# Beta globin gene cluster haplotypes of the beta thalassemia mutations observed in the Denizli province of Turkey

*Denizli yöresinde gözlenen beta talasemi mutasyonlarına ait beta globin gen ailesi haplotipleri*

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## Abstract

**Objective:** Our aim was to identify the beta globin gene cluster haplotypes for the beta thalassemia mutations in Turkey on a regional level. Beta thalassemia mutations included in this study were IVS-I-110 (G>A), FSC 8/9 (+G), IVS-II-1 (G>A), IVS-I-5 (G>C), IVS-I-1 (G>A), IVS-I-6 (T>C), and FSC 8 (-AA).

**Methods:** We studied 22 unrelated patients with β-thalassemia major and 72 unrelated healthy subjects from our Department's DNA bank. Haplotype analysis was done by polymerase chain reaction (PCR)-based restriction enzyme digestion for the beta globin gene cluster of the following polymorphic restriction sites: Hinc II 5' to ε, Hind III 5' to Gγ, Hind III in the IVS-II 5' to Aγ, Hinc II in pseudo β, Hinc II 3' to pseudo β, Ava II in β, and Hinf I 3' to β. Associated haplotypes for the normal control samples (72 individuals, 144 chromosomes) were determined by Arlequin 3.1 software with unknown gametic phase.

**Results:** According to the results obtained, the most frequent beta globin gene cluster haplotypes in the normal population were (+----++), (+----+-), (-+-++++), and (+-----+), with frequencies of 28.6%, 17.2%, 9.8%, and 8.3%, respectively. IVS-I-110 mutation was linked with the haplotypes (+----++) and (+-----+). Observed haplotypes were (+----++) for FSC 8/9 (+G), (-+-+++-) for IVS-II-1 (G>A), (-+-++-+ and -+-++++) for IVS-I-5 (G>C), (+----+- and +------) for IVS-I-1 (G>A), (-++---+) for IVS-I-6 (T>C), and (+-----+) for FSC 8 (-AA).

**Conclusion:** Our region shows the Mediterranean character for the beta thalassemia mutations. According to the obtained results, IVS-I-110 (G>A) mutation linked with haplotype VII (+-----+), IVS-I-5 (G>C) mutation with haplotype IV (-+-++-+), and codon 8/9 (+G) with haplotype I (+----++) were shown for the first time in the Turkish population. The linkage of haplotype (+------) with the IVS-I-1 (G>A) mutation is reported for the first time in the published literature. In the Denizli province of Turkey, beta globin gene cluster haplotypes of the normal population are strongly associated with the haplotypes of I (+----++), V (+----+-) and IX (-+-++++), respectively. *(Turk J Hematol 2009; 26: 129-37)*

**Key words:** Beta thalassemia, beta globin haplotypes, mutation

*Received: November 3, 2008* *Accepted: June 27, 2009*

## Özet

**Amaç:** Bu çalışmamızda, Denizli yöresinde gözlenen beta talasemi mutasyonları ile beta globin haplotip ilişkileri incelenmiştir. Çalışmada IVS-I-110 (G>A), FSC 8/9 (+G), IVS-II-1 (G>A), IVS-I-5 (G>C), IVS-I-1 (G>A), IVS-I-6 (T>C) and FSC 8 (-AA) mutasyonları ve haplotip ilişkileri irdelenmektedir.

**Metodlar:** Çalışmada 22 akraba olmayan homozigot beta talasemi mutasyonu taşıyan hasta ile 72 akrabalık ilişkisi bulunmayan sağlıklı birey yer almaktadır. Haplotip analizi PCR tabanlı enzim kesimine dayalı biçimde, Hinc II 5'-ε, Hind III 5'-Gγ,

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Hind III IVS-II 5'-Aγ, Hinc II-pseudo β, Hinc II 3'-pseudo β, Ava II-β, Hinf I 3'-β olmak üzere yedi odakta gerçekleştirilmiştir. Normal sağlıklı kontrol örnekleri için ilişkili haplotipler Arlequin 3.1 yazılımı ile elde edilmiştir.
**Bulgular:** Çalışmamızda elde edilen sonuçlara göre, Denizli yöresindeki normal popülasyon ile ilgili haplotipler (+----++) (% 28.6), (+----+-) (%17.2), (-+-++++) (% 9.8)ve (+-----+) (% 8.3) olarak belirlenmiştir. IVS-I-110 (G>A) mutasyonun (+----++) ve (+-----+) haplotipleri ile ilişkili olduğu görülmüştür. Diğer mutasyonların ise; (+----++) FSC 8/9 (+G), (-+-+++-) IVS-II-1 (G>A), (-+-++-+ ve -+-++++) IVS-I-5 (G>C), (+----+- ve +------) IVS-I-1 (G>A), (-++---+) IVS-I-6 (T>C) ve (+-----+) for FSC 8 (-AA) şeklinde olduğu gözlenmiştir.
**Sonuç:** Sonuç olarak yöremizin beta globin gen ailesi haplotipleri açısından Akdeniz tipi karakter ortaya koyduğu gösterilmektedir. Elde edilen sonuçlara göre, IVS-I-110 (G>A) mutasyonunun haplotip VII (+-----+), IVS-I-5 (G>C) mutasyonunun haplotip IV (-+-++-+), Kodon 8/9 (+G) mutasyonunun haplotip I (+----++) ile ilişkili oldukları Türkiye'de ilk kez bildirilmektedir. IVS-I-1 (G>A) mutasyonunun (+------) mutasyonu ile ilişkili olduğu ise literatürde ilk kez gösterilmiştir. Çalışmada Denizli yöresindeki normal popülasyondaki haplotiplerin ise sırası ile haplotip I (+----++), haplotip V (+----+-) ve haplotip IX ( -+-++++) ile yüksek oranda ilişkili olduğu gözlenmektedir. *(Turk J Hematol 2009; 26: 129-37)*
**Anahtar kelimeler:** Beta talasemi, beta globin haplotipi, mutasyon

*Geliş tarihi: 03 Kasım 2008 Kabul tarihi: 27 Haziran 2009*

## Introduction

Beta thalassemia is the most common inherited blood disorder in Turkey as well as in several other Mediterranean countries, and represents a major public health problem in several countries. Beta thalassemia is characterized by reduced or absent beta globin gene expression. At present, more than 200 different mutations resulting in a $\beta^0$- or $\beta^+$ -thalassemia phenotype have been reported in different parts of the world (http://globin.cse.psu.edu). Beta thalassemia, alpha thalassemia and sickle cell anemia are also the most common hemoglobinopathies in Turkey. Although the overall frequency of beta thalassemia in Turkey is 2%, there are significant regional differences [1]. The incidence of beta thalassemia in the Denizli province is between 2.6-3.7% as reported by different researchers [2-4]. In Turkey, beta thalassemia mutations show a heterogeneous character, and do not present a specific distribution pattern that would aid in the identification of any ethnic background [1]. For this reason, hemoglobinopathies should be investigated in detail at the regional level. Since different molecular techniques have been developed, the mutations should also be studied for their molecular genetic background and mutation mechanisms.

The beta globin gene cluster is located at human chromosome 11 (region 11p15.5) including five genes arranged in the order 5'-ε-Gγ-Aγ-ψβ-δ-β-3'. Beta thalassemia mutations along the beta globin gene cluster were studied using restriction enzymes on a limited number of polymorphic sites in order to define the RFLP (restriction fragment length polymorphism) haplotypes. Studies demonstrated a non-random association of specific RFLP haplotypes with specific beta thalassemia mutations. Among the Mediterranean population, the association between specific haplotypes and specific mutations is very high, but not definite, although some specimens are predominantly associated with some mutations [5-7]. Beta globin gene cluster haplotypes are a useful tool for the determination of genetic structure and origin of the populations, including the possible associations with mutations and hereditary diseases like thalassemias and abnormal hemoglobins of interest [8].

Hemoglobinopathies also have a heterogeneous character in the Denizli province of Turkey [9,10]. The beta globin gene cluster haplotypes associated with abnormal hemoglobins (like Hb D-Los Angeles, Hb G-Coushatta, Hb Beograd, Hb Yaizu, etc.) were investigated both in Denizli and Turkey [8,11,12]. In this study, we aimed to determine the beta globin gene cluster haplotypes and their association with the beta thalassemia mutations in the Denizli province of Turkey on a regional level.

## Materials and Methods

Blood samples were collected in EDTA vacutainers and DNA was obtained from peripheral blood using the standard phenol-chloroform procedure. Written informed consent was obtained from these individuals and/or from their parents for DNA analysis, and the samples were deposited in the Pamukkale University Medical Faculty Biophysics Department DNA Bank (Denizli, Turkey) as anonymous samples for further investigations. The Hemoglobinopathy Control Program is done by the Turkish Ministry of Health Denizli Hemoglobinopathy Laboratory. Beta thalassemia carriers are diagnosed at the premarital stage, and since 2004, the couples at risk have been monitored for possible prenatal diagnosis. We studied DNA samples of 22 unrelated patients with beta thalassemia major and 72 unrelated healthy subjects from our Department's DNA bank. All samples are from the Denizli province. The mutations of the beta thalassemia cases from the DNA bank were already previously determined by Strip Assay (Vienna Lab) and confirmed by DNA sequencing of the entire beta globin gene.

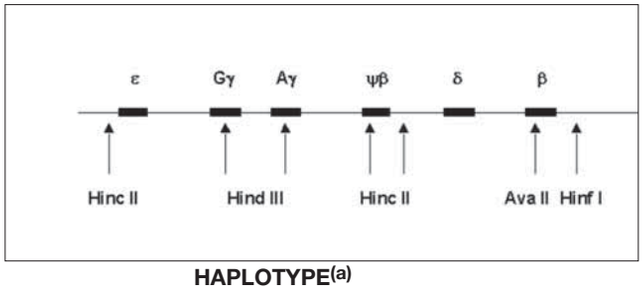
Haplotype analysis was done by polymerase chain reaction (PCR)-based restriction enzyme digestion for the beta globin gene cluster of the following polymorphic restriction sites: Hinc II 5' to ε, Hind III 5' to Gγ, Hind III in the IVS-II 5' to Aγ, Hinc II in ψβ, Hinc II 3' to ψβ, Ava II in β, and Hinf I 3' to β. The PCR products containing each of these polymorphic sites were amplified by PCR and digested with the appropriate restriction enzyme. Restriction enzymes used for the haplotype analysis were purchased from Sibenzyme (Novosibirsk, Russia), Bioron (Ludwigshafen, Germany) and New England Biolabs, Inc (Beverly, MA, USA) [8,13]. The sequences of the oligonucleotide primers for the seven polymorphic sites were as previously published [14].

Associated haplotypes for the normal control samples (72 individuals, 144 chromosomes) were determined by Arlequin 3.1 software with unknown gametic phase [15].

## Results

Beta thalassemia mutations included in this study were IVS-I-110 (G>A) (24 chromosomes), FSC 8/9 (+G) (2 chromosomes), IVS-II-1 (G>A) (2 chromosomes), IVS-I-5 (G>C) (4 chromosomes), IVS-I-1 (G>A) (2 chromosomes), IVS-I-6 (T>C) (6 chromosomes) and FSC 8 (-AA) (4 chromosomes). For the control group, in total 72 healthy individuals (144 chromosomes) were used. Beta globin gene cluster haplotype results for the normal individuals are shown in Table 1. Using these results, the associated beta globin gene cluster haplotypes were generated by the Arlequin 3.1 software to

**Table 1. Beta globin gene cluster haplotypes of the normal population in Denizli, Turkey**




| | HAPLOTYPE[a] | | | | | | | | HAPLOTYPE[a] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CASE | 1 | 2 | 3 | 4 | 5 | 6 | 7 | CASE | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 01 | +/- | +/- | -/- | +/- | +/- | +/- | +/+ | 37 | +/- | +/- | +/- | -/- | +/- | +/- | +/+ |
| 02 | +/+ | -/- | -/- | -/- | -/- | +/- | +/+ | 38 | +/+ | -/- | -/- | -/- | +/- | +/+ | -/- |
| 03 | +/- | +/- | -/- | +/- | +/- | +/- | +/+ | 39 | -/- | +/+ | +/- | +/- | +/- | +/- | -/- |
| 04 | +/- | +/- | +/- | -/- | +/- | +/- | +/+ | 40 | +/- | +/- | -/- | -/- | +/- | +/+ | +/+ |
| 05 | -/- | +/+ | +/- | +/- | +/+ | +/- | +/+ | 41 | +/+ | -/- | -/- | -/- | +/- | +/+ | +/+ |
| 06 | +/+ | -/- | -/- | -/- | -/- | +/+ | +/+ | 42 | +/- | +/- | -/- | +/- | +/- | +/+ | -/- |
| 07 | +/+ | -/- | -/- | -/- | -/- | +/+ | -/- | 43 | +/- | +/- | +/- | -/- | +/- | +/- | +/+ |
| 08 | +/+ | -/- | -/- | -/- | -/- | +/+ | +/- | 44 | +/+ | -/- | -/- | -/- | +/- | +/+ | +/- |
| 09 | -/- | +/- | -/- | +/- | +/+ | +/+ | +/+ | 45 | +/- | +/- | +/- | -/- | +/- | +/+ | +/+ |
| 10 | +/- | +/- | -/- | +/- | +/- | +/+ | +/+ | 46 | -/- | +/+ | +/- | +/- | +/+ | +/- | +/+ |
| 11 | -/- | +/+ | -/- | +/+ | +/+ | +/- | +/+ | 47 | +/- | +/- | -/- | +/- | +/- | +/- | +/- |
| 12 | -/- | +/- | -/- | +/- | -/- | +/- | +/+ | 48 | +/+ | -/- | -/- | -/- | -/- | +/+ | +/- |
| 13 | +/+ | -/- | -/- | -/- | -/- | +/+ | +/+ | 49 | +/- | +/- | -/- | +/- | +/- | +/+ | +/+ |
| 14 | +/+ | -/- | -/- | -/- | -/- | +/+ | +/+ | 50 | +/+ | -/- | -/- | -/- | -/- | +/+ | +/- |
| 15 | +/+ | -/- | -/- | -/- | +/+ | +/+ | -/- | 51 | +/- | +/- | -/- | +/- | +/- | +/+ | +/+ |
| 16 | +/+ | -/- | -/- | -/- | -/- | +/+ | +/+ | 52 | -/- | +/+ | -/- | +/+ | +/+ | +/+ | +/+ |
| 17 | -/- | +/+ | +/- | +/- | -/- | +/+ | +/+ | 53 | +/+ | -/- | -/- | -/- | -/- | +/- | +/+ |
| 18 | -/- | +/+ | +/+ | -/- | +/+ | +/- | +/+ | 54 | +/- | +/- | -/- | +/- | +/- | +/- | +/+ |
| 19 | +/- | +/- | -/- | +/- | +/- | +/+ | +/- | 55 | +/+ | -/- | -/- | -/- | -/- | +/+ | +/+ |
| 20 | +/+ | -/- | -/- | -/- | -/- | +/+ | +/- | 56 | +/+ | -/- | -/- | -/- | -/- | +/+ | +/- |
| 21 | -/- | +/+ | +/- | +/- | +/- | +/+ | +/+ | 57 | +/- | +/- | -/- | +/- | +/- | +/- | +/- |
| 22 | +/+ | -/- | -/- | -/- | -/- | +/- | +/+ | 58 | +/+ | +/- | -/- | +/- | +/- | -/- | +/+ |
| 23 | +/+ | -/- | -/- | +/- | -/- | +/+ | +/+ | 59 | +/- | +/- | +/- | -/- | +/- | +/+ | +/- |
| 24 | +/+ | -/- | -/- | -/- | -/- | +/- | +/- | 60 | +/- | +/+ | +/- | +/- | +/+ | +/- | +/- |
| 25 | +/+ | -/- | -/- | -/- | -/- | +/+ | +/- | 61 | +/- | +/- | +/- | -/- | +/- | +/+ | +/- |
| 26 | +/+ | -/- | -/- | -/- | -/- | +/+ | +/- | 62 | +/- | +/- | -/- | +/- | +/- | +/- | +/+ |
| 27 | +/- | +/- | +/- | -/- | -/- | +/- | +/- | 63 | +/+ | -/- | -/- | -/- | -/- | +/+ | +/- |
| 28 | +/+ | -/- | -/- | -/- | +/- | +/+ | +/+ | 64 | +/+ | -/- | -/- | -/- | -/- | +/- | +/- |
| 29 | +/- | +/- | +/- | -/- | +/- | +/+ | +/+ | 65 | -/- | +/+ | -/- | +/+ | +/+ | +/- | +/+ |
| 30 | +/- | +/- | -/- | +/- | +/- | +/+ | +/- | 66 | +/+ | -/- | -/- | -/- | -/- | +/- | +/- |
| 31 | -/- | +/+ | +/+ | -/- | +/+ | +/+ | +/+ | 67 | -/- | +/+ | -/- | +/+ | +/+ | +/+ | +/- |
| 32 | +/- | +/- | -/- | +/- | +/- | +/- | +/+ | 68 | +/+ | -/- | -/- | -/- | -/- | +/- | +/- |
| 33 | +/- | +/- | -/- | +/- | +/- | +/- | +/- | 69 | +/- | +/- | +/- | -/- | +/- | -/- | +/- |
| 34 | +/- | +/- | -/- | +/- | +/- | +/+ | +/+ | 70 | +/+ | -/- | -/- | -/- | -/- | +/+ | +/- |
| 35 | -/- | +/+ | -/- | +/+ | +/+ | +/- | +/- | 71 | -/- | +/+ | -/- | +/+ | +/+ | +/+ | +/- |
| 36 | +/- | +/- | +/- | -/- | +/- | +/- | +/- | 72 | +/+ | -/- | -/- | -/- | -/- | +/+ | +/- |

[a](1) ε-Hinc II, (2) Gγ-Hind III, (3) Aγ-Hind III, (4) 5'ψβ-Hinc II, (5) 3'ψβ-Hinc II, (6) Ava II in β, (7) 3'β-Hinf I

[b]Number of chromosomes analyzed is 144, from 72 individuals

obtain the associated haplotype structure for the normal population in the Denizli province (Table 2). According to the results obtained, the most frequent beta globin gene cluster haplotypes were (+----++), (+----+-), (-+-++++), (+-----+), with frequencies of 28.6%, 17.2%, 9.8% and 8.3%, respectively. Since all the beta thalassemia cases were homozygous for the specified mutation, the beta globin gene cluster haplotypes were determined directly from their laboratory results. Haplotypes for the homozygous cases are presented in Table 3. According to these results, IVS-I-110 mutation was linked with the haplotypes (+----++) and (+-----+). Observed haplotypes were (+----++) for FSC 8/9 (+G), (-+-+++-) for IVS-II-1 (G>A), (-+-++-+ and -+-++++) for IVS-I-5 (G>C), (+----+- and +------) for IVS-I-1 (G>A), (-++---+) for IVS-I-6 (T>C), and (+-----+) for FSC 8 (-AA).

## Discussion

Beta globin gene cluster haplotypes are being used to identify the genetic diversity of the specified population and inter-population relationships in both normal and mutated samples [16-18]. Although high frequency of a mutation in a specific population might be accepted as fingerprints of the population movements, the presence of a mutation may not be a reflection of the real biological phenomenon. Molecular characteristics of the beta thalassemia mutations like beta globin gene cluster haplotypes and frameworks might help such efforts by accumulating the data for the specific mutations rather than thalassemia phenotype. Comparisons of sequence variations in normal and mutant globin genes are termed as frameworks, and DNA polymorphism haplotype analysis in beta globin gene clusters is done with the goal of identifying distinctive ethnic mutations and/or tracing the origin and spread of the targeted mutations [19]. The beta globin gene cluster haplotypes of the abnormal hemoglobins, being also beta globin gene defects, were investigated both in Denizli and in Turkey [8]. In this study, we aimed to determine the beta globin gene cluster haplotypes for the beta thalassemia mutations in the Denizli province. Identification of the haplotypes

**Table 2. Beta globin gene cluster haplotypes in association with $\beta^A$ chromosomes in Denizli, Turkey**

| | Haplotype | | 5'-ε | Gγ | Aγ | 5'-ψβ | 3'-ψβ | 5'-β | 3'-β |
|---|---|---|---|---|---|---|---|---|---|
| | Frequency | s.d. | Hinc II | Hind III | Hind III | Hinc II | Hinc II | Ava II | Hinf I |
| 01 | 0.285668 | 0.043438 | + | - | - | - | - | + | + |
| 02 | 0.171933 | 0.038697 | + | - | - | - | - | + | - |
| 03 | 0.098397 | 0.034430 | - | + | - | + | + | + | + |
| 04 | 0.082626 | 0.027988 | + | - | - | - | - | - | + |
| 05 | 0.068167 | 0.027013 | - | + | - | + | + | - | + |
| 06 | 0.063835 | 0.025328 | - | + | + | - | + | + | + |
| 07 | 0.050212 | 0.022172 | - | + | + | - | + | - | + |
| 08 | 0.037376 | 0.021096 | - | + | - | + | + | + | - |
| 09 | 0.026482 | 0.015772 | + | - | - | - | + | + | - |
| 10 | 0.018032 | 0.012517 | - | + | - | + | - | + | + |
| 11 | 0.016374 | 0.013238 | + | - | - | - | + | + | + |
| 12 | 0.014069 | 0.009871 | - | + | + | - | - | - | - |
| 13 | 0.014028 | 0.010862 | + | + | - | + | + | + | - |
| 14 | 0.010754 | 0.009708 | - | + | + | - | - | + | + |
| 15 | 0.007147 | 0.006848 | - | - | - | - | - | - | + |
| 16 | 0.007007 | 0.009248 | + | + | - | + | + | + | + |
| 17 | 0.006992 | 0.006431 | + | - | - | + | - | + | + |
| 18 | 0.006992 | 0.006616 | - | + | - | - | + | + | + |
| 19 | 0.006944 | 0.006581 | - | - | - | - | + | + | + |
| 20 | 0.006944 | 0.007901 | + | + | - | + | + | - | + |
| Sum ➢1.000000 | | (*) Haplotypes were generated by Arlequin 3.1 software | | | | | | | |

s.d.: Standard deviation.

Arlequin settings for the calculations:

-Unknown gametic phase EM calculation

-Epsilon value: 1.000000e-07

-Initial conditions: 50

-Maximum number of iterations: 1000

-Bootstrap replicates: 1000

-Number of gene copies: 144

in the normal population was the first step of our study in order to compare with the beta thalassemia mutations. The second step was to determine the associated haplotypes with the beta thalassemia mutations. Premarital screening has been applied since 1995 in the Denizli province by the Turkish Ministry of Health Denizli Hemoglobinopathy Center. Prenatal diagnosis for the hemoglobinopathies has been applied since 2004 in our province under collaboration with the Denizli Hemoglobinopathy Center and Pamukkale University Medical Faculty (Departments of Biophysics, Hematology and Gynecology). According to our unpublished reports, the number of registered beta thalassemia major cases is around 100 in our province.

Normal population results are the first data to be discussed. In total, 144 normal chromosomes belonging to 72 individuals were included into this study. They were unrelated and all were from the Denizli province. Beta globin gene cluster haplotype analysis of these normal individuals is shown in Table 1. In Table 2, the Arlequin generated data is presented. According to our results, the most frequent haplotypes were (+----++), (+----+-) and (-+-++++), with frequencies of 28.6%, 17.2% and 9.8%, respectively. As far as 5'-haplotypes are concerned, five haplotypes accounted for 89% of these βA chromosomes. These haplotypes were (+----), (-+-++), (-++-+), (-++--) and (----+), with frequencies of 54.1%, 20.3%, 11.4%, 2.5% and 0.7%, respectively. There is limited data for the βA chromosomes in Turkey. Antonarakis et al. [20] stated that only three haplotypes were observed and that haplotype (+----) was the most frequent 5' haplotype among Turkish people, followed in order by (-+-++) and (-++-+). For the normal population, it can be concluded that (+----), (-+-++) and (-++-+) are the dominant 5' haplotypes, which accounted for 85.8% in our province, representing the Mediterranean character of the region. The other haplotypes were added into the gene pool with small contributions.

Beta thalassemia mutations included in this study were IVS-I-110 (G>A), FSC 8/9 (+G), IVS-II-1 (G>A), IVS-I-5 (G>C), IVS-I-1 (G>A), IVS-I-6 (T>C) and FSC 8 (-AA). IVS-I-110 (G>A) is the most prevalent beta thalassemia mutation in our province as well as in Turkey [9]. We selected homozygous cases for this study to be able to determine their associated beta globin gene cluster haplotypes directly from their PCR-based restriction analysis results. Otherwise, family studies and larger sample size would be needed to identify their associated haplotypes. According to the Ministry of Health Denizli Hemoglobinopathy Center, the number of registered beta thalassemia patients is about 100. Many of them are relatives. We also excluded the relatives and double/compound heterozygous cases.

IVS-I-110 (G>A) is the first beta thalassemia mutation to be discussed. The results obtained for this mutation are shown in Table 3. According to the results, most chromosomes (18/24) are associated with the Mediterranean haplotype I [+----++]. On the other hand, haplotype VII [+-----+] is another haplotype associated with the IVS-I-110 (G>A) mutation in the Denizli province. IVS-I-110 (G>A) mutation is mostly associated with haplotype I in Turkey as well as in world populations [21-31]. Haplotypes II, IV and IX were also reported from Turkey [21]. Haplotype VII is reported for the first time in Turkey in this study. Haplotype VII was reported in relation with the IVS-I-110 (G>A) mutation only from Brazilian cases among the world populations [29]. A genetic connection between the Brazilian and Turkish samples is unknown. More molecular data is necessary for clarification of this issue.

The FSC 8 (-AA) mutation was originally detected in a Turkish patient [31]. Agouti et al. [32] stated that the occurrence of this mutation in Ottomans was proven by Filon et al. [33] in the archaeological remains of a child who was homozygous for FSC 8 mutation. This mutation was observed in Middle Eastern countries and found in very low frequencies among the Mediterranean populations, except Morocco, with the frequency of 22.8% [32]. Most FSC 8 mutations are associated with haplotype IV in Greece, Morocco, Israel and Turkey [21,27,32,34]. In Algeria, this mutation is linked with haplotype IX [35]. This mutation is also associated with haplotype IV in the Israeli Arab population [34]. Haplotype VI and haplotype VII were also reported from Morocco [27,32]. Although most FSC 8 mutations were associated with haplotype IV, one case with haplotype VII was previously reported from Turkey [21]. We also observed haplotype VII in the Denizli province (Table 3). Although this mutation is reported from Middle Eastern countries, FSC 8 (-AA) mutation is not observed in the Palestinian population [24]. On the other hand, the FSC 8 (-AA) anomaly is the most frequent allele in Morocco, while it is very rare in Tunisia [36]. Lemsaddek et al. [27] discussed this issue in the model of Morocco. Since the Ottoman Empire never included Morocco and three different haplotypes (IV, VI and VII)

**Table 3. Beta globin gene cluster haplotypes of the homozygous beta thalassemia cases in Denizli, Turkey**

| Mutation | Haplotype Loci | | | | | | | Hp |
|---|---|---|---|---|---|---|---|---|
| | 5'-ε Hinc II | Gγ Hind III | Aγ Hind III | 5'-ψβ Hinc II | 3'-ψβ Hinc II | 5'-β Ava II | 3'-β Hinf I | |
| IVS-I-110 (G>A) (n=6) | +/+ | -/- | -/- | -/- | -/- | +/- | +/+ | I / VII |
| IVS-I-110 (G>A) (n=6) | +/+ | -/- | -/- | -/- | -/- | +/+ | +/+ | I / I |
| Fsc 8/9 (+G) (n=1) | +/+ | -/- | -/- | -/- | -/- | +/+ | +/+ | I / I |
| IVS-II-1 (G>A) (n=1) | -/- | +/+ | -/- | +/+ | +/+ | +/+ | -/- | III / III |
| IVS-I-5 (G>C) (n=1) | -/- | +/+ | -/- | +/+ | +/+ | -/- | +/+ | IV / IV |
| IVS-I-5 (G>C) (n=1) | -/- | +/+ | -/- | +/+ | +/+ | +/- | +/+ | IV / IX |
| IVS-I-1 (G>A) (n=1) | +/+ | -/- | -/- | -/- | -/- | +/- | -/- | V / VIIa |
| IVS-I-6 (T>C) (n=3) | -/- | +/+ | +/+ | -/- | -/- | -/- | +/+ | VI / VI |
| Fsc 8 (-AA) (n=2) | +/+ | -/- | -/- | -/- | -/- | -/- | +/+ | VII / VII |

Hp: Associated haplotype

are present in Morocco, they could not draw reliable conclusions, but they also hypothesized that this mutation did not originate in Turkey.

The substitution (G>C) at IVS-I position 5 was previously reported in Chinese and Asian-Indian populations and is widespread in Arab countries. This mutation is practically non-existent in Northern Africa. However, the mutation in Arabs is found on a beta globin haplotype distinct from the Asian-Indian one, and is therefore believed to have an independent origin [37-39]. Several mutations described in Eastern Turkey are thought to be of Asian-Indian origin (e.g., FSC-8/9, IVS-I-5, Cd15, and others) [40,41]. The IVS-I-5 (G>C) mutation is the most frequent mutation in the United Arab Emirates (UAE) constituting 60.2% of the entire beta thalassemia chromosomes in the indigenous UAE population. This mutation is thought to have been introduced to UAE via gene migration from Baluchistan, Pakistan and the Indian subcontinent [42]. For the UAE cases, no haplotype was reported confirming this approach. Beta thalassemia alleles for the IVS-I-5 (G>C) mutation are mostly associated with haplotype VII [38]. In the previous studies, this mutation was found to be linked to RFLP haplotype IX from Turkey [21] and in a Lebanese homozygote, and thus was suggested to have a different origin [28,43]. IVS-I-5 (G>C) mutation is linked with haplotypes I and V in Brazilian beta thalassemia patients [29]. In India, this mutation is the most common beta thalassemia allele, at a frequency of 45%. IVS-I-5 (G>C) mutation is considered to be the oldest beta thalassemia allele in India [44,45]. Most Indian alleles are associated with haplotype VII [44]. Italia et al. [46] also observed that this mutation is linked with four different haplotypes in India as [-+-++-+], [+------+], [------+] and [-++-+-+]. In our case, we observed one haplotype IX and three haplotype IV out of four chromosomes carrying IVS-I-5 (G>C) mutation (Table 3). As regards beta globin gene cluster haplotypes, our results showed that the Indian-Asian connection is valid for the IVS-I-5 (G>C) mutation linked with haplotype IV [-+-++-+]. Furthermore, a Mediterranean connection is observed with haplotype IX. One should also consider that our normal population is strongly linked with 5'-haplotype [-+-++] in the Denizli province (Table 2).

IVS-I-1 (G>A) mutation is found mostly in Berbers in Algeria associated with haplotypes I, III, V in Italy, and IX, and is also observed in Morocco in haplotypes IV, V and IX [26]. This mutation is found in association with haplotypes III, V and IX in Portugal, haplotypes II, III and IV, and haplotype V in Brazil [25, 27,29]. In Lebanon, IVS-I-1 (G>A) is the second most frequent beta thalassemia mutation, with an incidence of 15.0% and is linked with the beta globin gene cluster haplotype V [28]. This mutation was found to be predominant in Hungary and Czechoslovakia (Czech Republic and Slovakia), with frequencies of 29.4% and 45.2%, respectively; there is a possibility that it may have originated in Eastern Europe [28,47,48]. Unfortunately, the beta globin gene cluster haplotype data regarding the Hungarian and Czechoslovakian (Czech Republic and Slovakia) samples is not published. We observed haplotypes V and VIIa in our samples in the Denizli province (Table 3). Haplotype V was also reported from Turkey previously [21]. It should also be considered that our haplotypes are associated with the 5'-haplotype [+----] of our normal population, with a frequency of 54.1%. As a general conclusion, IVS-I-1 (G>A) mutation is associated with haplotype V and has a Mediterranean character in the Denizli province gene pool with dominance in the normal population.

The IVS-I-6 (T>C) mutation is widespread in the Mediterranean basin, with a frequency of <10% except in the West Bank Palestinian Authority (48.5%) and Republic of Macedonia (18.1%), Morocco (14.8%), Egypt (17.6%), and Lebanon (14.4%) [24,28,36,49,50]. Beta globin gene cluster haplotypes of this mutation are linked with haplotypes VI and VII in Israeli and Palestinian Arab populations [24,34,51]. This mutation was also observed in a Samaritan (a genetic isolate in Israel for at least 2700 years) family linked with haplotype VI [33]. The IVS-I-6 (T>C) mutation is linked with haplotypes VI and VII in Morocco as described in Portugal, whereas in Algeria, Tunisia, Egypt and Brazil, it is linked with haplotype VI, and in Italy with haplotypes VI, IV, VII and X [25-27,29,32]. In Lebanon, the linkages of the haplotypes with the IVS-I-6 (T>C) mutation differ according to religious groups (Sunnis, Shiites, Druze, Maronites, Orthodox and Catholics). Haplotype VI is observed in all groups in the Lebanese population. Haplotype VII is linked with the mutation in Sunnis and Shiites. On the other hand, haplotype I is also observed only in Sunnis and Catholics [28]. In Turkey, most IVS-I-6 (T>C) mutations are linked with haplotype VI, but VII was also reported in one homozygous case [21]. All chromosomes carrying the IVS-I-6 (T>C) mutation are associated with haplotype VI in the Denizli province (Table 3). It should also be considered that our haplotypes are in association with the 5'-haplotype [-++--] of our normal population, with a frequency of 2.5%. This mutation is linked with haplotype VI as observed in all Mediterranean populations and was introduced to the Denizli province gene pool by gene flow throughout history.

FSC 8/9 (+G) mutation was firstly described in Asian populations [19,38]. FSC 8/9 (+G) is endemic in the northwestern regions of India and Pakistan and found only in the Muslim Indian population [44]. Several mutations (like FSC 8/9, IVS-I-5 etc) described in eastern Turkey are also thought to be of Asian Indian origin [41]. Although Persians had very little impact on the structure of the Anatolian population, the development of extensive trade routes at that time might have facilitated the introduction of beta thalassemia mutations common in Asian-Indian populations into Anatolia through Eastern parts [41,52]. As far as the beta globin gene cluster haplotypes are concerned, this mutation is linked with haplotype I in the Shiite population in Lebanon [28], the Palestinian population [24] and in Koreans [53,54]. No published haplotype data is available for the Turkish population. According to our results, haplotype I is associated with the FSC 8/9 (+G) mutation in the Denizli province as well as in the world populations (Table 3).

The last mutation to be discussed is IVS-II-1 (G>A) mutation in the Denizli province. This mutation is also observed in the Mediterranean populations and is known as Mediterranean type. IVS-II-1 (G>A) mutation is detected in all Arab countries except Tunisia and Algeria, has a high frequency in North Jordan (20%), and is the most common mutation in Kuwait (29%) [39]. Although Zahed et al. [39] stated that this mutation is absent in Tunisia, it was also reported from Tunisia [36,55]. For the Algerian cases, Boudrahem-Addour et al. [35] reported

that the IVS-II-1 (G>A) mutation is linked with haplotype III. Haplotype III is mostly linked with this mutation observed in Morocco [26,32], Italy [22,25] and Lebanon [28]. In Israel, haplotype I (Druze ethnic group and Jews), haplotype V (Jews) and haplotype III (Arabs) were found to be associated with this mutation [34]. In the Palestinian population, haplotype I was reported in connection with IVS-II-1 (G>A) [24]. In Turkey, haplotypes III and V were reported in linkage with this mutation [21]. In our province, we observed only haplotype III, which is mostly found in the Mediterranean populations (Table 3).

In conclusion, we analyzed beta globin gene cluster haplotypes in association with the beta thalassemia mutations at a provincial level in Turkey. Basically, our region shows the Mediterranean characterization for the beta thalassemia mutations. We report haplotype VII in association with IVS-I-110 (G>A) mutation for the first time in Turkey. This haplotype was reported only from Brazil, and the genetic connection between Turkish and Brazilian haplotypes is not known at present. Molecular research of the beta thalassemia mutations at a regional/provincial level will contribute to an understanding of the mutation mechanisms and future possible molecular therapeutic approaches. While considering the personalization of the therapeutic approaches, molecular vision becomes a more critical issue in hemoglobinopathy research. The relationships with beta globin gene cluster haplotypes and other single nucleotide polymorphisms (SNPs) with Hb F induction by hydroxyurea (HU) treatment is a controversial issue for beta thalassemia therapy. Although there are many association studies published [56-60], an exact conclusion cannot be drawn and the matter remains under discussion. This might have arisen from the complexity of the genome while interaction with many gene(s) and gene products other than beta globin locus. Aleubouyeh et al. [58] stated that the obtained data imply the importance of genetic surveys helping to identify those patients who may be appropriate candidates for the present and novel therapeutic interventions. Beta thalassemias should be studied in more detail including beta globin gene cluster haplotypes and SNPs and also at a genomic level in order to understand and develop novel therapeutic strategies. Liu et al. [61] suggested that high density SNP mapping might be required to be able to define beta globin gene cluster haplotypes in correlation with different clinical phenotypes in sickle cell disease. A similar approach could be valid also for the beta thalassemias. Our results are the first reported beta globin gene cluster haplotypes at a provincial level in Turkey. Such studies should be done more extensively and in collaboration with other research institutions on a national level to develop novel diagnostic and therapeutic strategies and also to clarify the mutation mechanisms of the beta thalassemia mutations in Turkey.

**Acknowledgement**

This work was supported by Pamukkale University Research Fund project, Nos 2005SBE001, 2005SBE002, 2006SBE004 and 2008SBE001.

## References

1. Altay Ç. The frequency and distribution pattern of β-thalassemia mutations in Turkey. Turk J Hematol 2002;19:309-15.
2. Keskin A, Turk T, Polat A, Koyuncu H, Saracoglu B. Premarital screening of beta-thalassemia trait in the province of Denizli, Turkey. Acta Hematol 2000;104:31-3.
3. Bolaman Z, Enli Y, Köseoğlu M, Koyuncu H, Aslan D. Prevalence of beta thalassemia trait in Denizli. Turk J Hematol 2001;18:85-8.
4. Sözmen M, Uysal Z, Yeşil N, Akar N, Arcasoy A. Denizli'de anormal hemoglobin ve hemoglobin A2 yüksekliği ile karakterize beta talasemi taşıyıcılığı araştırması. Ankara Tıp Fakültesi Mecmuası 1990;43:959-64.
5. Kazazian HH Jr, Orkin SH, Markham AF, Chapman CR, Youssoufian H, Waber PG. Quantification of the close association between DNA haplotypes and specific beta-thalassaemia mutations in Mediterraneans. Nature 1984;310:152-4.
6. Antonarakis SE, Orkin SH, Kazazian HH, Goff SC, Boehm CD, Waber P, Sexton JP, Ostrer H, Fairbanks VF, Chakravarti A. Evidence for multiple origins of the βE globin gene in Southeast Asia. Proc Natl Acad Sci USA 1982;79:6608-11.
7. Smith RA, Ho PJ, Clegg JB, Kidd JR, Thein SL. Recombination break points in the human beta globin gene cluster. Blood 1998;11:4415-21.
8. Öztürk O, Atalay A, Köseler A, Özkan A, Koyuncu H, Bayram J, Demirtepe S, Aksoy K, Atalay EÖ. Beta globin gene cluster haplotypes of abnormal hemoglobins observed in Turkey. Turk J Hematol 2007;24:146-54.
9. Yıldız S, Atalay A, Bağcı H, Atalay EÖ. Beta thalassemia mutations in Denizli province of Turkey. Turk J Hematol 2005;22:19-23.
10. Atalay EÖ, Koyuncu H, Turgut B, Atalay A, Yıldız S, Bahadir A, Köseler A. High incidence of Hb D-Los Angeles [β121(GH4) Glu>Gln] in Denizli province, Aegean region of Turkey. Hemoglobin 2005;29:307-10.
11. Atalay A, Koyuncu H, Köseler A, Özkan A, Atalay EÖ. Hb Beograd [β121(GH4)Glu>Val, GAA>GTA] in the Turkish population. Hemoglobin 2007;31:491-3.
12. Atalay EÖ, Atalay A, Koyuncu H, Öztürk O, Köseler A, Özkan A, Demirtepe S. Rare hemoglobin variant Hb Yaizu observed in Turkey. Med Princ Pract 2008;17:321-4.
13. Bahadır A, Köseler A, Atalay A, Koyuncu H, Akar E, Akar N, Atalay EÖ. Hb D Los Angeles [beta121(GH4)Glu>Gln] and Hb Beograd [beta121(GH4)Glu>Val]: implications for their laboratory diagnosis and genetic origins. Turk J Hematol 2009;26:17-20.
14. Falchi A, Giovannoni L, Vacca L, Latini V, Vona G, Varesi L. β-Globin gene cluster haplotypes associated with β-thalassemia on Corsica Island. Am J Hematol 2005;78:27-32.
15. Excoffier LG, Laval LG, Schneider S. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 2005;1:47-50.
16. De Lugo MV, Rodriguez-Larralde A, De Guerra C. Beta globin gene cluster haplotypes as evidence of African gene flow to the northeastern coast of Venezuela. Am J Hum Biol 2003;15:29-37.
17. Alcantara LC, Van Dooren S, Goncalves MS, Kashima S, Costa MCR, Santos FLN, Bittencourt AL, Dourado I, Filho AA, Covas DT, Vandamme AM, Galvao-Castro B. Globin haplotypes of human T-cell lymphotropic virus type I-infected individuals in Salvador, Bahia, Brazil, suggest a post-Columbian African origin of this virus. JAIDS 2003;33:536-42.
18. Currat M, Trabuchet G, Rees D, Perrin P, Harding RM, Clegg JB, Langaney A, Excoffier L. Molecular analysis of the beta globin gene cluster in the Niokholo Mandenka population reveals a recent origin of the beta S Senegal mutation. Am J Hum Genet 2002;70:207-23.
19. Wong C, Antonarakis SE, Goff SC, Orkin SH, Boehm CD, Kazazian HH. On the origin and spread of β-thalassemia: recurrent observation of four mutations in different ethnic groups. PNAS 1986;83:6529-32.

20. Antonarakis SE, Boehm CD, Giardina PJV, Kazazian HH. Nonrandom association of polymorphic restriction sites in the β-globin gene cluster. PNAS 1982;79:137-41.
21. Diaz-Chico JC, Yang KG, Stoming TA, Efremov DG, Kutlar A, Kutlar F, Aksoy M, Altay C, Gurgey A, Kilinc Y, Huisman THJ. Mild and severe beta thalassemia among homozygotes from Turkey: identification of the types by hybridization of amplified DNA with synthetic probes. Blood 1988;71:248-51.
22. Pirastu M, Saglio G, Camaschella C, Loi A, Serra A, Bertero T, Gabutti W, Cao A. Delineation of specific beta thalassemia mutations in high-risk areas of Italy: a prerequisite for prenatal diagnosis. Blood 1988;71:983-8.
23. Zahed L, Demont J, Bouhass R, Trabuchet G, Hanni C, Zalloula P, Perrin P. Origin and history of the IVS-I-110 and codon 39 beta thalassemia mutations in the Lebanese population. Hum Biol 2002;74:837-47.
24. El-Latif MA, Filon D, Rund D, Oppenheim A, Kanaan M. The $\beta^+$-IVS-I-6 (T>C) mutation accounts for half of the thalassemia chromosomes in the Palestinian populations of the mountain regions. Hemoglobin 2002;26:33-40.
25. Ferrara M, Matarese SMR, Francese M, Borrelli B, Perrotta A, Meo A, La Rosa MA, Esposito L. Role of polymorphic sequences 5' to the Gγ gene and 5' to the β gene on the homozygous β thalassemic phenotype. Hemoglobin 2003;27:167-75.
26. Lemsaddek W, Picanco I, Seuanes F, Mahmal L, Benchekroun S, Khattab M, Nogueira P, Osorio-Almeida L. Spectrum of beta thalassemia mutations and Hb F levels in the heterozygous Moroccan population. Am J Hematol 2003;73:161-8.
27. Lemsaddek W, Picanco I, Seuanes F, Nogueira P, Mahmal L, Benchekroun S, Khattab M, Osorio-Almeida L. The beta thalassemia mutation/haplotype distribution in the Moroccan population. Hemoglobin 2004;28:25-37.
28. Makhoul NJ, Wells RS, Kaspar H, Shbaklo H, Taher A, Chakar N, Zalloua PA. Genetic heterogeneity of beta thalassemia in Lebanon reflects historic and recent population migration. Ann Hum Genet 2005;69:55-66.
29. Martins JTN, Bordin S, De Albuquerque DM, Saad STO, Costa FF. Dnase I hypersensitive site 3' to the beta globin gene cluster containing two TAA insertions and a G>A polymorphism is predominantly associated with the beta+ thalassemia IVS-I-6 (T>C) mutation. Hemoglobin 2005;29:85-9.
30. Knott M, Ramadan KMA, Savage G, Jones FGC, El-Agnaf M, McMullin MF, Percy MJ. Novel and Mediterranean beta thalassemia mutations in the indigenous Northern Ireland population. Blood Cells Mol Dis 2006;36:265-8.
31. Orkin SH, Goff SC. Nonsense and frameshift mutations in β-thalassemia detected in cloned β-globin genes. J Biol Chem 1981;256:9782-4.
32. Agouti I, Badens C, Abouyoub A, Khattab M, Sayah F, Barakat A, Bennani M. Genotypic correlation between six common β-thalassemia mutations and the Xmn I polymorphism in the Moroccan population. Hemoglobin 2007;31:141-9.
33. Filon D, Faerman M, Smith P, Oppenheim A. Sequence analysis reveals a beta thalassemia mutation in the DNA of skeletal remains from the archaeological site of Akhziv, Israel. Nat Genet 1995;9:365-8.
34. Filon D, Oron V, Kricheevski S, Shaag A, Shaag Y, Warren TC, et al. Diversity of β-globin mutations in Israeli ethnic groups reflects recent historic events. Am J Hum Genet 1994;54:836-43.
35. Boudrahem-Addour N, Zidani N, Carion N, Labie D, Belhani M, Beldjord C. Molecular heterogeneity of beta thalassemia in Algeria: how to face up to a major health problem. Hemoglobin 2009;33:24-36.
36. Fattoum S, Messaoud T, Bibi A. Molecular basis of β-thalassemia in the population of Tunisia. Hemoglobin 2004;28:177-87.
37. Cheng TC, Orkin SH, Antonarakis SE, Potter MJ, Sexton JP, Markham AF, et al. β-Thalassemia in Chinese: use of in vivo RNA analysis and oligonucleotide hybridization in systematic characterization of molecular defects. Proc Natl Acad Sci USA 1984;81:2821-5.
38. Kazazian HH, Orkin SH, Antonarakis SE, Sexton JP, Boehm CD, Goff SC, Waber PG. Molecular characterization of seven β-thalassemia mutations in Asian Indians. EMBO J 1984;3:593-6.
39. Zahed L. The spectrum of β-thalassemia mutations in the Arab populations. J Biomed Biotech 2001;1:129-32.
40. Öner AF, Özer R, Üner A, Arslan Ş, Gümrük F. Beta thalassemia mutations in the east of Turkey. Turk J Haematol 2001;18:239-42.
41. Tadmouri GO, Garguier N, Demont J, Perrin P, Basak AN. History and origin of beta thalassemia in Turkey: sequence haplotype diversity of beta globin genes. Hum Biol 2001;73:661-74.
42. Baysal E. Hemoglobinopathies in the United Arab Emirates. Hemoglobin 2001;25:247-53.
43. Chehab FF, Der Kaluostian V, Khouri FP, Deeb SS, Kan YW. The molecular basis of β-thalassemia in Lebanon: application to prenatal diagnosis. Blood 1987;69:1141-5.
44. Bandyopadhyay A, Bandyopadhyay S, Chowdhury MD, Dasgupta UB. Major beta-globin gene mutations in Eastern India and their associated haplotypes. Hum Hered 1999;49:232-5.
45. Kukreti R, Dash D, Vineetha KE, Chakravarty S, Das SK, De M, Talukder G. Spectrum of β-thalassemia mutations and their association with allelic sequence polymorphisms at the β-globin gene cluster in an Eastern Indian population. Am J Hematol 2002;70:269-77.
46. Italia K, Jain D, Gattani S, Jijina F, Nadkarni A, Sawant P, Nair S, Mohanty D, Ghosh K, Colah R. Hydroxyurea in sickle cell disease-a study of clinico-pharmacological efficacy in the Indian haplotype. Blood Cells Mol Dis 2009;42:25-31.
47. Ringelhann B, Szelenyi JG, Horanyi M, Svobodova M, Divoky V, Indrak K, Hollan S, Marosi A, Laub M, Huisman TH. Molecular characterization of beta thalassemia in Hungary. Hum Genet 1993;92:385-7.
48. Indrak K, Brabec V, Indrakova J, Chrobak L, Sakalova A, Jarosova M, Cermak J, Fei YJ, Kutlar F, Gu YC, Baysal E, Huisman THJ. Molecular characterization of beta thalassemia in Czechoslovakia. Hum Genet 1992;88:399-404.
49. Efremov GD. Thalassemias and other hemoglobinopathies in the Republic of Macedonia. Hemoglobin 2007;31:1-15.
50. Hussein G, Fawzy M, El Serafi T, Ismail EF, El Metwally D, Saber MA, Giansily M, Schwed JF, Pissard S, Martinez PA. Rapid detection of β-thalassemia alleles in Egypt using naturally or amplified created restriction sites and direct sequencing: a step in disease control. Hemoglobin 2007;31:49-62.
51. Rund D, Oron-Karni V, Filon D, Goldfarb A, Rachmilewitz E, Oppenheim A. Genetic analysis of β-thalassemia in Israel: diversity of mechanisms and unpredictability of phenotype. Am J Hematol 1997;54:16-22.
52. Tadmouri GO, Başak AN. β-thalassemia in Turkey: a review of the clinical, epidemiological, molecular and evolutionary aspects. Hemoglobin 2001;25:227-39.
53. Lee YJ, Park SS, Kim JY, Cho HI. RFLP haplotypes of β-globin gene complex of β-thalassemic chromosomes in Koreans. J Korean Med Sci 2002;17:475-8.
54. Park SS, Lee YJ, Kim JY, Joo SI, Hattori Y, Ohba Y, Cho HI. β-Thalassemia in the Korean population. Hemoglobin 2002;26:135-45.

55. Chouk I, Ben Daoud B, Mellouli F, Bejaoui M, Gerard N, Dellagi K, Abbes S. Contribution to the description of the β-thalassemia spectrum in Tunisia and the origin of mutation diversity. Hemoglobin 2004;28:189-95.
56. Patrinos GP, Grosveld FG. Pharmacogenomics and therapeutics of hemoglobinopathies. Hemoglobin 2008;32:229-36.
57. Yavarian M, Karimi M, Bakker E, Harteveld CL, Giordano PC. Response to hydroxyurea treatment in Iranian transfusion-dependent β-thalassemia patients. Haematologica 2004;89:1172-8.
58. Aleubouyeh M, Moussavi F, Haddad-Deylami H, Vossough P. Hydroxyurea in the treatment of major β-thalassemia and importance of genetic screening. Ann Hematol 2004;83:430-3.
59. Dedoussis GVZ, Mandilara GD, Boussiu M, Loutradis A. Hb F production in β-thalassemia heterozygotes for the IVS-II-1 G>A β°-globin mutation. Implication of the haplotype and the Gγ-158 C>T mutation on the Hb F level. Am J Hematol 2000;64:151-5.
60. De Angioletti M, Lacerra G, Pagano L, Alessi M, D'Avino R, Manca L, Carestia C. β-thalassemia -87 C>G: relationship of the Hb F modulation and polymorphisms in compound heterozygous patients. Br J Haematol 2004;126:743-9.
61. Liu L, Muralidhar S, Singh M, Sylvan C, Kalra IS, Quinn CT, Onyekwere OC, Pace BS. High-density SNP genotyping to define beta globin locus haplotypes. Blood Cells Mol Dis 2009;42:16-24.