

## Beta-Thalassemia Haplotypes in Southwest of Iran

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### Abstract

**Background:** Thalassemia is a widespread disease affecting people across various ethnicities and regions. In comparison to previous studies conducted in different regions of Iran, such as those in Lorestan and Sistan-Baluchestan, this study highlights unique mutation patterns prevalent in the southwestern population, emphasizing the genetic heterogeneity in this region. The identification of common mutations of beta-thalassemia in various ethnic groups within the nation is regarded as a practical solution for thalassemia prevention and prenatal diagnosis.

**Materials and Methods:** In this retrospective observational study, the medical records of 545 patients with various types of beta-thalassemia (silent, minor, intermediate, and major), referred to the center at Baqaei 2 hospital over a 14-year period (2008–2022), were examined. The age range of patients spanned from a 2-month-old fetus to a 34-year-old individual. Their mutations and thalassemia types were determined and confirmed using molecular methods, including PCR-ARMS (polymerase chain reaction-amplification refractory mutation system) and sequencing. The results were analyzed using SPSS software.

**Results:** The study examined 545 patients and identified 81 types of mutations. The most frequent mutations observed were CD36-37(-T)/N, IVSII-1/N, and IVS1-110(G>A). The study also noted population heterogeneity, reflected in the wide range of mutations found in the region. Among the patients, 6 had the silent form of beta-thalassemia, 488 had the minor form (464 patients and 24 fetuses), 9 had the intermediate form (8 patients and 1 fetus), and 42 had the major form (26 fetuses and 16 adults).

**Conclusion:** The identification of prevalent beta-thalassemia mutations facilitates disease control and prevention programs and is crucial for the identification of various beta-thalassemia gene mutations. This should be re-evaluated periodically. Observing a wide range of beta-thalassemia genotypes in the southwestern region of Iran suggests gene flow; thus, identifying these genotypes is instrumental in preventing and controlling the disease.

**Keywords:** beta thalassemia, Mutation, Iran

### Introduction

Beta-thalassemia is one of the most prevalent autosomal recessive genetic disorders globally. This disease has a high prevalence in Mediterranean, Middle Eastern, Transcaucasian, Central Asian, Indian Subcontinent, and Far East populations and is relatively common in African populations. The highest incidence rates have been reported in Cyprus (14%), Sardinia (12%), and Southeast Asia.

The prevalence of thalassemia in these regions is likely due to selective pressure from *Plasmodium falciparum* malaria, as the distribution of beta-thalassemia corresponds closely with malaria-endemic areas. Notably, carriers of beta-thalassemia exhibit relative protection against *Plasmodium falciparum* invasion.

Beta-thalassemia results from the reduced or absent production of beta globin chains in the hemoglobin tetramer (Hb), which comprises two alpha globin chains and two beta globin chains ( $\alpha_2\beta_2$ ). There are three

forms of this type of thalassemia: beta-thalassemia carrier, thalassemia intermedia, and thalassemia major (1). The beta globin protein is produced through the expression of a structural gene located on the short arm of chromosome 11 (11p15.4) (2). Beta-thalassemia results from reduced or absent synthesis of the beta globin chains in the hemoglobin tetramer. Individuals in the beta-thalassemia carrier state, characterized by heterozygosity for beta-thalassemia, are clinically asymptomatic but display specific blood characteristics. Thalassemia major is a severe form of anemia requiring regular blood transfusions. Thalassemia intermedia encompass a heterogeneous group of thalassemia disorders with a severity ranging from asymptomatic carriers to those with severe, transfusion-dependent forms. To date, approximately 300 beta-thalassemia alleles have been identified. Unlike alpha-thalassemia, where deletions in the  $\alpha$ -globin gene cluster account for most mutations, the majority of beta-thalassemia cases are caused by mutations involving one or a limited number of nucleotides in the  $\beta$  gene or its flanking regions (3). The beta globin gene sequence contains approximately 1,600 base pairs, encoding 146 amino acids and comprising three exons separated by two introns. Over 95% of all beta-thalassemia mutations worldwide are point mutations in the beta globin gene, with a small percentage attributed to gene deletions. Point mutations include single nucleotide substitutions as well as the addition or deletion of a few nucleotides, affecting gene expression during transcription, RNA processing, or RNA translation. To date, more than 200 significant mutations in the beta globin gene have been identified as causes of the beta-thalassemia phenotype. Identifying beta globin gene mutations is crucial for specific diagnostic and

management programs, such as the prenatal diagnosis (PND) of beta-thalassemia (1).

Over the past two decades, various methods have been employed to identify mutations in the beta globin gene. Among these, Reverse Blot Dot Hybridization (RDB) has been used extensively to identify known mutations of the beta globin gene (2, 3). However, this technique covers only 17 types of common mutations, leaving rare and unknown mutations undetected (1). According to various studies conducted in Iran, more than 60 different types of mutations in the beta globin gene have been identified. Identifying each mutation in practical experiments can present significant challenges (4). The exon of the beta globin gene, along with its upstream regions, is among the most frequently mutated areas affecting beta-thalassemia. Various methods are available for detecting beta-thalassemia mutations. While direct sequencing is effective in identifying unknown mutations, its application can be problematic in developing countries due to high costs and longer processing times (1). Several mutation screening techniques can be used before sequencing, such as PCR-ARMS (PCR-amplification refractory mutation), which is widely used in detecting common and known mutations (5-8). However, the RDB (Reverse Dot Blot) strip detection technique, which relies on single nucleotide changes in the primers, can sometimes result in false positive or negative responses (1).

Mutations of the human beta globin gene have been extensively studied. In isolated and small geographical populations, one or more mutations tend to be common (5). Conversely, in regions with dynamic, ethnically, and genetically heterogeneous populations, a large number of rare and unknown mutations are present alongside common ones (4). Our research in the

southwest of Iran indicates that about 17 types of mutations in the beta globin gene are responsible for beta-thalassemia. In Iran as a whole, more than 60 types of mutations have been reported in this gene. This study examines the mutations reported between 2008 and 2022 in the country's southwestern region.

## Materials and Methods

### Data collection

This retrospective study reviewed patient records from the Thalassemia Department at Baqaei 2 Hospital. The records of all patients treated between 2008 and 2022 were evaluated. A checklist was used to collect data on the following variables: age, sex, type of thalassemia, and type of mutation.

### Molecular methods

The molecular techniques used included Reverse Dot Blot Hybridization (RDB), Restriction Fragment Length Polymorphism (RFLP), and sequencing. These methods were applied to analyze the samples. Finally, the data was analyzed using SPSS software. This study was approved by Jundishapur University of Ahvaz under project number TH-0109, with the ethics code IR.AJUMS.REC.1401.270.

## Statistical analysis

The statistical analysis was performed by STATA software to calculate the frequency and percent of each genetic type of beta-thalassemia.

## Results

### Demographic Data

Of the 545 patients, 488 had the minor form of beta-thalassemia (approximately 89.5%), 9 had the intermediate form (1.65%), and 42 had the major form (7.7%). Among these, 239 were female (43.85%), 225 were male (41.28%), and 81 were fetuses (14.86%). Ages ranged from 2 months to 34 years, with most cases falling within the pediatric age group (see Table I).

### Genomic Data

The most commonly observed mutations included CD36-37(-T)/N, IVSII-1/N, and IVS1-110(G>A). CD36-37(-T)/N was particularly prevalent in fetuses, accounting for 112 cases. The patients included in the study were examined for genotype using molecular methods and sequencing. The genotypes are shown in Table II. This study also examined silent mutations in beta-thalassemia; six patients had silent mutations, and nine patients had intermediate mutations, which are detailed in Table III. Beta-thalassemia minor gene mutations observed in 488 patients are presented in Table IV.

Table I: Basic characteristics of beta-thalassemia patients referred to the treatment center of Bagai 2 Hospital (2008-2022)

|     |        | silent    | minor        | intermediate | major       |
|-----|--------|-----------|--------------|--------------|-------------|
|     |        | N=6       | N=488        | N=9          | N=42        |
| Age |        |           | 26.66 (7.55) | 9 (2.83)     | 10.95(7.24) |
| SEX | Female | 3 (50.0%) | 239(48.7%)   | 5 (55.6%)    | 6(14.2%)    |
|     | Male   | 3 (50.0%) | 225 (46.2%)  | 3 (33.3%)    | 9 (21.5%)   |
|     | Fetus  | 0 (0.0%)  | 24 (5.1%)    | 1 (11.1%)    | 26 (64.3%)  |

Table II: Gene diversity in types of beta thalassemia referring to Begai Hospital Treatment Center 2 (2008-2022)

| Gene code                              | Type of beta thalassemia |       |              |       |       |
|----------------------------------------|--------------------------|-------|--------------|-------|-------|
|                                        | silent                   | minor | intermediate | major | Total |
| IVSI-1(G-A)/ Normal                    | 0                        | 12    | 0            | 0     | 12    |
| CD36-37(-T)/ Normal                    | 0                        | 112   | 0            | 0     | 112   |
| Initiation codon CD(T>C)/ Normal       | 0                        | 10    | 0            | 0     | 10    |
| IVS1-110(G>A)/ Normal                  | 0                        | 39    | 0            | 0     | 39    |
| 28/ Normal                             | 0                        | 13    | 0            | 0     | 13    |
| IVSII-1/ Normal                        | 0                        | 83    | 0            | 0     | 83    |
| 101(C-T)/IVSII-I(G-A)                  | 0                        | 0     | 1            | 0     | 1     |
| 25 del/Normal                          | 0                        | 8     | 0            | 0     | 8     |
| IVSI-6(T-C)/IVSI-6(T-C)                | 0                        | 0     | 4            | 0     | 4     |
| IVSI-5(G-C)/ Normal                    | 0                        | 16    | 0            | 0     | 16    |
| CD41/42[-TTCT]/ Normal                 | 0                        | 2     | 0            | 0     | 2     |
| CD36/37[-T]/CD36/37[-T]                | 0                        | 0     | 0            | 7     | 7     |
| Fr 8-9/C36-37                          | 0                        | 0     | 0            | 1     | 1     |
| IVSI-6(T-C)/ Normal                    | 0                        | 19    | 0            | 0     | 19    |
| CD44(-C)/ Normal                       | 0                        | 2     | 0            | 0     | 2     |
| CD88(-AA)/ Normal                      | 0                        | 17    | 0            | 0     | 17    |
| silian ( $\delta^\circ\beta$ )/ Normal | 0                        | 5     | 0            | 0     | 5     |
| CD30/ Normal                           | 0                        | 4     | 0            | 0     | 4     |
| IVSI.5(G>C)/ Normal                    | 0                        | 4     | 0            | 0     | 4     |
| CD22[7bp-del]/ Normal                  | 0                        | 1     | 0            | 0     | 1     |
| Fr8-9(+G)/ Normal                      | 0                        | 8     | 0            | 0     | 8     |
| CD77(CAC>GAC)/ Normal                  | 0                        | 2     | 0            | 0     | 2     |
| CD39/ Normal                           | 0                        | 18    | 0            | 0     | 18    |
| CD44/ Normal                           | 0                        | 11    | 0            | 0     | 11    |
| 101(C-T)/ Normal                       | 6                        | 0     | 0            | 0     | 6     |
| CD17(A>T)/ Normal                      | 0                        | 1     | 0            | 0     | 1     |
| CD67/ Normal                           | 0                        | 1     | 0            | 0     | 1     |
| IVSI-6(T>A)/IVSI-I 110(G>A)            | 0                        | 0     | 1            | 0     | 1     |
| CD57(A>T)/ Normal                      | 0                        | 1     | 0            | 0     | 1     |
| IVSII[G-C]/ Normal                     | 0                        | 1     | 0            | 0     | 1     |
| CD5(-CT)/ Normal                       | 0                        | 6     | 0            | 0     | 6     |
| CD15/ Normal                           | 0                        | 8     | 0            | 0     | 8     |
| IVSII-848/ Normal                      | 0                        | 4     | 0            | 0     | 4     |
| 5'UTR+20(C-T)&IVSII-745(C-G)-Cisform/  | 0                        | 5     | 0            | 0     | 5     |

|                                                        |          |            |          |           |            |
|--------------------------------------------------------|----------|------------|----------|-----------|------------|
| Normal                                                 |          |            |          |           |            |
| CD82-83(-G)/CD82-83(-G)                                | 0        | 0          | 0        | 1         | 1          |
| IVSI-25/ Normal                                        | 0        | 6          | 0        | 0         | 6          |
| IVSII-1/IVSII-1                                        | 0        | 0          | 0        | 6         | 6          |
| CD14/42(-CTTT)/ Normal                                 | 0        | 2          | 0        | 0         | 2          |
| IVSII-6/IVSII-6                                        | 0        | 0          | 1        | 0         | 1          |
| IVSII-1/CD36-37(-T)                                    | 0        | 0          | 0        | 1         | 1          |
| CD82/83(-G)/ Normal                                    | 0        | 6          | 0        | 0         | 6          |
| IVSII-745/ Normal                                      | 0        | 6          | 0        | 0         | 6          |
| IVSII-745/IVSII-745                                    | 0        | 0          | 0        | 2         | 2          |
| IVSI-10/ Normal                                        | 0        | 1          | 0        | 0         | 1          |
| CD39/CD36/37                                           | 0        | 0          | 0        | 1         | 1          |
| IVSII-110/CD39                                         | 0        | 0          | 0        | 1         | 1          |
| CD8-9/ Normal                                          | 0        | 1          | 0        | 0         | 1          |
| CD 8[-AA]/ Normal                                      | 0        | 7          | 0        | 0         | 7          |
| IVSI-110/IVSI-110                                      | 0        | 0          | 2        | 0         | 2          |
| CD83(-G)/ Normal                                       | 0        | 6          | 0        | 0         | 6          |
| IVSI-6/CD83(-G)                                        | 0        | 0          | 0        | 1         | 1          |
| IVSI-5/IVSI-5                                          | 0        | 0          | 0        | 2         | 2          |
| CD74/75(-C)/ Normal                                    | 0        | 1          | 0        | 0         | 1          |
| CD41/42/ Normal                                        | 0        | 1          | 0        | 0         | 1          |
| CD5[-CT]/CD36/37                                       | 0        | 0          | 0        | 1         | 1          |
| CD8/9/ Normal                                          | 0        | 13         | 0        | 0         | 13         |
| IVSII-5(G-C)/ Normal                                   | 0        | 2          | 0        | 0         | 2          |
| CD8/9/CD8/9                                            | 0        | 0          | 0        | 2         | 2          |
| CD20/ Normal                                           | 0        | 2          | 0        | 0         | 2          |
| CD36/37/CD20                                           | 0        | 0          | 0        | 1         | 1          |
| Hb Monroe (CD30 G>C)/ Normal                           | 0        | 2          | 0        | 0         | 2          |
| IVSII/ Normal                                          | 0        | 2          | 0        | 0         | 2          |
| IVSI-1/ Normal                                         | 0        | 5          | 0        | 0         | 5          |
| IVSII.1/IVSI.6                                         | 0        | 0          | 0        | 1         | 1          |
| IVSI-128/ Normal                                       | 0        | 1          | 0        | 0         | 1          |
| IVSI-1/IVSI-1                                          | 0        | 0          | 0        | 2         | 2          |
| 30[T>A]/CD5[-CT]                                       | 0        | 0          | 0        | 1         | 1          |
| CD81-82/ Normal                                        | 0        | 1          | 0        | 0         | 1          |
| IVSII-1/CD30                                           | 0        | 0          | 0        | 1         | 1          |
| 101(C-T) &[5'UTR+20(C-T)&IVSII-745(G-C)] in Cis format | 0        | 1          | 0        | 0         | 1          |
| 88[C>A]/IVSII.1                                        | 0        | 0          | 0        | 1         | 1          |
| 88(C>A)/CD15(G>A                                       | 0        | 0          | 0        | 3         | 3          |
| CD36-37(-T)/CD8(-AA)                                   | 0        | 0          | 0        | 1         | 1          |
| Hb Ern2/ Normal                                        | 0        | 2          | 0        | 0         | 2          |
| IVSII.1[G>A]/IVSII745[C>G]                             | 0        | 0          | 0        | 1         | 1          |
| IVSII-1/CD36                                           | 0        | 0          | 0        | 1         | 1          |
| CD59[G>A]/ Normal                                      | 0        | 1          | 0        | 0         | 1          |
| CD5/CD8(-AA)                                           | 0        | 0          | 0        | 1         | 1          |
| IVSII-110/ Normal                                      | 0        | 7          | 0        | 0         | 7          |
| Fr8-9/Fr8-9                                            | 0        | 0          | 0        | 1         | 1          |
| CD121(Hb-D)/IVSI-6                                     | 0        | 0          | 0        | 1         | 1          |
| <b>Total</b>                                           | <b>6</b> | <b>489</b> | <b>9</b> | <b>42</b> | <b>545</b> |

Table III: Gene diversity in types of silent and intermediate beta thalassemia referred to the treatment center of Bagai 2 Hospital (2008-2022).

| Gene code                   | Type of beta thalassemia |        |      |       |
|-----------------------------|--------------------------|--------|------|-------|
|                             | Silent                   | Female | Male | Fetus |
| 101(C-T)/ Normal            | 6                        | 3      | 3    | 0     |
| <b>Total</b>                | <b>6</b>                 |        |      |       |
| Gene code                   | Type of beta thalassemia |        |      |       |
|                             | intermediate             | Female | Male | Fetus |
| 101(C-T)/IVSII-I(G-A)       | 2                        | 1      | 1    | 0     |
| IVSI-6(T-C)/IVSI-6(T-C)     | 1                        | 1      | 0    | 0     |
| IVSI-6(T>A)/IVSI-I 110(G>A) | 4                        | 1      | 2    | 1     |
| IVSII-6/IVSII-6             | 1                        | 1      | 0    | 0     |
| IVSI-110/IVSI-110           | 1                        | 1      | 0    | 0     |
| <b>Total</b>                | <b>9</b>                 |        |      |       |

Table IV: Gene diversity in types of beta thalassemia minor referred to the treatment center of Baqaei 2 Hospital (2008-2022)

| Genomic variation                                | Type of beta thalassemia |        |      |       |
|--------------------------------------------------|--------------------------|--------|------|-------|
|                                                  | minor                    | Female | Male | Fetus |
| IVSI-1(G-A)/ Normal                              | 12                       | 4      | 8    | 0     |
| CD36-37(-T)/ Normal                              | 112                      | 56     | 50   | 6     |
| Initiation codon CD(T>C)/ Normal                 | 10                       | 5      | 5    | 0     |
| IVS1-110(G>A)/ Normal                            | 39                       | 22     | 15   | 2     |
| 28/ Normal                                       | 14                       | 8      | 5    | 1     |
| IVSII-1/ Normal                                  | 83                       | 44     | 35   | 4     |
| 25 del/Normal                                    | 7                        | 3      | 4    | 0     |
| IVSI-5(G-C)/ Normal                              | 20                       | 11     | 9    | 0     |
| CD41/42[-TTCT]/ Normal                           | 2                        | 2      | 0    | 0     |
| IVSI-6(T-C)/ Normal                              | 19                       | 6      | 13   | 0     |
| CD44(-C)/ Normal                                 | 2                        | 1      | 1    | 0     |
| CD88(-AA)/ Normal                                | 17                       | 5      | 11   | 1     |
| silian ( $\delta^\circ\beta$ )/ Normal           | 5                        | 2      | 3    | 0     |
| CD30/ Normal                                     | 4                        | 3      | 1    | 0     |
| CD22[7bp-del]/ Normal                            | 1                        | 1      | 0    | 0     |
| Fr8-9(+G)/ Normal                                | 7                        | 5      | 2    | 0     |
| CD77(CAC>GAC)/ Normal                            | 2                        | 1      | 1    | 0     |
| CD39/ Normal                                     | 18                       | 7      | 7    | 4     |
| CD44/ Normal                                     | 11                       | 5      | 6    | 0     |
| CD17(A>T)/ Normal                                | 1                        | 1      | 0    | 0     |
| CD67/ Normal                                     | 1                        | 0      | 1    | 0     |
| CD57(A>T)/ Normal                                | 1                        | 1      | 0    | 0     |
| IVSI[G-C]/ Normal                                | 1                        | 0      | 1    | 0     |
| CD5(-CT)/ Normal                                 | 6                        | 3      | 3    | 0     |
| CD15/ Normal                                     | 8                        | 5      | 3    | 0     |
| IVSII-848/ Normal                                | 4                        | 2      | 2    | 0     |
| 5'UTR+20(C-T)&IVSII-745(C-G)-<br>Cisform/ Normal | 5                        | 3      | 2    | 0     |
| IVSI-25/ Normal                                  | 6                        | 3      | 3    | 0     |
| CD14/42(-CTTT)/ Normal                           | 2                        | 0      | 1    | 1     |
| CD82/83(-G)/ Normal                              | 6                        | 0      | 6    | 0     |
| IVSII-745/ Normal                                | 6                        | 3      | 2    | 1     |
| IVSI-10/ Normal                                  | 1                        | 0      | 1    | 0     |
| CD8-9/ Normal                                    | 1                        | 0      | 1    | 0     |

|                                                        |    |            |   |   |
|--------------------------------------------------------|----|------------|---|---|
| CD 8[-AA]/ Normal                                      | 7  | 4          | 2 | 1 |
| CD83(-G)/ Normal                                       | 6  | 3          | 2 | 1 |
| CD74/75(-C)/ Normal                                    | 1  | 1          | 0 | 0 |
| CD41/42/ Normal                                        | 1  | 1          | 0 | 0 |
| CD8/9/ Normal                                          | 13 | 6          | 6 | 1 |
| IVSII-5(G-C)/ Normal                                   | 2  | 2          | 0 | 0 |
| CD20/ Normal                                           | 2  | 1          | 1 | 0 |
| Hb Monroe (CD30 G>C)/ Normal                           | 2  | 1          | 1 | 0 |
| IVSII/ Normal                                          | 2  | 1          | 1 | 0 |
| IVSI-1/ Normal                                         | 5  | 2          | 3 | 0 |
| IVSI-128/ Normal                                       | 1  | 1          | 0 | 0 |
| CD81-82/ Normal                                        | 1  | 1          | 0 | 0 |
| 101(C-T) &[5'UTR+20(C-T)&IVSII-745(G-C)] in Cis format | 1  | 1          | 0 | 0 |
| Hb Ernz/ Normal                                        | 2  | 1          | 1 | 0 |
| CD59[G>A]/ Normal                                      | 1  | 0          | 1 | 0 |
| IVSII-110/ Normal                                      | 7  | 1          | 5 | 1 |
| <b>Total</b>                                           |    | <b>488</b> |   |   |

## Discussion

This study provides a comprehensive overview of beta-thalassemia mutations in the southwestern region of Iran, an area characterized by high ethnic and genetic diversity. The identification of 81 different mutations enhances our understanding of genetic heterogeneity in the region and has important implications for public health initiatives. These findings offer valuable data to inform prenatal screening, genetic counseling, and targeted interventions for at-risk populations. The insights gained from this study improve our ability to prevent the birth of children with severe forms of beta-thalassemia, thereby reducing the burden of the disease on families and healthcare systems. The study evaluated 545 patients with beta-thalassemia and identified 81 different mutations, reflecting population heterogeneity in the southwestern region. For example, in the Alborz region and the Caspian Sea region in northern Iran, the CD30/N mutation has been reported (9), and this study found it in 4 minor patients and 4 fetuses. In the present study, the most commonly observed mutations in the beta-globin gene were CD36-37(-T)/N, IVSII-1/N, and IVS1-110(G>A)/N. According to Thein et al.'s study (2013), the Kurdish-Iranian mutation is considered

both Mediterranean and Iranian in origin (10, 11). Six patients in this 14-year study had the silent mutation 101(C-T)/N (Mediterranean), but this mutation was not found in any fetuses. The 101(C-T)/N mutation was the first base mutation identified in the beta-thalassemia gene (10–11) and is one of the most common forms of beta-thalassemia in the Mediterranean population. The substitution of G for A creates an alternative AG receptor (19 bp 5') to the normal IVS1 AG receptor. In vitro expression studies revealed that 80% to 90% of transcripts preferentially use this alternative splicing site (12). This may be due to a premature termination codon in the 19-bp portion of the conserved intronic sequence (13). This mutation primarily leads to a slight decrease in  $\beta$ -globin gene expression. Carriers exhibit a normal hematologic profile without microcytosis and borderline hemoglobin A2 levels, which can result in missed screening diagnoses. When combined with one of the classic beta-thalassemia mutations, this mutation results in beta-thalassemia intermedia (14). Among thalassemia minor patients, 48.7% were women, 46.2% were men, and 1.5% were fetuses. The most commonly observed mutation, CD36-37(-T)/N, was also the most frequent in fetuses

and thalassemia minor patients. According to Behfar et al. (2011), it is the most common mutation in Lorestan province (15). The second most common mutation was IVS1-110(G>A)/N, a Mediterranean mutation, and the third was IVSII-1/N, similar to the findings of Mehrzad's 2020 study in Sistan and Baluchistan (16). Notably, IVSII-1/N is the most prevalent mutation in the Gilan and Mazandaran provinces (17). This study identified a limited number of mutations in minor fetuses, with the most commonly observed being CD36-37(-T)/N, IVSII-1/N, and CD39/N. The CD39/N mutation is more prevalent in the Brazilian population (18). In the current study, 9 patients had thalassemia intermedia. The most frequently observed mutations were IVSI-6(T-C)/IVSI-6(T-C) and IVSI-110/IVSI-110 in both patients and fetuses. However, in the study by Saleh-Gohri et al. in Kerman, the IVSI-6(T>A)/IVSI-110(G>A) mutation had the lowest prevalence (19). The IVSI-6(T-C)/IVSI-6(T-C) mutation is Mediterranean in origin (20), while the IVSI-110/IVSI-110 mutation shows a decreasing prevalence from east to west, and its origin is thought to be in Iran (21). Among the 42 beta-thalassemia major patients, the CD36-37(-T)/CD36-37(-T) and IVSII-1/IVSII-1 mutations were observed in both adults and fetuses. Another mutation, 88(C>A)/CD15 (G>A), was seen only in adults and not in fetuses. This mutation has also been reported in India (22). Some beta-thalassemia major mutations were observed exclusively in fetuses, including IVSII-1/CD36-37(-T), CD39/CD36-37, IVSII-110/CD39, IVSI-6/ CD83 (-G), and IVSI-5/IVSI-5. The identification of various beta-thalassemia gene mutations, which should be re-evaluated periodically, is essential. This includes screening and identifying mutation types in thalassemia patients' families, identifying common

mutations, and preventing the birth of individuals with these mutations. This also helps reduce the high costs associated with diagnosing and treating the disease. Given the diversity of beta-thalassemia mutations in southwestern Iran, the presence of numerous distinct mutations in the beta-globin gene group may reflect the historical formation of the population in this region.

## Conclusion

The identification of prevalent beta-thalassemia mutations facilitates disease control and prevention programs and is crucial for the identification of various beta-thalassemia gene mutations. This should be re-evaluated periodically. Observing a wide range of beta-thalassemia genotypes in the southwestern region of Iran suggests gene flow; thus, identifying these genotypes is instrumental in preventing and controlling the disease. Altogether, these findings specify that, even though Silymarin reduces inflammatory factors, it can promote coagulation by increasing VWF and FVIII activities and inhibit fibrinolysis by suppressing TPA-1 production, thereby making thrombosis probable. This drug should, therefore, be cautiously prescribed for patients prone to thrombosis.

## Ethical considerations

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## Authors' contributions

Roya Salehi Kahyesh and Bijan Keykhaei Dehdezi: Conceptualization, Supervision, Project Administration

Ladan Mafakhar, Mahmoud Meniati, and Emir Yiğit Perk: Data Curation, Investigation

Saeed Beytaraf: Formal Analysis, Data Analysis

Arta Farhadi Kia: Writing – Original Draft, Writing – Review & Editing

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## Conflict of interest

The authors declare no conflict of interest related to this research.

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