Identification of β-globin haplotypes linked to sickle hemoglobin (Hb S) alleles in Mazandaran province, Iran

Faeghe Aghajani1, Mohammad Reza Mahdavi1*, Mehrnoush Kosaryan1, Mehrad Mahdavi2, Mohaddase Hamidi1 and Hossein Jalali1

1Thalassemia Research Center, Mazandaran University of Medical Sciences, Sari, Iran
2Sina Mehr Research Center, Sari, Iran

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Carrier frequency of the βS allele has been reported to be 0.19% in Mazandaran province, northern Iran. Haplotype analysis of the βS allele helps trace the origin of its encoded hemoglobin (Hb) variant, Hb S, in a region. The aim of this study was to investigate the haplotypes associated with βS alleles in Mazandaran province. Capillary electrophoresis was carried out to detect individuals suspected to have a βS allele(s). DNA analysis (PCR-RFLP) was used for final confirmation. To identify 5’ to 3’ β-globin gene cluster haplotypes associated with βS alleles, family linkage analysis was applied. Six polymorphic sites (HincII 5’ to ε, XmnI 5’ to Gγ, HindIII in Gγ, HindIII in Aγ, HincII 3’ to ωβ and AvaII in β) were investigated using the PCR-RFLP method. Five different haplotypes were linked to βS alleles, while βA alleles were associated with nine haplotypes. Among the βS alleles, 53.9% were associated with the Benin (----++) haplotype, and the Arab-Indian (++---+) haplotype had the second-highest frequency (23%). Unlike southern provinces, where the Arab-Indian haplotype is prominent, the Benin haplotype is the most frequent haplotype in northern Iran, and this may represent a founder effect. Since the Benin haplotype does not carry the XmnI polymorphism 5’ to the Gγ gene, which is responsible for high expression of Hb F, a severe form of sickle cell disease can be anticipated in patients that are homozygous for the βS allele in the northern region.

Key words: βS allele, haplotype, PCR-RFLP

Sickle cell disease is a common health problem in Africa, the Middle East, India and Mediterranean countries (Schneider et al., 1976; Pielet et al., 2013). The highest carrier frequency of the βS gene, reaching 40%, was reported from some tribal groups in India and across equatorial Africa (Schneider et al., 1976). The carrier frequency of the disease is 1%–2% on the North African coast and in South Africa (Jastaniah, 2011), about 7% to 9% among African-Americans (Schneider et al., 1976; Heller et al., 1979) and 4.6% in Turkey (Basak and Tuzman, 2011). In the south of Iran, 1.5% of the population are carriers of the βS gene, whereas the frequency is about 0.19% in Mazandaran province in the north of Iran (Habibzadeh et al., 2000; Valizadeh et al., 2012). Patients with sickle cell disease display a wide spectrum of clinical manifestations ranging from mild anemia to severe forms of the disease, including lethality. Different levels of fetal hemoglobin expression and the simultaneous presence of α-thalassemia and β-globin cluster-linked haplotypes are among the factors affecting hematological indices of patients and subsequently the severity of the disorder (Figueiredo et al., 1996; Rahimi et al., 2003).

The βS allele has been reported in association with several haplotypes, which can be distinguished by specific patterns of restriction endonuclease sites in the β-globin gene cluster. Benin, Senegal, Bantu, Cameroon and Arab-Indian are the most common haplotypes linked to the βS allele. These haplotypes are named after the geographical areas where they are most prevalent. Haplotype analysis of the βS allele helps us to trace the origin of this hemoglobin variant in a region (Pagnier et al., 1984; Serjeant, 1994; Loggetto, 2013).

The aim of this study was to investigate the haplotypes associated with βS alleles in Mazandaran province, Iran. The study was approved by the Ethics Committee of Mazandaran University of Medical Sciences and all the participants formally consented to take part in the study, which was performed during 2012–2013.

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* Corresponding author. E-mail: Mahdavi899@gmail.com
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Premarital screening for thalassemia is compulsory in Iran. For this study, blood samples from 2,580 patients from laboratories or health centers in Mazandaran, who had been found during premarital screening to have reduced hematological indices, were investigated at the Fajr laboratory. Complete blood count (CBC) and hemoglobin (Hb) electrophoresis, using a capillary device (Minicap, Sebia, France), were carried out for each sample.

Samples from individuals suspected to be Hb S or Hb D carriers underwent alkaline (pH 8.4–8.6) or citrate agar gel electrophoresis (pH 6.1), solubility testing, and sickling testing, to differentiate Hb D from Hb S cases, which both display peaks in the same zone in capillary electrophoresis.

DNA analysis was performed for final identification of the Hb S cases. Subjects with Hb S completed a questionnaire; all of them were born in Mazandaran.

Genomic DNA was extracted from peripheral blood samples using a QIAamp DNA mini kit (Qiagen, Germany). To verify the Hb S mutation, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was applied. A 444-bp segment of the ω-globin gene was amplified using specific primers (5′-TAGGTTGGCACAATCTACTC-3′ and 5′-TTAGGGTTGCCATAAAGC-3′). Amplified PCR fragments were digested with MSII restriction enzyme and subjected to electrophoresis on a 2% agarose gel. In normal alleles the PCR product remained intact. was cut into two fragments, while in mutated alleles it was cut into two fragments, while in mutated alleles it remained intact.

To identify 5′ to 3′ ω-globin gene cluster haplotypes associated with βs alleles using PCR-RFLP, the presence of the following polymorphic sites was investigated: HincII 5′ to ϵ, XmnI 5′ to ϵγ, HindIII in ϵγ, HindIII in ϵγ, HindII in 3′ to ϵβ and AvaII in β (Cabeda et al., 1999; Rahimi et al., 2003), and family linkage analysis was carried out.

<table>
<thead>
<tr>
<th>Haplotype (5′→3′)</th>
<th>βs alleles (frequency)</th>
<th>RBC (×10⁶/ml)</th>
<th>Hb (g/dl)</th>
<th>Hct (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dl)</th>
<th>Hb A1 (%)</th>
<th>Hb A2 (%)</th>
<th>Hb S (%)</th>
<th>HbF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>----++ (Benin)</td>
<td>7 (53.9%)</td>
<td>4.49</td>
<td>11.9</td>
<td>33.6</td>
<td>74.9</td>
<td>26.5</td>
<td>35.4</td>
<td>57.4</td>
<td>2.8</td>
<td>39.8</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.80</td>
<td>11.9</td>
<td>36.5</td>
<td>76.0</td>
<td>24.8</td>
<td>32.6</td>
<td>66.0</td>
<td>3.2</td>
<td>30.8</td>
<td>&lt; 0.5</td>
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<tr>
<td></td>
<td></td>
<td>4.99</td>
<td>11.9</td>
<td>36.6</td>
<td>73.3</td>
<td>23.8</td>
<td>32.5</td>
<td>63.3</td>
<td>3.6</td>
<td>33.3</td>
<td>&lt; 0.5</td>
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<tr>
<td></td>
<td></td>
<td>4.99</td>
<td>11.8</td>
<td>35.6</td>
<td>73.6</td>
<td>23.5</td>
<td>33.5</td>
<td>62.7</td>
<td>3.7</td>
<td>34.3</td>
<td>&lt; 0.5</td>
</tr>
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<td></td>
<td></td>
<td>4.77</td>
<td>12.1</td>
<td>36.9</td>
<td>77.4</td>
<td>25.4</td>
<td>32.8</td>
<td>63.0</td>
<td>3.1</td>
<td>33.9</td>
<td>&lt; 0.5</td>
</tr>
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<td></td>
<td>5.20</td>
<td>13.1</td>
<td>39.0</td>
<td>76.5</td>
<td>26.0</td>
<td>34.0</td>
<td>57.6</td>
<td>3.5</td>
<td>38.9</td>
<td>&lt; 0.5</td>
</tr>
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<td></td>
<td></td>
<td>4.87</td>
<td>11.1</td>
<td>38.2</td>
<td>75.1</td>
<td>24.6</td>
<td>32.8</td>
<td>55.1</td>
<td>2.3</td>
<td>35.3</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>++++ (Arab-Indian)</td>
<td>3 (23.0%)</td>
<td>5.28</td>
<td>12.9</td>
<td>39.2</td>
<td>74.2</td>
<td>24.4</td>
<td>32.9</td>
<td>59.3</td>
<td>2.3</td>
<td>38.4</td>
<td>&lt; 0.5</td>
</tr>
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<td></td>
<td></td>
<td>5.30</td>
<td>11.6</td>
<td>34.8</td>
<td>65.7</td>
<td>21.9</td>
<td>33.3</td>
<td>0.0</td>
<td>3.7</td>
<td>58.2</td>
<td>38.1</td>
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<td>5.66</td>
<td>12.0</td>
<td>36.1</td>
<td>63.7</td>
<td>21.2</td>
<td>32.2</td>
<td>58.2</td>
<td>3.5</td>
<td>32.7</td>
<td>5.6</td>
</tr>
<tr>
<td>++ (Senegal)</td>
<td>1 (7.7%)</td>
<td>4.89</td>
<td>13.2</td>
<td>38.2</td>
<td>78.9</td>
<td>27.2</td>
<td>33.6</td>
<td>57.2</td>
<td>2.7</td>
<td>39.0</td>
<td>1.1</td>
</tr>
<tr>
<td>++ (CAR)</td>
<td>1 (7.7%)</td>
<td>4.98</td>
<td>12.8</td>
<td>39.1</td>
<td>69.1</td>
<td>24.2</td>
<td>33.2</td>
<td>60.6</td>
<td>3.0</td>
<td>36.1</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>++++</td>
<td>1 (7.7%)</td>
<td>4.80</td>
<td>13.2</td>
<td>38.1</td>
<td>79.4</td>
<td>27.5</td>
<td>34.6</td>
<td>60.0</td>
<td>2.2</td>
<td>37.8</td>
<td>&lt; 0.5</td>
</tr>
</tbody>
</table>

*This case is a compound heterozygote for βs/βthal alleles.

Mazandaran is a northern province of Iran with four million inhabitants. Previous studies showed that around 10% of the Mazandaran population are carriers of β-thalassemia and 15% of neonates have at least one of the common α-globin gene mutations, but the Hb S variant has a very low incidence (0.19%) (Mahdavi et al., 2013; Jalali et al., 2014).

The first report of sickle cell anemia in Iran was a mild form of the disease and patients, in comparison to sickle patients reported from other parts of the world, had a relatively high Hb F content (18%) (Haghshenass et al., 1977).

Haplotype analysis of the βs allele among 64 individuals in the south of Iran (Rahimi et al., 2003) showed that this variant is associated with 10 different haplotypes; the Arab-Indian haplotype was the most common one (69.1% in patients homozygous for βs), followed by the Bantu A2 haplotype (10.6% in patients homozygous for βs). Another study in 50 patients with homozygous sickle cell anemia in Khuzestan province (southeast Iran) revealed that the Arab-Indian haplotype is the most frequent one (38%), while Benin (18%), Senegal (16%), Bantu (16%) and Cameroon (12%) were other common haplotypes (Keikhaei et al., 2012). In central Iran and in 23 sickle
cell trait subjects, only the Arab-Indian haplotype was reported in association with the β⁰ allele (Rahgozar et al., 2000). The high prevalence of the Arab-Indian haplotype in south and southeast Iran is suggested to be a result of gene flow to/from the Arabian Peninsula or India. The present study showed that although the Benin haplotype has a low frequency in southern provinces, it is the most frequent one in Mazandaran province. This may represent a founder effect phenomenon. Moreover, in the present study, the CAR (Central African Republic) haplotype and an atypical haplotype (+---+++ ) were observed in association with β⁰ alleles, and these haplotypes have not previously been reported from Iran. Taking these points into consideration, we conclude that these alleles have different genetic origins from those reported in cases from the south of Iran.

Haplotype analysis of Hb D-Los Angeles in Mazandaran province (Mahdavi et al., 2015) showed that more than 90% of the β⁰ alleles shared the same haplotypic background. This may represent a founder effect for that hemoglobin variant in the region. On the other hand, the Benin haplotype (the most frequent one) in Mazandaran province was observed in association with 53% of the β⁰ alleles. Moreover, the β⁰ alleles were identified as being linked to three different haplotypes, but five different haplotypes were recognized in association with β⁰ alleles in the present study. In comparison to the β⁰ allele, these results show the geographical diversity of the β⁰ allele's origin.

A previous study has shown that among sickle cell patients carrying haplotypes with Xmn1 (+/+) polymorphism at position −158(C→T) of the O⁰γ gene, the O⁰γ gene is expressed at a high level, resulting in Hb F induction and leading to milder presentation of the disease (Rahimi et al., 2003). Generally, the cases reported from India, eastern provinces of Saudi Arabia, Qatar, Oman, Azerbaijan, Baluchistan, Iraq, Afghanistan and southern Iran show milder clinical manifestation than cases from Africa (Keikhai et al., 2012). The present study showed that in Mazandaran province, around 70% of the β⁰ alleles are not associated with Xmn1 polymorphism, which means that β⁰-homozygous individuals are likely to suffer more severe forms of sickle cell anemia.

REFERENCES


