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## SHORT COMMUNICATION

## IDENTIFICATION OF THREE NOVEL MUTATIONS [−41 (A>C), codon 24 (−G), and IVS-I-109 (−T)], IN A STUDY OF β-THALASSEMIA ALLELES IN THE ISFAHAN REGION OF IRAN

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□   *β-Thalassemia (β-thal) is one of the most common autosomal recessive disorders in Iran, with more than 15,000 registered cases of thalassemia major in the country. Iran has a multiethnic society and knowledge of the mutation spectrum and regional distribution is an essential requirement for health planning and a prenatal diagnosis program. We have determined the spectrum of mutations in patients from the Isfahan region of Iran. A study of 190 chromosomes revealed 24 different mutations, including three novel ones: −41 (A>C), IVS-I-109 (−T) and codon 24 (−G). The most common mutation was IVS-II-1 (G>A) (20.5%), followed by IVS-I-5 (G>C) (11%). The findings for the Isfahan region confirm the extremely heterogeneous nature of the molecular basis of β-thal in Iran. The results show that a strategy of using the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) for 14 of the most common mutations and DNA sequencing for the rare mutations can be used for prenatal diagnosis of β-thal in this region.*

**Keywords**  β-Thalassemia (β-thal), Novel mutations, Isfahan, Iran

Hemoglobin (Hb) disorders present a significant health complication in 71% of 229 countries worldwide and $\beta$-thalassemia ($\beta$-thal) is the most common single gene disorder in Iran (1). More than 200 different $\beta$-thal mutations have been identified worldwide and the mutations are regionally specific, with each population having a characteristic spectrum consisting of a smaller number of common mutations and a larger number of rare ones (2). The Iranian community is comprised of a complex mixture of different ethnic groups and therefore a high heterogeneity of $\beta$-thal mutations is expected, with much variation between different geographical regions.

A $\beta$-thal prevention program was launched in Iran in 1992, and after extensive efforts and consultations by the experts in the field, the clerical approval of induced abortion in cases diagnosed with $\beta$-thal major ($\beta$-TM) was issued in 1997 (3). The heterogeneity of the disease causing mutations across the country creates a serious challenge to the development of an appropriate mutation-based molecular diagnostic procedure, and thus, the spectrum of $\beta$-thal mutations has been studied in many different regions of Iran (4–11). However, no studies have been reported from the Isfahan Province located in the central region of Iran.

We report here the distribution and molecular spectrum of $\beta$-thal alleles in the Isfahan Province of Iran. DNA samples were prepared from peripheral blood samples taken from unrelated patients with either homozygous $\beta$-thal or $\beta$-thal trait, and a total of 190 chromosomes were analyzed for $\beta$-thal mutations. Samples were screened first by the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) for 16 known $\beta$-thal mutations previously reported in Iranian patients, and then, if none of these mutations were identified, each sample was subjected to DNA sequence analysis of the $\beta$-globin gene to look for rare or novel mutations. The ARMS-PCR analysis for 14 known mutations was carried out as previously described (12) and for two other reported Iranian mutations, codon 44 (–C) and −88 (C>A), a new set of ARMS primers were developed using the standard ARMS-PCR amplification conditions (12). The mutant ARMS-PCR primer sequence for the detection of the mutation −88 was 5′-TCA CTT AGA CCT CAC CCT GTG GAG CCA CGA-3′, and to detect the mutation <u>at</u> codon 44 the mutant ARMS-PCR primer sequence was 5′-TAC CCT TGG ACC CAG AGG TTC TTT GAG TGT-3′.

DNA analysis results revealed a total of 24 different $\beta$-thal mutations in the Isfahan population, including three novel ones (Table 1). Fourteen of the mutations in Table 1 were detected by ARMS-PCR, with a combined frequency of 78% of all alleles found. The other 12 alleles (totaling 22%) were diagnosed by DNA sequencing. The most frequent mutation was IVS-II-1 (G>A) (20.5%), followed by IVS-I-5 (G>C) (11%), IVS-I-1 (G>A) (10.1%), codon 8 (–AA) (9.0%) and codons 36/37 (–T) (7.9%). The remaining mutations occurred at lower frequencies, ranging from 5.8 to 1.1%. Twelve

**TABLE 1** Spectrum of β-Thalassemia Mutations in Isfahan Province, Iran

| Mutation | Number of alleles | Frequency (%) |
|---|---|---|
| −41 (A>C) | 1 | 0.5 |
| −32 (C>A) | 1 | 0.5 |
| −30 (T>A) 2 | 2 | 1.1 |
| −28 (A>C) | 2 | 1.1 |
| Codon 8 (−AA) | 17 | 9.0 |
| Codons 8/9 (+G) | 7 | 3.7 |
| Codon 16 (−C) | 2 | 1.1 |
| Codons 22/23/24 (−7 bp) | 7 | 3.7 |
| Codon 24 (−G) | 1 | 1.1 |
| IVS-I-1 (G>A) | 19 | 10.1 |
| IVS-I-2 (T>C) | 1 | 0.5 |
| IVS-I-5 (G>C) | 21 | 11.0 |
| IVS-I-6 (T>C) | 6 | 3.2 |
| IVS-I-109 (−T) | 5 | 2.7 |
| IVS-I-110 (G>A) | 11 | 5.8 |
| IVS-I (−25 bp) | 4 | 2.1 |
| Codons 36/37 (−T) | 15 | 7.9 |
| Codon 39 (C>T) | 8 | 4.2 |
| Codons 41/42 (−TCTT) | 2 | 1.1 |
| Codon 44 (−C) | 9 | 4.8 |
| Codon 47 (+A) | 1 | 0.5 |
| Codons 82/83 (−G) | 7 | 3.7 |
| IVS-II-1 (G>A) | 39 | 20.5 |
| IVS-II-745 (C>G) | 2 | 1.1 |
| Unknowns | 0 | 0.0 |
| Total chromosomes | 190 | 100.0 |

of the mutations had been observed before in Iranian patients in previous studies from other regions of Iran. Thus, this is the first report in the Iranian population of the remaining nine known mutations reported elsewhere. Two alleles for IVS-II-745 (C>G) were observed, both of which were linked *in cis* to a 5′UTR (5′ untranslated region) +20 (C>T) transition, as previously found for this allele in Iranian patients (8). Also in the rare mutation category were four mutations in the promoter region: −28 (A>C), −30 (T>A), −32 (C>A) and the novel mutation −41 (A>C). The patient with −28 was homozygous for this mutation and was severely affected.

The promoter region mutation −41 has not been reported before (Figure 1A). This novel mutation is located in a β-globin direct repeat element (βDRE). These elements are a highly conserved sequence of a 10 bp repeat that lies just upstream from the TATA box of the promoter region with a consensus sequence of 5′-AGGGCAG(G)AGC-3′. Using mutations that alter both copies of the direct-repeat motif, Stuve and Myers (13) demonstrated that this sequence is required for maximal transcription levels from the β-globin promoter in erythroid cells and plays a role in transcription of the β-globin gene during MEL cell differentiation. Therefore,
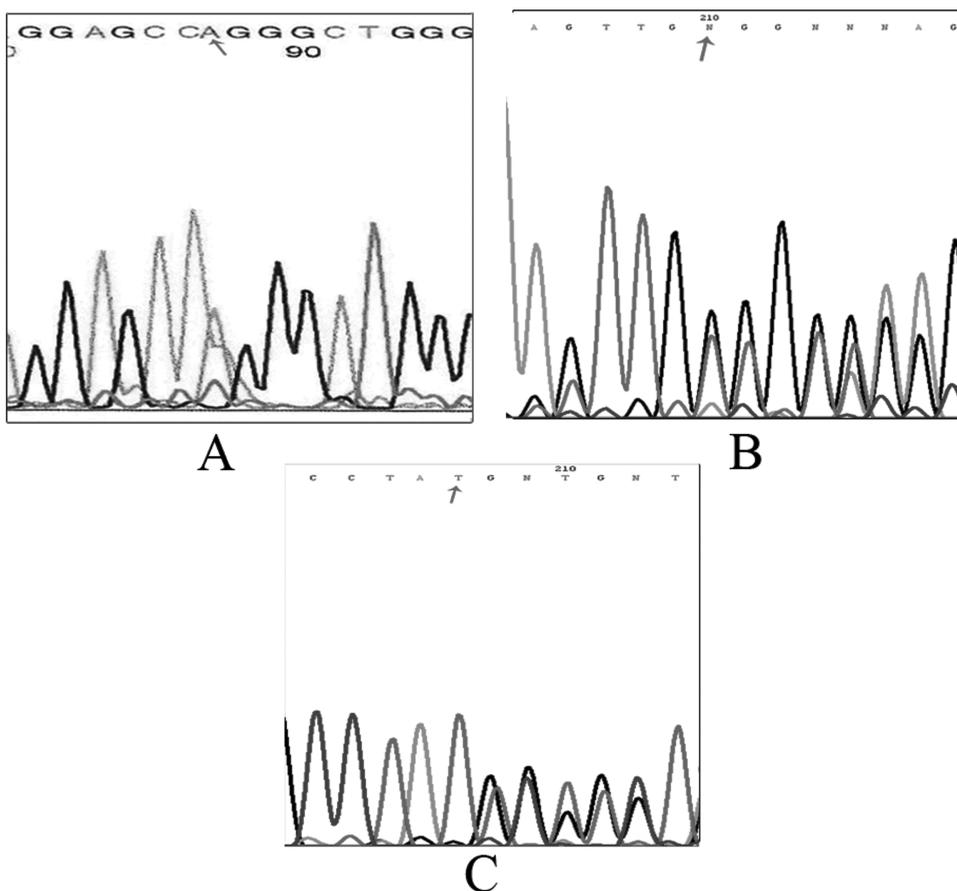
**FIGURE 1** Nucleotide sequence analyses showing three novel mutations: (A) −41 (A>C); (B) codon 24 (−G); (C) IVS-I-109 (−T).

the −41 mutation found in our study, being included in the conserved promoter direct repeat element, should count as a $\beta$-thal mutation.

The novel mutation, codon 24 (−G), was identified in one patient with $\beta$-TM who was doubly heterozygous for the IVS-II-1 mutation and the new mutation at codon 24 (Figure 1B). This deletion mutation in exon 1 is a frameshift mutation, leading to termination of globin synthesis at a new stop codon in exon 2 at codon 61 (TGA), and thus is a $\beta^0$-thal type mutation.

The third novel mutation was a single nucleotide deletion, IVS-I-109 (−T), located 22 nucleotides downstream from the acceptor splice site junction of IVS-I and exon 2 (Figure 1C). This mutation was found in three individuals with $\beta$-thal trait and in two patients with $\beta$-TM. The two affected patients were doubly heterozygous for this mutation and another known $\beta$-thal mutation, IVS-I-1 and IVS-I-2 (T>C). In the carriers, no other mutations

were found by sequencing, which could account for the phenotype. The mutation shortens the intervening sequence by one nucleotide, but does not create an alternative splice site like its neighboring mutation IVS-I-110 (G>A). However, there are some hints regarding the presence of important regulatory elements in the first intron of the β-globin gene affecting transcription and splicing. A substitution of T>C at position 108 has been reported in several patients, including in one Iranian case, with differing interpretations. In one case it was classified as a silent polymorphism (14), but in another patient it was interpreted as a novel β-thal mutation with the possibility of reducing splicing efficiency due to the activation of a cryptic branch site during splicing of pre-mRNA (15). In addition, taking into consideration the results of Alibert et al. (16) who have done an extensive in vitro study on pre-mRNA splicing, we can clearly claim the important role played by this mutation in development of a thalassemia phenotype. They concluded that during early events in formation of spliceosomes three functional branch points are used, one is the usual A residue at −37 and two U residues at −17 and −22 bp downstream of the 3′ splice junction of IVS-I and exon 2. Deletion of the −22 U residue in our patients with IVS-I-109 abolishes this functional branch point and consequently results in reduction of transcription and/or abnormal splicing.

This study of the spectrum of β-thal mutations found in the Isfahan region confirms the findings reported for other regions that demonstrate β-thal in Iran is highly heterogeneous. It also confirms the finding that distribution of mutations is not uniform throughout the country, due to the fact that the Iranian population represents a mixture of various ethnic groups. Isfahan Province is located in the central region of Iran and the spectrum of mutations is different to that reported before for patients from this region and other parts of the country. Our studies of patients from Isfahan Province bring the total number of reported Iranian β-thal mutations to 43. The identification of the spectrum of mutations in Isfahan Province will help improve the screening of couples at risk for β-thal and prenatal diagnosis in this region. The results show that a strategy of using ARMS-PCR for 16 common mutations and DNA sequencing for the rare mutations can be used for prenatal diagnosis of β-thal in this region.

**Declaration of Interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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