, 56°C for 30 sec and 72°C for 30 sec were performed, followed by a final 5-min extension at 72°C in a thermal cycler (BIO-RAD thermo cycler, Germany). As negative control, a PCR reaction without genome was achieved to control for cross-contamination. The expected length of the PCR product is 1824 bp.

***Gel electrophoresis*:** The amplified products were visualized by staining with ethidium bromide after electrophoresis on 1.5% agarose gel to control for accurate size and specificity. Electrophoresis was carried out for 2-3 hr on the gel-Scan 2000 (Corbert Research) according to the manufacturer's instructions. The data were collected automatically and analyzed by GeneScan software (Corbert Research).

***Sequencing*:** The PCR products were cycle sequenced with the primers that are listed in table 1 in two separate reactions for each PCR product using the ABI PRISM Big Dye Primer Cycle Sequencing Ready Reaction Kit (Applied Biosystems)as described in company's manufacture.

RESULTS

In this study 202 patients (404 alleles) have been analyzed; from these 102 individuals were with Arabian background. In total, we detected 30 different mutations. One hundred and twenty two patients possessed homozygote mutations and the other 80 patients were compound heterozygote. The most frequent mutation with 21.3% was the splice site mutation IVSII-1 (G>A). We could not find any mutation in seven patients that were diagnosed with beta- thalassemia major, despite the full length sequencing of the beta globin gene. In table 2 all the detected mutations are listed.

Table 2. The detected mutations in this study are listed in the order of frequency of occurrence in the analyzed group

No.	Mutation	% (n/n)
1	IVS II-1 (G>A)	21.30 (86/404)
2	IVS I-1-110 (G>A)	17.80 (72/404)
3	cd 36/37 - T	16.00 (65/404)
4	IVSI-5 (G>C)	6.90 (28/404)
5	cd 5 -CT	5.50 (22/404)
6	IVSI-6 (T>C)	4.25 (15/404)
7	IVS I-1 (G>A)	3.25 (13/404)
8	cd39 (C>T)	3.00 (12/404)
9	cd 8+G	2.50 (10/404)
10	IVSI- del 17 nt.	2.00 (8/404)
11	IVS-II-848 (C>A)	2.00 (8/404)
12	IVSII-745 (T>C)	2.00 (8/404)
13	Cd 44-C	1.50 (6/404)
14	-28 (A>C)	1.00 (4/404)
15	-88 (C>A)	1.00 (4/404)
16	Cd 8 -AA	1.00 (4/404)
17	cd 82/83 –G	0.75 (3/404)
18	Cd 22/23/24 - AAGTTGG	0.75 (3/404)
19	IVSI-130 (G>C)	0.50 (2/404)
20	Cd 88 +T	0.50 (2/404)
21	cd 54 –T	0.50 (2/404)
22	5UTR+20 (C>T)	0.50 (2/404)
23	cd6 (A>T)	0.50 (2/404)
24	IVSI del 24 nt.	0.25 (1/404)
25	IVSI-2 (T>G)	0.25 (1/404)
26	cd 6 –A	0.25 (1/404)
27	cd 30	0.25 (1/404)
28	Cd 31 -C	0.25 (1/404)
29	Cd 50 (A>C)	0.25 (1/404)
30	Cd 15 (G>A)	0.25 (1/404)
32	No mutation has been found	3.20 (14/404)

DISCUSSION

We could detect a wide spectrum of mutations in the beta-globin gene from beta-thalassemia diagnosed patients. From 30 detected mutations, 12 mutations affected appropriate splice sites between introns 1 and 2 and between introns 2 and 3. Nine mutations caused deletion (1, 2 or 3 nucleotides) and 2 single nucleotide insertional mutations led to premature beta-globin chain that in the homozygote mode may initiate severe form of beta-thalassemia. The other detected mutations were nonsense

mutations and nucleotide changes in the promoter region of the beta-globin gene.

From the molecular point of view, beta-thalassemia is a disease with restricted mutations (4 to 10 mutations) in the populations (14). However, because of the composed and multi-ethnic population in Iran, especially in southwest Iran, the distribution of mutations in Iran's ethnic groups is very different (7,15). The detection of 30 mutations with broad scale of frequency confirms the mentioned hypothesis. On the other hand, we must consider that just 3 of the 30 detected mutations in this work, represented more than 50% of the total mutations and therefore belong to the common nucleotide changes within the beta-globin gene in Khuzestan province (Southwest Iran).

The heterogeneity of mutations may be explained with the closeness of Khuzestan province with many Arab countries in the Persian Gulf and the frequent traveling between the nations in this region. For example, the most frequent mutation in this study, the IVSII-1, is with 21 percent the common mutation in Kuwait, too (16). The mentioned mutation is observed in all other Arab countries as well, but in Algeria and Tunisia, it is the most common mutation (16). In other reports from Iran, IVSII-1 mutation (37%) was the most common mutation (17). Recently, there are reports that the six mutations: IVSI-110, IVSII-1, IVSI-1, IVSI-5, cd36/37 (delT) and IVSI-25 bp with 50% are the common mutations in southwest of Iran (13). The second common mutation in this study was the IVSI-110 with 17.8%, being the frequent mutation in the west and southwest of Iran. The mentioned mutation has originated from Mediterranean and its frequency seems to increase from east to west of Iran (14). The IVSI-110 has a wide distribution in eastern Mediterranean and Arabic countries with a frequency of 12-38% (16). The third common mutation in the present study is Cd36-37 [-T], which was not reported in most Arabic countries, but possesses high prevalence in Saudi Arabia (15). It seems also that Khuzestan province holds a wide spectrum of mutations in the HBB gene.

Finally, we could not find any mutation in 7 patients that were diagnosed with severe beta-thalassemia. We recommend searching the LCR (Locus Cluster Region) of the globin genes for putative mutations in these individuals. As has been reported previously, the LCR region or even the delta-globin gene can harbor mutations which cause beta-thalassemia major in particular cases as very rare events (18).

CONCLUSION

We conclude that our data will be very important as serving a basis for further prenatal and postnatal molecular diagnosis, at least in the province of Khuzestan.

REFERENCES

1. Bain BJ. Haemoglobinopathy diagnosis. 1st ed. London, UK: Blackwell; 2001.p.1083-1084
2. Schrier SL. Pathobiology of thalassemic erythrocytes. ***Curr Opin Hematol*** 1997; 4(2): 75-78.
3. Benito A, Villegas A, Perez-Cano R, Bernal R. Beta thalassemia in south-western Spain: high frequency of IVS I-1 mutation. ***Br J Haematol*** 1996; 92(2): 336-338.
4. Weatherall DJ, Clegg JB. The thalassaemia syndromes. 4th ed. Oxford, UK: Blackwell; 2001.p.154-162.
5. Olivieri NF. The ß thalassemias. ***New Engl J Med*** 1999; 341: 99-109.
6. Basalic R, Gavrila L. Application of molecular diagnosis technologies in Beta-thalassemia: enzymatically amplified DNA and restriction site analysis. ***Roum Biotechnol Lett*** 2001; 6(1): 63-70.
7. Mahboudi F, Zeinali S, Merat A, Delmaghani S, Mostafavipour K, Moghadam Z, et al. The molecular basis of beta thalassemia mutations in Fars province, Iran. ***Iran J Med Sci*** 1996; 21(3): 99-104.

8. Bottardi S, Aumont A, Grosveld F, Milot E. Developmental stage-specific epigenetic control of human beta globin gene expression is potentiated in hematopoietic progenitor cells prior to their transcriptional activation. ***Blood*** 2003; 102(12): 3989-3997.
9. Karimi M, Alavian Ghavanini A, Kadivar MR. Regional mapping of the gene frequency of beta thalassemia in Fars province, Iran during 1997-1998. ***Iran J Med Sci*** 2000; 25(1): 134-137.
10. Hardison RC, Chui DH, Giardine B, Riemer C, Patrinos G, Anagnou N, et al. Hb Var: A relational database of human hemoglobin variants and thalassemia mutations at the globin gene server. ***Hum Mutat*** 2002; 19(3): 225–233.
11. Habibzadeh F, Yadollahie M, Merat A, Haghshenas M. Thalassemia in Iran: an overview. ***Arch Iranian Med*** 1998; 1(1): 27-34.
12. Old JM. DNA based diagnosis of the hemoglobin disorders. In: Steinberg MH, Forget BG, Higgs DR, Nagel RL, (eds). Disorders of hemoglobin: genetics, pathophysiology and clinical management. 2nd ed Cambridge: Cambridge University Press; 2001.p.941-957.
13. Najmabadi H, Karimi Nejad R, Sahebjam S, Pourfarzad F, Teimourian SH, Sahebjam F, et al. The beta thalassemia mutation spectrum in the Iranian population. ***Hemoglobin*** 2001; 25(3): 285-296.
14. Karimi M, Yarmohammadi H, Farjadian S, Zeinali S, Mohgaddam Z, Cappellini MD, et al. Beta thalassemia intermedia from southern Iran: IVSII-1(G>A) is the prevalent thalassemia intermedia allele. ***Hemoglobin*** 2002; 26(2): 147-154.
15. Zahed L. The spectrum of beta thalassemia mutations in the Arab populations. ***J Biomed Biotech*** 2001; 1(3): 129-132.
16. El-Hazmi MAF, Wasy AS. The heterogeneity of molecular basis of beta thalassemia among Arabs. ***Bahrain Med Bull*** 1998; 20(1): 14-17.
17. Grosveld F, Dillon N, Higgs D. The regulation of human globin gene expression. ***Clin Haematol*** 1993; 6(1): 31-55.
18. Najmabadi H. Teimourian Sh. Khatibi T, Neishabury M, Pourfarzad F, Jalil Nejad S, et al. Amplification refractory mutation system (ARMS) and reverse hybridization in the detection of beta Thalassemia mutations. ***Arch Iranian Med*** 2001; 4(4): 165-170.