

Detection of Rare and Unknown Mutations in β - thalassaemia Traits in Iran

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Abstract

Beta-thalassaemia, by its high frequency and heterogeneity, constitutes a real problem of health in Iran. About 13 beta globin mutations encompass 70-90% of mutations spectrum in Iran, the rest are rare or unknown. In this study six mutations of the codon IVS1-130(G-C), Fr16 (-C), codon35 (-C), fr23/24(-G), codon8 (+G) and codon 20 (GTG-GAG) were recognized and added to spectrum of beta globin mutations in Iran, Using ARMS/PCR and DNA sequencing. Three latter cases are reported for first time.

Keywords: *Beta-thalassaemia, Unknown mutations, Iran*

Introduction

β -thalassaemia is a heterogeneous group of autosomal recessive disorders that results in β -chain hemoglobin. Due to climatic, geographic and ecological conditions, β -thalassaemia is one of the most frequent hemoglobinopathies and single gene disorders in Iran (1, 2, 3, 4, 5, 6, 7, 8). It is the first priority for the Iranian Ministry of Health concern with genetic disorders. Prevention of β -thalassaemia implies knowledge of molecular spectrum occurring in the population at risk. This knowledge is necessary when a prevention protocol is applied to multiethnic population (6, 7, 8). So the detection of all mutations of β -thalassaemia in each population is the major goal in prevention, and is especially helpful for prenatal diagnosis. Up to now more than 200 different mutations in the β -globin gene have been reported (3). About 13 mutations encompass 70 to 90 percent of mutations spectrum in Iran (1, 2, 6). These mutations are called common β -globin mutation in Iran; the others (10-30%) are rare or unknown.

Materials and Methods

25 patients and carriers of β -thalassaemia who had been referred to clinics for detection of their mutation, but their mutation had not been detected, were selected. DNA was extracted from the collected peripheral blood by proteinase K and Isopropanol method (9). The concentration of extracted DNA was determined by spectrophotometry. ARMS/PCR (10) was done by primers codon 15 N, codon 15M, Ivs1-130m/common D, control A, control B. PCR reaction mixture was tris (pH=8/3) 10mM, KCL 50mM dntp mixture 200mM, 10x buffer containig 50Mm Mg, Taq DNA polymerase 0/5 unit DNA 50ng /ml, 10pmol primers. Time and temperature were, initiation denaturing 94°C for 2 minutes and one cycle, denaturing 94°C, annealing

57°C and extension 72°C for one minute and 30 cycles. The ARMS/PCR products were electrophoresed on 2% Agars gel. They were stained with etidium bromid. To Screen PCR products on acryl amid gel, RCR reaction mixture (11) was the same for ARMS/PCR except primers. Primers were common C and IVS1 – I normal (Table1). PCR products were electrophoresed on 8 acryl – amid gel. They were stained with AGNO3. The PCR products in which there were shift on acrylamid gel, amplified by ARMS / PCR for mutation of framshift 16 using of primers of Common C, Fr 16N or Common C, Framshift 16M. Using primers of Common D and Codon 8 normal, Common, Ivs1-1 normal two regions on beta globin gene were amplified by PCR. PCR products were sequenced by automated DNA sequencer based on Sanger method (12).

Results

ARMS/PCR was used to screen mutations of Ivs1-130 (G→C) and codon 15 (TGG → TGA). Four cases of Ivs1-130 were detected. They were carriers for β -thalassaemia. No cases were detected for mutation of codon 15. Figure1 shows the results of ARMS/ PCR. Using two primers of Ivs1-1 N and common C, a 272bp fragment from nucleotid of -18 in region promoter to end of exon one was amplified. Using two primers of codon 8 N and common D, a 490 bp fragment from nucleotid of 24 of exon 1 to nucleotid of 79 of second intron was amplified. It is noted that the major of beta globin mutaions are located in these part of gene. Using of two primers of Ivs1-1 and common C with usual PCR, a shift was seen and after it ARMS/PCR proved which mutation of Fr16 is. Two cases of Fr16 were detected (Figures 1and 2). Using DNA sequencing, four mutations of codon 8(+G), codon 35(-C), Fr23/24(-G), codon20 (GTC→GAC) were detected (Fig 3).

Table 1: The sequences of primers

Primers	Sequences
Common C	5 ACATCACCCCTGTGGAGCCAC3
Common D	5GAGTCAAGGTCGAGAGATGCAAGGA3
IVSI-I Normal	5TTAAACCTGTCTTGTAACCTTGATACGAAC3
Codon8 Normal	5ACACATCGTTGCACCTGACTCCTGAGCAGA3
IVSI-130 Normal	5CTGCCTATTGGTGTATTTTCCCACCCTTAG3
IVSI-130 Mutant	5CTGCCTATTGGTGTATTTTCCCACCCTTAC3
Fr 16 Normal	5TCACCACCAACTTCATCCACGTTACGTTTC3
Fr 16 Mutant	5TCACCACCAACTTCATCCACGTTACGTTG3
Control A	5CAATGTATCATGCCTCTTTGCACC3
Control B	GAGTCAAGGCTGAGAAGATGCAGG3

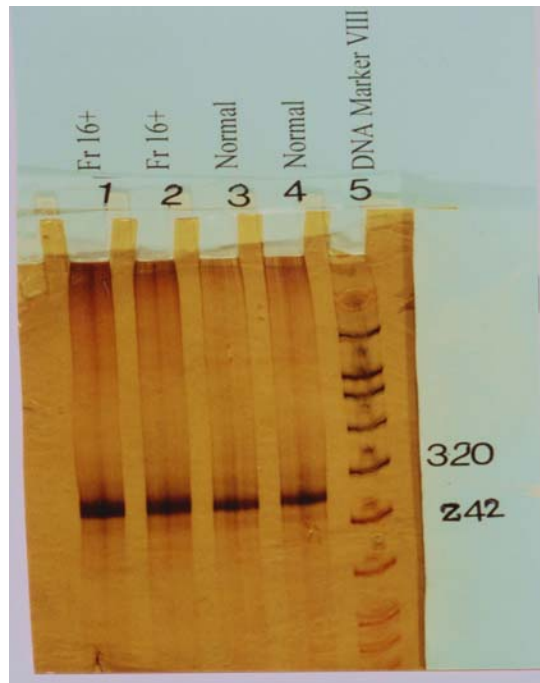


Fig. 1: Electrophoresis of PCR products on 8% acrylamide gel which were amplified by common C and IVSI-1 normal. Line 1 and 2 are positive for fr 16 but line 4 is normal. Line 5 for VIII DNA size marker. There are shift in lines 1 and 2.

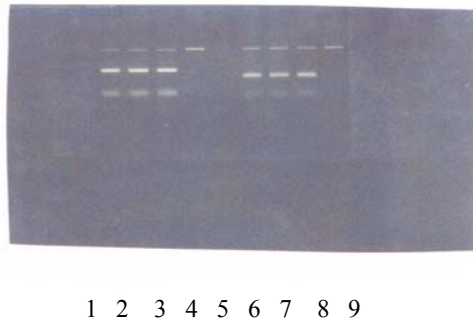


Fig. 2: Electrophoresis of PCR products on 2% agarose gel which are amplified by allele specific primers of normal and mutations of *ivs1-130* and *Fr 16*. Lines 1 to 4 are for *ivs1-130*. Line 1 and 2 are for one person and line 3 and 4 for others. Line 1 and 3 are for normal alleles and line 2 and 4 are for mutant alleles. Both of line 1, 2 and 3 have 301bp but line 4 has not. So the person on the left (line 1, 2) is positive for *Ivs1-130* and is heterozygote. The person on the right (lines 3, 4) is normal for this mutation. Lines 6-9 are for *Fr 16*. Left person (lines 6, 7) is positive for *Fr 16* but right person (lines 8, 9) is not. Bands of 861bp are products of control primers of A and B, which are seen above all lines

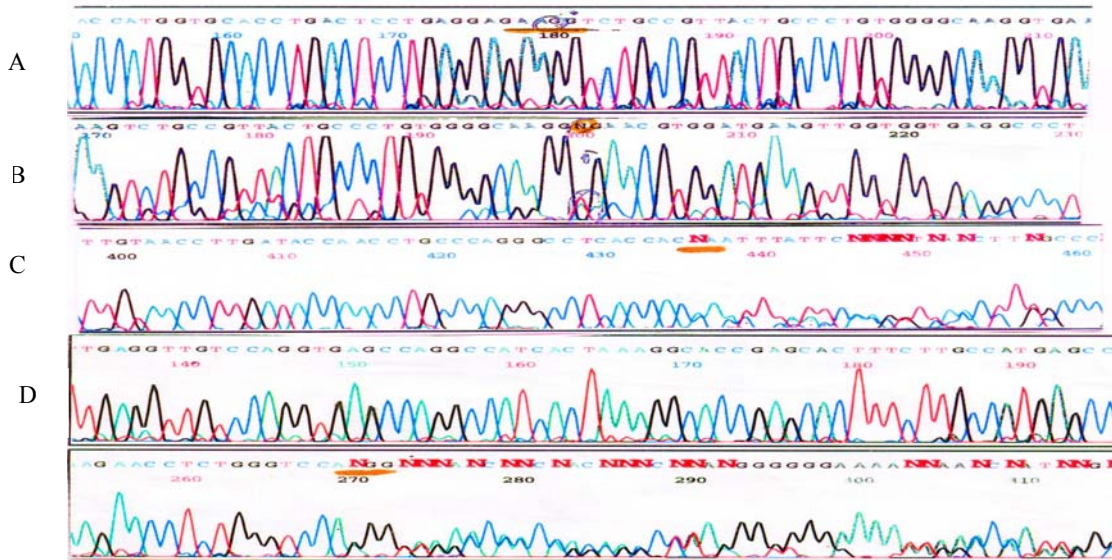


Fig. 3 (from top to bottom): Sequence obtained from automated DNA sequencing which are sequenced by common C and common D primers:

A- There is an insertion of G in codon 8. B- There is a transition of T→A in codon 20 which is caused valin codon converted to glutamic acid. There are two peaks in this site. One of them belongs to A and the other to T. So this person is heterozygote for this mutation. C- Occurrence of mutation caused change and overlap of nucleotide in this case. according to peaks there are two conditions CACCAACTT and CACAACCTT, normal and mutation alleles respectively, i.e C is deleted. Because primer of common D (reverse primer) has been used, in fact G is deleted. Position of this mutation is between codon 23/24 (frame shift 23/24). D- Occurrence of frame shift mutation caused change and overlap of nucleotide in upstream of codon 35. In this case according to peaks, There are two conditions, CAAGGTA and CAAGGGTA (mutant and normal allele respectively), i.e G is deleted because primer of common D (reverse primer) has been used for DNA sequencing, in fact C is deleted. The person is heterozygote for this mutation.

Discussion

β -thalassemia disease has high heterogeneity in molecular level. Up to now, more than 200 different mutations in β globin gene have been reported (3), and in each population and race only a little of them cause disease (1, 2, 3, 5, 6, 9). Based on the study which had been initiated since 1991 (1, 2, 3, 4, 6, 7, 8) the majority of common mutations have been recognized in Iran. The origins of these mutations are Mediterranean, Middle-East and Asian-Indian (3, 4, 13, 14). In all of these studies, a number of mutations always have been unknown. The rate of unknown mutations was about 3 to 10 percent depend on the country. In this study 25 patients and carriers of unknown β -thalassemia mutations from different parts of Iran were selected randomly. The spectrum mutations of β -thalassemia are similar to neighboring countries (3, 13, 14). So for this reason, we selected Ivs1-130 (G→C), codon 15, and Fr16 (-C) for screening. Ivs1-130 had been reported in Turkey (14), this mutation causes change in the splice junction site, so affects in splicing process. Fr16 (-C) had been reported in 1984 in Asian-Indian population, and also in Iran (15). This is a kind of frameshift mutation. Fr16 (-C) mutation was detected by electrophoresis of PCR production on 8 percent acrylamid gel compared to normal sample. This method is similar to SSCP but here double strand of DNA is electrophoresed. Using DNA sequencing, four kinds of mutations were detected. These were codon 35(-C), codon 8(+G), Fr23/24(-C) and codon 20 (GTG→GAG). All of these mutations are kind of frameshift except codon 20 (GTG→GAG). Codon 35(-C) was detected in 1989 in Malay population (16). Mutations of Fr23/24(-G), (+G), codon 8(+G) and codon 20 (GTG→GAG) are new mutations and have not reported up to now. It is noted that codon 8(+G) is different from the mutation of frameshift 8/9(+G) which is common in Iran. In the whole, the mutations of 10 cases (40 %) from 25 cases were detected. The others were not detected by these methods.

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