β-Globin Gene Cluster Haplotypes in Iranian Patients with β-Thalassemia

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Abstract

Introduction: β-globin gene cluster haplotypes are useful in diagnosis of particular molecular defects in β-thalassemia, prenatal diagnosis of β-thalassemia, and elucidating population affinities.

Methods: β-globin gene cluster haplotypes were studied in 150 β-thalassemia minor and 52 healthy individuals from the Fars province of Iran. DNA was extracted from leukocytes of whole blood by phenol-chloroform. Haplotype was determined by PCR-RFLP technique.

Results: There were 26 out of 150 with homozygous haplotypes. Haplotype I was found as the most prevalent haplotype among both patients and normal individuals. Out of 26 patients bearing homozygous haplotypes, 12 (46.2%) had typical haplotype I and 3 (11.5%) had atypical haplotype I. The prevalence of haplotype I in normal control subjects was around 43% (45 out of 104 βA chromosomes). The second prevalent haplotype was haplotypes V (15.4%) and III (15.4%) for homozygous patients and controls, respectively. The most frequent mutation in patients was IVS II.1 (G→A) that was not linked to a single haplotype. IVS I.110 (G→A) mutation was linked to haplotype I. Mutation in codon 30 (G→A) was associated with haplotype V.

Conclusion: Being Haplotype I the most prevalent haplotype in β-thal and βA chromosomes, implies that β-thalassemia mutations might have arisen in the chromosomal background common in the population, rather than due to selection pressure or gene flow (migration). Patients with haplotype IX had the highest HbF levels compared to other haplotypes.

Key words: β-globin gene, haplotypes, β-thalassemia, Iran

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Introduction

β-thalassemia is a single gene disorder with high prevalence (around 10%) in northern and southern Iran. The frequency of minor and major β-thalassemia in the Fars province of Iran was found to be 6.9% and 0.72%, respectively. [(1)] The spectrum of β-thalassemia mutations in the Fars province of Iran have been studied by Merat et al[(2)] and Karimi et al.[(3)] Orkin et al[(4)] found a strong linkage disequilibrium between polymorphic sites across the β-globin gene cluster (haplotypes) and β-thalassemia mutations. A limited number of haplotypes are found in each population, and each haplotype is usually associated with one specific type of thalassemia. When mutation analysis fails, haplotype analysis is useful in identifying a β-thal chromosome, with an accuracy of about 90% if the family is informative.[(5)]

Despite the few studies on types of β-thalassemia mutations in southwestern Iran, RFLP haplotypes of β-thalassemia chromosomes have not been yet determined. The aim of the present study is to find the haplotype background for β-thalassemia chromosomes among minor β-thalassemia patients from the Fars province and to compare them with the haplotype background of βA chromosomes from normal individuals in the region.

Methods

The patient group was comprised of 150 unrelated minor β-thalassemia individuals (105 females and 45 males) from the Fars province of Iran. The control group consisted of 52 healthy individuals (26 females and 26 males) with normal blood indices from the same area.

The levels of Hb A2 and HbF were determined by DEAE-52 micro-column chromatography and alkaline denaturation,[6,7] respectively. Hemoglobin electrophoresis was carried out on cellulose acetate at pH 8.6 and citrate agar at pH 6.6.[8] For haplotyping, DNA was extracted from leukocytes of whole blood by phenol-chloroform (9). Haplotype was determined by the pattern of six restriction sites through the β-globin gene cluster using primers described by Old (10) and by one pair for 3' to β gene by BamH I given by Lee et al.[11] The polymorphic sites were, 5' to ε gene by Hind II, within IVS 2 of the Gγ and Aγ genes by Hind III, within and 3' to ψβ by Hind II, IVS 2 of the β gene by Ava II, and 3' to the β gene by BamH I as shown in Figure 1.[10] The haplotype classification of Orkin[4] has been adopted.

Initially, Reverse Dot Blot (RDB) technique, with strips containing probes for the most common Mediterranean and Indian β-thalassemia alleles, previously reported for Iranian populations was used for screening β-thalassemia mutations.[12,13] The RDB membrane hybridization was carried out at 42°C and signals were revealed by the streptavidin horse radish peroxidase conjugate.[12,13] When RDB technique failed to detect any mutation, further chromosomal analysis was performed by Denaturing Gradient Gel Electrophoresis (DGGE) as described by Ghanem et al.[14]

A chi-square analysis was used to compare all studied haplotypes between patients and control group. To compare frequency of each haplotype in patients with corresponding haplotype in normal individuals Z test was used. The statistical analyses were executed by SPSS 11.5 statistical program. A P value of less than 0.05 was considered significant.

Results

Patterns of some agarose gel electrophoreses for haplotyping are illustrated in Figure 2. The distribution and frequency of haplotypes found in β-thalassemia minor patients are depicted in Table 1. Twenty three out of 150 patients had homozygous typical haplotypes as shown in Table 2. Three patients (2%) out of 150 had atypical haplotype I as homozygous state. Two of which had the profile -----++ of restriction sites lacking the site for Hind II enzyme 5́ to ε gene, and one patient had the +-+--++ pattern having the site for Hind III within IVS II Aγ gene which is absent in typical haplotype I (Table 2).

As pedigree analysis was not possible, we were unable to determine the haplotypes belonging to β-thal or βA chromosome in the patients. However, the presence of 23 patients bearing homozygous haplotypes provided the necessary information about the frequency of various haplotypes linked to β-thalaassemia. As Table 2 shows, haplotype I was the most prevalent haplotype (46.2%) among 52 β-thal chromosomes followed by haplotype V (15.4%), haplotype I Atypical (11.5%), haplotype II, III and IX each with 7.7% and haplotype VIII with 3.8% of the total haplotypes. Distribution and frequency of β-globin gene cluster haplotypes for 104 βA chromosomes are depicted in Table 2. Similar to β-thal chromosomes, the frequency of haplotype I was the highest (43.3%) among βA chromosomes. The magnitude of these frequencies, for other haplotypes including atypicals, is also shown in Table 2.

Ten different mutations were found, the mutation IVSII.1 (G: A) accounted for 39.5% of studied thalassemic alleles followed by 25bp deletion 3́ IVSI (18.5%). Mutations found in patients and the corresponding haplotypes are depicted in Table 3.

Discussion

β-globin gene cluster haplotypes are useful in diagnosis of particular molecular defects in β-thalassemia, prenatal diagnosis of β-thalassemia, and elucidating population affinities.[15] The linkage of β-thalassemia mutation with specific haplotypes has been reported previously.[4] In β-thalassemia chromosomes from Mediterranean patients[4] haplotype I was the most prevalent haplotype (47%) followed by haplotypes II (17%),V (12%) and III (8%). In the present study haplotype I was the most prevalent haplotype among all β-thalassemia minor patients including those with the homozygous haplotype (46.2%), and in normal indi-

viduals (43.3%). However, the second prevalent haplotype was haplotypes V (15.4%) and III (15.4%) for homozygous patients and controls, respectively. Being Haplotype I the most prevalent haplotype in β-thal and βA chromosomes, implies that β-thalassemia mutations might have arisen in the chromosomal background common in the population, rather than due to selection pressure or gene flow (migration). Further, there was no significant difference between the frequency of haplotypes in patients and normal individuals except for haplotype V which was significantly higher in patients compared to control. The IVS II. 1 (G→A) mutation has been reported as the most prevalent mutation in southern Iran.[1,2] Also, in the present study the analysis of 23 β-thal chromosomes from β-thalassemia minor patients showed the IVS II.1 (G→A) to be the most frequent mutation (around 48%).

IVS II 1 (G→A) mutation in the Mediterranean, is linked to haplotype III and V.[16] However, the same haplotype is generally associated with different forms of β-thalassemia in different populations,[5] and rarely, mutations are found on few β-gene frameworks within an ethnic group.[16] In the present study IVS II 1 (G→A) mutation was associated with combination of various haplotypes, predominantly with haplotype III. Although it was not possible to determine the haplotype(s) linked to this mutation, it is evident that the mutation is associated with more than one haplotype background. The IVS I.110 (G→A) mutation is the most common cause of β-thalassemia in Mediterranean countries.[17] In Lebanon haplotype I was associated with IVS I.110 (G→A) mutation.[17] In the present study IVS I.110 (G→A) mutation similar to Lebanon is linked to haplotype I. Mutation Cd 30 (G→A) was found linked to haplotype V in a patient bearing a homozygous haplotype. In 11 of our patients with IVS II.1 (G→A) mutation, the following haplotypes were found: II/III, I/III, I/III Atypical (having the site for Hind III within IVS II Aγ gene that is absent in the typical haplotype III), I/VIII, I Atypical/I Atypical (lacking the site for Hind II enzyme 5́ to ε gene), I/IX, and I/II. Although it was not possible to determine the haplotype(s) linked to this mutation, it is evident that the mutation is associated with more than one haplotype background. In one patient IVS I-110 was associated with atypical haplotype I. High levels of HbF was found in two patients with haplotype IX/IX (4.7%) and two other patients with haplotype IX/IX Atypical (having the site for Hind III within IVS II Aγ gene which is absent in the typical haplotype IX) (3.2%) may be explained by the presence of haplotype IX, that is known to be all most identical to Senegal haplotype linked to Xmn I site 5́ to Gγ gene.[18] The presence of this site is associated with increase Gγ gene expression and HbF level.[19] Among the Asian β-thalassemia homozygotes, the inheritance of a β-thalassemia chromosome with 5' haplotype IX is clearly associated with a milder clinical phenotype and a sufficient increase in Hb F to modify β+-thalassemia.[20]

Briefly, we found 6 typical and 2 atypical haplotypes among beta thalassemia patients which among them haplotype I was the predominant haplotype. There was no significant difference related to frequency of haplotypes, except haplotype V, between patients and normal individuals. IVS II 1 (G→A) mutation was associated with combination of various haplotypes, predominantly with haplotype III and IVS I.110 (G→A) mutation was associated with haplotype I. Patients with haplotype IX/IX had higher levels of Hb F which is associated with a milder clinical phenotype.

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Table 1: Distribution of β-haplotypes among all β-thalassemia minor patients.

Haplotype	Number of patients (%)
I/I	12 (8)
Atypical I/Atypical I	3 (2)
I/II,I/III, I/IV,I/V, I/VI,I/VII, I/IVIII, I/IX, I/Atypical	90 (60)
II/II	2 (1.3)
II/III, II/IV, II/VII, II/IX	9 (6)
III/III	2 (1.3)
III/IV, III/V III/IX	11 (7.3)
V/V	4 (2.7)
VIII/VIII	1 (0.7)
VIII/VI	1 (0.7)
IX/IX	2 (1.3)
Atypical/ Atypical	13 (8.7)
Total	150

Table 2: Frequency of haplotypes among 26 patients bearing homozygous haplotype and 52 normal individuals.

Haplotype	βthal chromosomes (%)	βA chromosomes (%)	P value
I (+----++)	24 (46.2)	45 (43.3)	0.7
II (-++-+++)	4 (7.7)	5 (4.8)	0.48
III (-+-+++-)	4 (7.7)	16 (15.4)	0.17
IV (-+-++-+)	-	5 (4.8)	-
V (+----+-)	8 (15.4)	4 (3.8)	0.02*
VI (-++---+)	-	3 (2.9)	-
VII (+-----+)	-	5 (4.8)	-
VIII (-+-+-+-)	2 (3.8)	1 (1)	-
IX (-+-++++)	4 (7.7)	13(12.5)	0.36
I Atypical (-----++)	4 (7.7)	5 (4.9)	0.48
I Atypical (+-+--++)	2 (3.8)	-	-
Atypical (------+)	-	1 (0.9)	-
Atypical (-+---++)	-	1 (0.9)	-
Total	52	104	

* The β-haplotypes are constructed from polymorphic sites as Hind II-ε, Hind III- Gγ, Hind III- Aγ, Hind II-ψβ, Hind II- 3' ψβ, Ava II- β and BamHI- 3'β.

* Eight of normal individuals (15.4%) had homozygous haplotypes, seven with homozygous haplotype I and one with homozygous haplotype IX.

Table 3: Haplotypes and mutations found in some of the patients.

Number of patients	Mutations	Haplotypes
4	IVS II.1 G:A	I/III
2	IVS II.1 G:A	I/III Atypical
1	IVS II.1 G:A	I/II
1	IVS II.1 G:A	I/IX
1	IVS II.1 G:A	I Atypical/I Atypical
1	IVS II.1 G:A	I/VIII
1	IVS II.1 G:A	II/III
1	Cd 30 G:A	V/V
1	IVS I.110 G:A	I/I Atypical
2	25 bp deletion Ǵ IVSI	I/IX
1	25 bp deletion Ǵ IVSI	I/III
1	25 bp deletion Ǵ IVSI	I/IV
1	Fs 8 (-AA)	IV/IX
1	IVS I.5 G:C	I/II
1	Cd 5 (-CT)	I/V
1	IVS II.745 C:G	I/IV

Figure- 1: Locations of seven regions for β-globin gene cluster treated with four restriction enzymes.

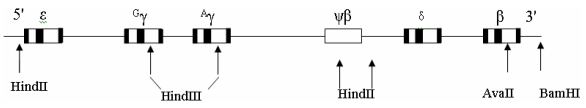
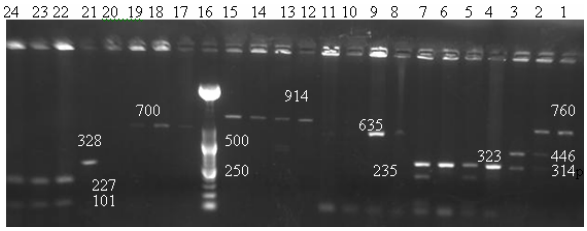


Figure 2: An agarose gel electrophoresis pattern of some of RFLP products of β gene cluster. Lane 1 shows undigested PCR product of Hind II 5' to ε. Lanes 2 and 3 are DNAs which are +/- and +/+ for Hind II 5' to ε, respectively. Lane 4 shows undigested PCR product of Hind III within IVS II Gγ. Lanes 5,6 and 7 are DNAs which are +/-, -/- and +/- for Hind III within IVS II Gγ, respectively. Lane 8 shows undigested PCR product of Hind III within IVS II Aγ. Lane 9 is DNA which is -/- for Hind III within IVS II Aγ. Lane 12 shows undigested PCR product of Hind II 3' ψβ. Lanes 13, 14 and 15 are DNAs which are -/- for Hind II 3' ψβ. Lane 16 indicates DNA molecular weight marker with 50 base pair ladder. Lane 17 shows undigested PCR product of for Hind II within ψβ. Lanes 18 and 19 are DNAs which are -/- for Hind II within ψβ. Lane 21 shows undigested PCR product of Ava II within β. Lanes 22,23 and 24 are DNAs which are +/+ for Ava II within β.



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