The Spectrum of β -thalassemia Mutations in Isfahan Province of Iran

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Abstract

Background: β-thalassemia is a common autosomal recessive disorder resulting from over 200 different mutations of beta globin genes. The aim of the present study was to identify the distribution and frequency of the most common β-thalassemia mutations among the population of Isfahan Province in central Iran.
Methods: The data presented here were derived from a total of 114 β-thalassemia chromosomes of 18 affected patients and 78 unrelated carriers identified in our screening program. Furthermore, 23 pregnant women were analyzed among couples with a PND request for β-thalassemia. Allele identification was carried out using routine Reverse Dot Blot, ARMS, and genomic sequencing.
Results: The most common mutation, IVS-II-I, followed by FSC-36-37, IVS-I-5, FSC-8-9, IVS-I-110, IVS-I,3'-end; -25bp, IVS-II-745, FSC-8, Cd-39, FSC-22-24, IVS-I-1, Cd-44, IVSII-2,3 (+11/-2), IVS-I-6, and FSC-16, respectively. The present study not only provides a guide for distribution and frequency of both recurrent and uncommon mutations, but also for the first time, reports a rare β-thalassemia mutation, IVSII-2, 3 (+11/-2), in the Isfahan province of Iran.
Conclusion: The information presented here could greatly facilitate screening for β-thalassemia and prenatal diagnosis in the province of Isfahan.

Key words: *β -thalassemia, β-globin mutation, Reverse dot blot, Isfahan, Iran.*

Introduction

β-thalassemia is a common autosomal recessive disorder resulting from over 200 different mutations of β-globin gene. It results mainly from mutations either decreasing ($β^+$) or eliminating ($β°$) the expression of β-globin gene (1). Furthermore β-thalassemia is the most common inherited blood disorder in Iran. An estimated of three million carriers with an extensive spectrum of mutations live in Iran (2). Previous studies have shown that the mutational basis of β-thalassemia in Iran is heterogeneous, and that more than 60 different mutations are prevalent across different geographic regions (2-11). With regards to the prevalence of the disease itself, β-thalassemia is extensively investigated among the general Iranian population, and particularly in the provinces of Isfahan.

Therefore the knowledge of the whole spectrum of mutations in the region would be regarded as a pre-requisite for defining a specific policy for carrier screening, genetic counseling, and prenatal diagnosis.
The aims of this study were to identify the detailed distribution and frequency of the most common and rare β-thalassemia mutations in the population of Central Iran.

Materials and Methods

A total of 96 blood samples taken from unrelated β-thalassemia major patients (n=18) and carriers (n=78) from the province of Isfahan were studied (114 β-thalassemia chromosomes). Besides, the β-thalassemia patients and carriers, a total of 93 samples including 23 chorion villus

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sampling (CVS) of the pregnant women and 70 unrelated healthy individuals, as negative control were tested.

Analysis of prenatal diagnoses (PNDs) was done on the 23 pregnant women among the couples who had requested PND for diagnosis of β-thalassemia. It should be noted that patients investigated in this study were unrelated and different from that studied earlier (12, 13). An informed consent was obtained in all cases. Peripheral blood samples of the patients, the affected child, and any normal child (negative controls) were collected in EDTA for delineation of the β-thalassemia mutations. Genomic DNA was extracted by standard salting out extraction method as described previously (14, 15). Briefly, the red blood cells were lysed and the DNA was purified from the white blood cells by ethanol precipitation. For prenatal diagnosis, chorionic villus sampling during week 10 to 15 of gestation was used for identification of mutations (16). The samples were tested for the most common β-thalassemia mutations among the Iranian population (3-10) by using reverse dot blot (RDB) (17). Briefly, two oligonucleotides were used for each mutation, one containing the wild type sequence and the other containing the mutated sequence. The β globin regions of exons 1 and 2 were amplified from extracted DNA in a single PCR reaction using two biotin-labled primers and hybridized to strips containing the wild type and mutant-specific probes for eighteen mutations. The results of RDB were verified by amplification refractory mutation system (ARMS) (18). Briefly, two oligo-nucleotide primers with a complementary to the sequence of a specific mutation and normal sequence, coupled with a common primer is used in two PCR reactions. The presence of an amplified product in the first reaction indicated the mutation while its absence suggests presence of the normal DNA sequence at that specific site. The remaining mutations were detected by sequencing of amplified β-globin genes and analyzing the results of automated fluorescence detection based DNA sequencing (ABI PRISM ™ 310, PE Biosystems).

Results

The prevalence of β-thalassemia mutations among the province of Isfahan in Central Iran is shown in Table 1. In this study we reported the presence of 15 β-thalassemia mutations (98%), and 3 unknown mutations (2.0%) in 192 unrelated chromosomes (96 blood samples). The most common mutation, IVS-II-1 (G-A) (27.9%), is followed, in order of frequency, by FSC-36-37(-T) (19.7%), IVS-I-5(G-C) (16.3%), FSC-8-9(+G) (6.8%), IVS-I-110(G-A) (4.8%), IVS-I,3'-end (-25bp) (4.8%), IVS-II-745(C-G) (4.8%), FSC-8 (-AA) (3.4%), C39(C-T) (2.7%), FSC-22-24 (-AAGTTGG)(1.4%), IVS-I-1(G-A) (1.4%), FSC-44(-C) (1.4%), IVSII-2,3 (+11/-2; nts ACGTTCT-CTGA were inserted and and nts 497-498 were deleted) (1.4%), IVS-I-6(T-C) (0.9%), FSC-16(-C) (0.9%). The first three mutations account for about 64% of all of the mutations. In the molecular analysis of prenatal diagnosis, 26.1% (6/23) of the fetuses were found to be normal, 47.8% (11/23) were identified as carriers, and 26.1% (6/23) were affected with β-thalassemia. The results of RDB were verified either with ARMS-PCR or sequencing (Figures 1 and 2) and ARMS (data not shown).
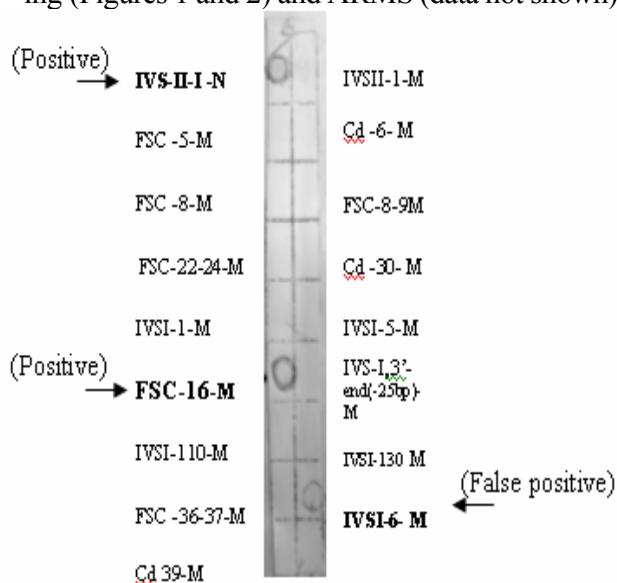

Fig. 1: A representative photograph of reverse dot blot showing the affected β̄-thalassemia minor with FSC-16 mutation (positive blot), an IVSII-1-normal allele (positive blot), and a false positive blot for IVSI-6 (high concentration of primer).
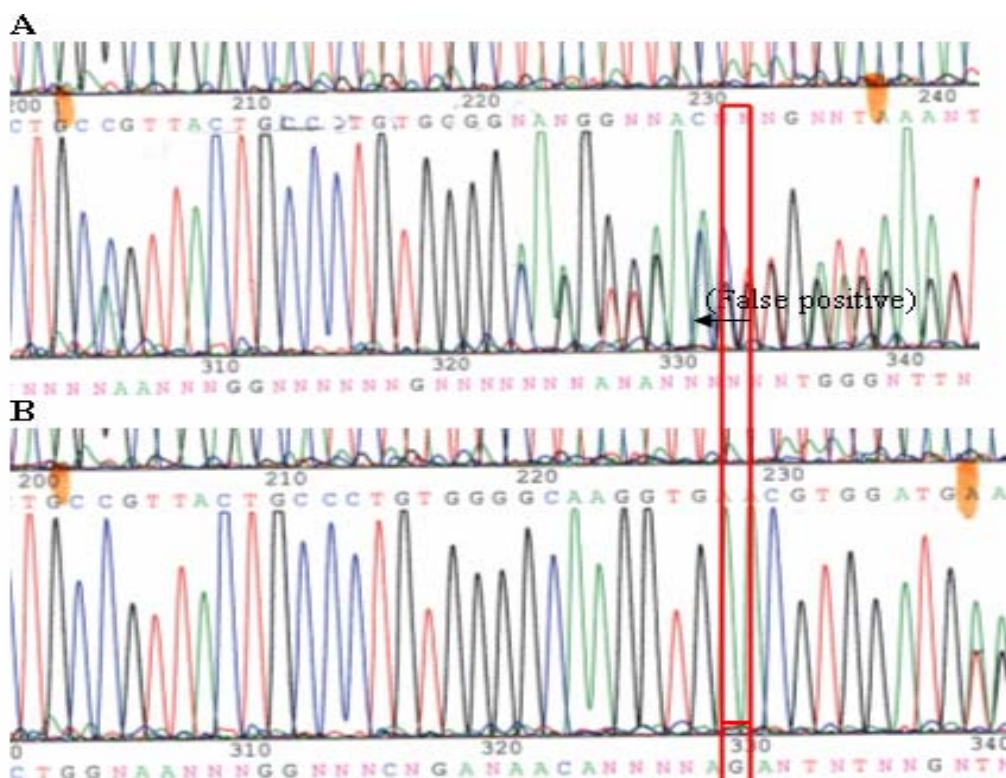
Fig. 2: Electropherogram sequences of the β globin gene with site of recessively inherited mutation, A) showing the FSC-16 (-C) frame shift mutation in cd 16 B) sequence of normal hemoglobin B (HBB) at the same site

Table 1: Frequency and regional distribution of β-thalassemia mutations in the province of Esfahan

Mutations	Type	Present study n(%)	Previous studies n(%) Ref. 12	Ref. 13	Central3 (%)	Distribution
IVSII-1 (G-A)	β	41 (27.9)	25 (29)	-	(28.0)	Mediterranean, US Blacks
FSC-36-37 (-T)	β	29 (19.7)	-	36 (17.5)	(4.3)	Kurd, Iranian
IVS-I-5 (G-C)	β	24 (16.3)	3 (3.4)	-	(9.3)	Asian Indian, SE Asian, Melanesian
FSC-8-9 (+G)	β	10 (6.8)	22 (26)	-	(11.7)	Asian Indian, Japanese
IVS-I-110 (G-A)	β	7 (4.8)	7 (8)	-	(5.1)	Mediterranean
IVS-I,3'end (-25bp)	β	7 (4.8)	-	4 (2)	(2.3)	Asian Indian, UAE
IVS-II-745 (C-G)	β	7 (4.8)	-	-	(2.3)	Mediterranean
FSC-8 (-AA)	β	5 (3.4)	-	17 (8.3)	(6.6)	Mediterranean
Cd-39 (C-T)	β	4 (2.7)	-	8 (3.9)	(1.9)	Mediterranean
FSC-22-24 (-AAGTTGG)	β	2 (1.4)	-	-	(0.8)	Turkish
IVS-I-1 (G-A)	β	2 (1.4)	6 (7)	-	(4.7)	Mediterranean
FSC-44 (-C)	β	2 (1.4)	-	9 (4.4)	(3.9)	Kurdish
IVSII-2,3 (+11/-2)	β	2 (1.4)	-	-	-	Iranian
IVS-I-6 (T-C)	β	1 (0.9)	-	6 (2.9)	(1.9)	Mediterranean
FSC-16 (-C)	β	1 (0.9)	-	2 (1)	(0.0)	Asian Indian
Cd-30 (G-C)	β	0 (0.0)	7 (8)	-	(1.9)	Mediterranean
FSC-5 (-CT)	β	0 (0.0)	-	-	(1.2)	Mediterranean
Unknown		3 (2.0)	16 (18.6)	123 (60)	(13.2)	
Total		147	86	205	257	

N: number, more information regarding these mutants can be obtained by HbVar database for hemoglobin variants and thalassemia mutations (19, 20)

Discussion

β-thalassemia is one of the most common genetic disorders in Iran (21). Despite the efforts to develop a treatment based on gene therapy or bone marrow transplantation, prenatal diagnosis followed by termination of the affected fetus still remains the best form of disease management. Several studies on the frequency of mutations associated with β-thalassemia in Iran have been carried out previously (2-11). Among the previous studies, only two references (12, 13) dealt adequately with the province of Isfahan. The present study was conducted in order to provide a more detailed analysis for this region. IVSII-1 is followed, in the order of frequency, by FSC-36-37, IVS-I-5, FSC-8-9, IVS-I-110, IVS-I,3'-end;-25bp, IVS-II-745, FSC-8,Cd-39, FSC-22-24, IVS-I-1, Cd-44, IVSII-2,3 (+11/-2), IVS-I-6, and FSC-16. Similar to the previous studies (all central provinces including Isfahan), we found IVSII-1 to be the most frequent mutation in the Isfahan province (12, 3). We also revealed the existence of three new mutations, IVS-II-745, FSC-22-24 and IVSII-2,3 (+11/-2). One of the rare mutations identified in this study, IVS-II-2,3 (+11/-2), has been reported earlier in an Iranian family with unknown regional origin (22,4) and recently in two families in the Northern provinces of Iran (6). A relative high frequency of a few mutations (IVS-II-1, FSC-36-37 and IVS-I-5) in Isfahan province, which account for about 64% of all of the known mutations, is identified in our study. This frequency is significantly higher than the rate reported previously for Isfahan and other Central Provinces of Iran (32.4% in reference 12 and 41.6% in reference 3). We also found some other notable differences in the regional frequency of some mutations, e.g. IVSI-5, FCS-8-9, compared with the previous studies in Isfahan province, about 3.0% and 26%, respectively (12, 13) (Table 1). However, the frequency of IVSI-5 and FCS-8-9 mutations in our study (about 16% and 7%, respectively) is similar to a previously reported study for the Central provinces of Iran, about 9% and 12%,

respectively (Table1). This is probably due to the bigger scope in the present study. Interestingly, the number of cases studied here is within the range of previous studies in Isfahan province (12, 13). However, the spectrum and frequency of total mutations analyzed here is broader than both of the previous analysis and it is about the same in previous study in central (with just an exception in FCS-36-37, IVSI-5 and FCS-8-9 mutations). There is a significant decrease in the frequency rate for unknown mutations in our study (2%). in comparison to the previous studies reported for Isfahan and Central provinces of Iran (18.6%, 60%, and 13%) (Table 1). ARMS, RDB, and sequencing techniques used in this study allowed a precise identification of mutations in parents, making PND straightforward, and less time consuming with almost 100% accuracy in pregnancies. Therefore, the present study provides a distribution and frequency guide for both recurrent and uncommon mutations in the province of Isfahan. In addition, for the first time, we report a rare β-thalassemia mutation, IVSII-2,3 (+11/-2), for this province. This could be highly useful for carrier detection and prenatal diagnosis. Therefore, we recommend that prenatal diagnosis in all high-risk families should be taken as an essential test that could help with a significant reduction in the burden of β-thalassemia in these regions.

Our efforts reported here could provide an important step for completing a catalogue of β-thalassemia mutations among the population residing in the province of Isfahan, which is an important central region of Iran.

Acknowledgements

The authors declare that they have no Conflict of Interests.

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