RFLP Haplotypes of β-Globin Gene Complex of β-Thalassemic Chromosomes in Koreans

Korea is in the low-prevalence area of β-thalassemia and the Korean population has relatively homogenous racial characteristics. Recently, we identified some causative mutations of the Korean β-thalassemia patients. In order to elucidate the genetic background of β-thalassemia alleles in Koreans, we determined the restriction fragment length polymorphism (RFLP)-haplotype and framework (FW) in nine β-thalassemia chromosomes of five different causative mutations by PCR-based method and family linkage study. The result that the haplotype and the framework linked to the initiation codon ATG→AGG mutation were →+++-+ and FW3A, respectively, in all of three families in this study suggests a common origin of this mutation at least in Koreans. A novel β-thalassemia mutation, codons 89/90 -TG, showed discrepancy between →+++-+ and FW1, which could be explained by gene conversion. A case of codons 8/9 +G frameshift mutation had +----++ and FW1. The linkage of the two β-thalassemia mutations, codon 17 AAG→TAG and codons 41/42 -TTCT, with specific haplotypes and frameworks common to the Koreans and the neighboring countries suggests that those mutations are influenced by the genetic flow from the south China.

Key Words: Haplotypes; Framework; β-Thalassemia; β-Globin gene; Korea

INTRODUCTION

A limited number of RFLP-haplotypes of the β-globin gene cluster have been demonstrated in some ethnic groups (1, 2) and Orkin et al. revealed a nonrandom association of specific β-thalassemic mutations with particular RFLP-haplotypes and frameworks (3). These polymorphisms were used as diagnostic tools for β-thalassemia, which is one of the most common genetic diseases worldwide, and for anthropological studies (4, 5).

The Korean population has relatively homogenous racial characteristics and Korea is in the low-prevalence area of β-thalassemia. The β-thalassemia gene frequency in Koreans is roughly assumed to be about 0.1% or less as in Japanese (6). An analysis of β-haplotypes is very useful for the genetic epidemiological study in this region, however, these characteristics have not been investigated in β-thalassemia chromosomes of the Korean population.

To elucidate the molecular background of the β-globin gene clusters in Koreans, we examined the haplotypes and frameworks of the β-thalassemia chromosomes by PCR-based method and family linkage study, and compared the results with those of other ethnic groups (7, 8).

MATERIALS AND METHODS

Subjects

Nine unrelated Korean β-thalassemia patients and their family members were included in this study. The patients had five types of β-thalassemic mutations: the initiation codon ATG→AGG, codon 17 AAG→TAG, codons 41/42 -TTCT, codons 8/9 +G, and codons 89/90 -TG, all of which were heterozygous mutations (9-11).

Analysis of the RFLP haplotypes and the frameworks

We determined the β-haplotypes by family linkage study using PCR and restriction enzyme digestions of seven polymorphic sites: Hind II 5' to g, Hind III in 6', Hind III in 8', Hind II 5' in and 3' to ψ6, Ava II in β, and Bam HI 5' to β (1-3, 7, 12) (Fig. 1). Oligonucleotide primers used for the amplification of the seven polymorphic sites are shown in Table 1. The β-haplotypes were constructed on the basis of appropriate family studies. The seven polymorphic restriction enzyme sites analyzed were clustered in two groups: 5' and 3' subhaplotypes. 5' subhaplotype consisted of the first five RFLP
sites located 5′ to the β-globin gene, and 3′ subhaplotype was made up of the last two RFLP sites located within the β-globin gene and 3′ to it (2, 13).

In order to assign the frameworks of the β-globin genes linked to the β-thalassemia mutation, we conducted a direct cloning of the amplified whole β-globin gene using TA cloning kits (Invitrogen Co. Carlsbad, CA, U.S.A.) and subsequently analyzed the sequences of the five polymorphic sites, a pseudogene, respectively. A relatively increased rate of recombination between β- and δ-globin genes has led to the subdivision of the full haplotype into 5′ and 3′ subhaplotypes.

**RESULTS**

Haplotypes of nine β-thalassemia chromosomes were determined (Table 2). Three cases of the initiation codon ATG→AGG mutation, which is a common β-thalassemic mutation in Koreans, were linked to the haplotype 6+ and FW3A. Two cases of codon 17 AAG→TAG nonsense mutation were linked to ++++ and FW3A, and a case of

![Image](image-url)

**Fig. 1.** Representative seven RFLP sites in β-globin gene cluster on chromosome 11. Filled boxes and a open box indicate functional and a pseudogene, respectively. A relatively increased rate of recombination between β- and δ-globin genes has led to the subdivision of the full haplotype into 5′ and 3′ subhaplotypes.

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**Fig. 2.** Frameworks of β-globin gene and their association with Bam HI site 3′ to β-globin gene.

| β-globin gene framework type | Number/β* alleles |
|-----------------------------|--------------------|
| Initiation codon ATG→AGG    |                   |
| Codon 17 A→T                | ++      (+)       |
| Codons 41/42-TTCT           | ++++    (+)       |
| Codons 8/9+G                | ++++    (+)       |
| Codons 89/90-TG             | ++++    (+)       |
| Total                       | 9                   |

* The presence (+) or absence (-) of seven RFLP sites along each allele is presented in 5′ to 3′ order: Hinc II 5′ to 5, Hind III in γ, Hind III in γ′, Hinc II 5′ in and 3′ to ϕβ, Ava II in β, and Bam HI 3′ to β.

**Table 1. Oligonucleotide primers used for PCR analysis of seven RFLP sites in β-globin gene cluster**

| RFLP site | Name (bp) | Primer sequence | Product size (bp) | After enzyme cut (bp) |
|-----------|-----------|-----------------|-------------------|-----------------------|
| Hinc II 5′ | RF1       | 5′-CCTCCCAAGTGAGAAGTATAAAGCAG-3′ | 710               | 425+285*               |
| Hinc II 5′ | RF2       | 5′-AGTCTTTTGGCTCAAGGCTGCTG-3′ | 710               | 425+285*               |
| Hind III 4′ | RF3      | 5′-CAATTGAAACATTGGGCTGAGTAT-3′ | 1,202             | 970+232, 273+697+232   |
| Hind III 4′ | RF4      | 5′-CCTCTTGTGCCCTGACAGCTATGT-3′ | 1,042             | 933+109, 252+681+109   |
| Hind III 4′ | RF5      | 5′-CAATTGAAACATTGGGCTGAGTAT-3′ | 996               | 996                   |
| Hind III 4′ | RF6      | 5′-CTTCTGTGCCCTGACAGCTATGT-3′ | 996               | 996                   |
| Hind III 4′ | RF7      | 5′-CAATTGAAACATTGGGCTGAGTAT-3′ | 996               | 996                   |
| Hind III 4′ | RF8      | 5′-CTTCTGTGCCCTGACAGCTATGT-3′ | 996               | 996                   |
| Hind III 4′ | RF9      | 5′-CAATTGAAACATTGGGCTGAGTAT-3′ | 996               | 996                   |
| Hind III 4′ | RF10     | 5′-CAATTGAAACATTGGGCTGAGTAT-3′ | 996               | 996                   |
| Ava II 3′ | RF11     | 5′-CAATTGAAACATTGGGCTGAGTAT-3′ | 996               | 996                   |
| Bam HI 3′ | RF12     | 5′-CAATTGAAACATTGGGCTGAGTAT-3′ | 996               | 996                   |

* Underlined fragments of PCR product after enzyme digestion indicate RFLPs.
\(\beta\)-thalassemia Haplotypes in Koreans

**DISCUSSION**

\(\beta\)-thalassemia is not common in countries of a temperate climate, such as Britain, northern Europe, Japan and Korea, possibly due to the absence of selection in favor of the \(\beta\)-thalassemia genes. We identified some \(\beta\)-thalassemia mutations previously and analyzed their genetic background in this study.

Shimizu et al. previously analyzed \(\beta\)-haplotypes and frameworks in healthy Koreans and identified the three common \(\beta\)-subhaplotypes: \(++++++\), \(++++++\), and \(+++++++\) (15-17). Similar findings were also observed in the studies of Japanese and Chinese (18-21).

Although the number of \(\beta\)-haplotypes and frameworks analyzed in \(\beta\)-thalassemia chromosomes was small, there were some clues of genetic flow of \(\beta\)-thalassemic alleles in the East Asia. The codon 17 AAG→TAG nonsense mutation and the codons 41/42 -TTCT are the two most common mutations in south China and the Southeast Asia (22). These results suggest a common origin of this mutation at least in Koreans. Unfortunately, no comparable haplotype data of the initiation codon mutation are available in the Chinese and the Japanese.

A novel \(\beta\)-thalassemic mutation found in a Korean patient, codons 89/90-GT, was associated with the haplotype \(++++++\) and FW1. As \(3\) subhaplotypes \(+\) and \(++\) have been known to correspond to FW1 and FW2, respectively, the discrepancy between the haplotype and framework of this mutation could be explained by gene conversion (23).

In conclusion, we identified the genetic background of \(\beta\)-thalassemia chromosomes in Koreans by family linkage analysis.

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