J Korean Med Sci 2002; 17: 475-8
ISSN 1011-8934

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*

Young-Joon Lee*†, Sung Sup Park*, Ji Yeon Kim*, Han-Ik Cho*

Department of Clinical Pathology*, Seoul National University College of Medicine, Seoul National University Hospital Clinical Research Institute, Seoul; Department of Clinical Pathology†, National Cancer Center, Koyang, Korea

Received : 16 July 2001
Accepted : 29 March 2002

Address for correspondence
Sung Sup Park, M.D.
Department of Clinical Pathology, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-gu, Seoul 110-744, Korea
Tel : +82.2-760-1702, Fax : +82.2-760-3206
E-mail : sparkle@plaza.snu.ac.kr

*This study was financially supported by the Korea Research Foundation made in the program year of 1996 (1996-F1001-F0081).

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: *Hin*c II 5′ to ε, *Hin*d III in $^{G}\gamma$, *Hin*d III in $^{A}\gamma$, *Hin*c II 5′ in and 3′ to ψβ, *Ava* II in β, and *Bam* HI 3′ 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 δ-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, codon 2 (CAC/CAT), IVS-II-16 (C/G), IVS-II-74 (G/T), IVS-II-81 (C/T) and IVS-II-666 (T/C), in the clones that carried the causative mutation (2, 3). Then we compared these cloning results with deduced frameworks of the β-thalassemia chromosomes from 3′ subhaplotype data, because the RFLPs of the *Ava* II site within the β-globin gene and the *Bam* HI site 3′ to the β-globin gene are closely linked to frameworks (3, 14) (Fig. 2).

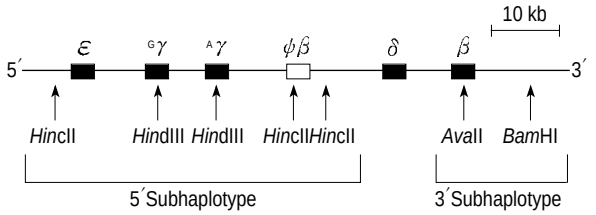

Fig. 1. Representative seven RFLP sites in β-globin gene cluster on chromosome 11. Filled boxes and a open box indicate functional genes and a pseudogene, respectively. A relatively increased rate of recombination between δ- and β-globin genes has led to the subdivision of the full haplotype into 5′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

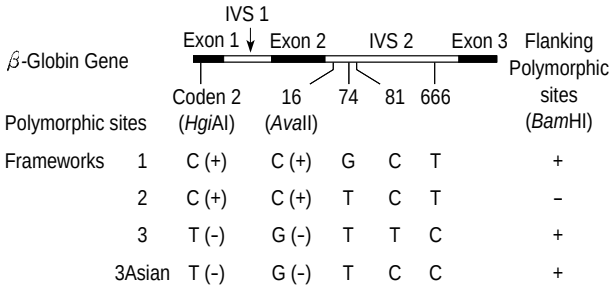

Fig. 2. Frameworks of β-globin gene and their association with *Bam* HI site 3′ to β-globin gene.

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 -+-++-+ and FW3A. Two cases of codon 17 AAG→TAG nonsense mutation were linked to +-----+ and FW3A, and a case of

Table 2. β-thalassemia mutations in Koreans and the associated frameworks and haplotypes

β-thalassemic mutation	β-haplotype*	β-globin gene framework type	Number β^{Thal} alleles
Initiation codon ATG→AGG			
	-+-++ -+	FW3A	3
Codon 17 A→T	+---- -+	FW3A	2
Codons 41/42-TTCT	+---- ++	FW1	1
	+---- -+	FW3A	1
Codons 8/9+G	+---- ++	FW1	1
Codons 89/90-TG	-++-+ +-	FW1	1
Total			9

*: The presence (+) or absence (-) of seven RFLP sites along each allele is presented in 5′ to 3′ order: *Hinc* II 5′ to ε, *Hind* III in $^{G}\gamma$, *Hind* III in $^{A}\gamma$, *Hinc* II 5′ in and 3′ to ψβ, *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	Primer sequence	Product size (bp)	After enzyme cut (bp)	
				RFLP (–)	RFLP (+)
Hinc II/5′ε	RF1	5′-CCTTCCCAGTGAGAAGTATAAGCAG-3′	710	710	425+285*
	RF2	5′-AGTCATTGGTCAAGGCTGACCTGTG-3′			
Hind III/$^{G}\gamma$	RF3	5′-CAATTGAAACATTTGGGCTGGAGTAG-3′	1,202	970+232	273+697+232
	RF4	5′-CCTCTTTAGGCATGCGTCAACACTT-3′			
Hind III/$^{A}\gamma$	RF5	5′-CAATTGAAACATTTGGGCTGGAGTAG-3′	1,042	933+109	252+681+109
	RF6	5′-TTTCTTAGGCATCCACAAGGGCTGT-3′			
Hinc II/ψβ	RF7	5′-GATGAGGGAACAGAAGTTGAGATAG-3′	996	996	371+625
	RF8	5′-GTTCTCTCTTTTCTTGCAGGATTGC-3′			
Hinc II/3′ψβ	RF9	5′-GTAGCATGAATGCTTGTGCATGTAG-3′	1,085	1,085	728+357
	RF15	5′-AAGGAGCACCCACTAGCTCACTGAA-3′			
Ava II/β	K7	5′-TTGGGGATCTGTCCACTCCTGAT-3′	963	963	191+772
	K12	5′-CCAGCCTTATCCCAACCATAAAATAA-3′			
*Bam*H I/3′β	SF1	5′-GCCCACATCACCAAGGCAAT-3′	1,520	1,520	292+1,228
	SF2	5′-GCTCTACGGATGTGTGAGAT-3′			

*: Underlined fragments of PCR product after enzyme digestion indicate RFLPs.

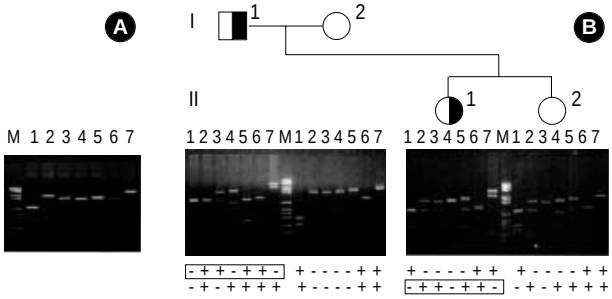

Fig. 3. PCR-based haplotyping of a family with the β-thalassemia mutation of codons 89/90 -TG. (A) A picture of PCR products at seven RFLP sites: The numbers 1 to 7 correspond to seven RFLP sites of *Hin*c II 5′ to ε, *Hin*d III in $^{G}\gamma$, *Hin*d III in $^{A}\gamma$, *Hin*c II 5′ in and 3′ to $\psi\beta$, *Ava* II in β, and *Bam* HI 3′ to β in 5′ to 3′ order. (B) The family pedigree and pictures of their restriction enzyme-treated PCR products. The proband (I-1) and his daughter (II-1) are heterozygous for the mutation and the mutation-associated haplotype, indicated in the box, was determined by the family study. M: molecular size marker VI (Boerhinger Mannheim, Germany).

codons 8/9 +G frameshift mutation had +----++ and FW1. Two cases of codons 41/42 -TTCT frameshift mutation had different haplotypes and frameworks, +----++ with FW1 and +-----+ with FW3A, respectively. A novel β-thalassemic mutation, codons 89/90 -TG, had FW1 and -++-++-, and the family pedigree and pictures of RFLP were shown in Fig. 3.

DISCUSSION

β-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 β-thalassemia genes. We identified some β-thalassemia mutations previously and analyzed their genetic background in this study.

Shimizu et al. previously analyzed β-haplotypes and frameworks in healthy Koreans and identified the three common 5′ β-subhaplotypes: +----, -++-+, and -+-++ (15-17). Similar findings were also observed in the studies of Japanese and Chinese (18-21).

Although the number of β-haplotypes and frameworks analyzed in β-thalassemia chromosomes was small, there were some clues of genetic flow of β-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. The codon 17 AAG →TAG was linked to the haplotype of +---- -+ and FW 3A in two families in this study, and also in the Chinese and the Thailand (22). The codons 41/42 -TTCT was associated with two predominant haplotypes (+---- ++, +-- ---+) in this study, as in the south China and the Southeast Asia (22). These findings suggest that the two mutations might be influenced by the genetic flow from the south China.

Molecular epidemiology of the initiation codon ATG→AGG mutation is interesting. The haplotype and framework linked to the mutation were -+-++-+ and FW3A, respectively, in all of three families in this study. 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 β-thalassaemic 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 β-thalassemia chromosomes in Koreans by family linkage analysis.

ACKNOWLEDGMENTS

We wish to thank all of the patients and their family members who participated in this study; Professor Yuzo Ohba and Dr. Yukio Hattori of Yamaguchi University for their helpful advice; and Professor Yasuyuki Fukumaki of Kyushu University for his comments.

REFERENCES

1. Kan YW, Lee KY, Furbetta M, Angius A, Cao A. *Polymorphism of DNA sequence in the β-globin gene region: Application to prenatal diagnosis of β^0 thalassemia in Sardinia. N Engl J Med 1980; 302: 185-8.*
2. Antonarakis SE, Boehm CD, Giardina PJ, Kazazian HH Jr. *Nonrandom association of polymorphic restriction sites in the β-globin gene cluster. Proc Natl Acad Sci USA 1982; 79: 137-41.*
3. Orkin SH, Kazazian HH Jr, Antonarakis SE, Goff SC, Boehm CD, Sexton JP, Waber PG, Giardina PJ. *Linkage of β-thalassaemia mutations and β-globin gene polymorphisms with DNA polymorphisms in human β-globin gene cluster. Nature 1982; 296: 627-31.*
4. Kan YW, Dozy AM. *Evolution of the hemoglobin S and C genes in world populations. Science 1980; 209: 388-91.*
5. Trabuchet G, Elion J, Baudot G, Pagnier J, Bouhass R, Nigon VM, Labie D, Krishnamoorthy R. *Origin and spread of β-globin gene mutations in India, Africa, and Mediterranea: Analysis of the 5′ flanking and intragenic sequences of β^S and β^C genes. Hum Biol 1991; 63: 241-52.*
6. Ohba Y, Hattori Y, Harano T, Harano K, Fukumaki Y, Ideguchi H,

Cho HI, Park SS. *β-thalassemia mutations in Japanese and Koreans. Hemoglobin 1997; 21: 191-200.*

7. Sutton M, Bouhassira EE, Nagel RL. *Polymerase chain reaction amplification applied to the determination of β-like globin gene cluster haplotypes. Am J Hematol 1989; 32: 66-9.*
8. Varawalla NY, Fitches AC, Old JM. *Analysis of β-globin gene haplotypes in Asian Indians: origin and spread of β-thalassemia in the Indian subcontinent. Hum Genet 1992; 90: 443-9.*
9. Koo MS, Kim SI, Cho HI, Hattori Y, Yamashiro Y, Hoshitani M, Ohba Y, Miyaji T, Yamamoto K, Yamamoto K. *A β-thalassemia mutation found in Korea. Hemoglobin 1992; 16: 313-20.*
10. Lee J, Shin HC, Yang SH, Park S, Kim BK, Kim NK, Cho HI. *Two Korean familial cases of β-thalassemia minor. Korean J Intern Med 1994; 46: 704-11.*
11. Park HS, Park SS, Lee YJ, Yoon KY, Joo SI, Cho HI. *β-thalassemia mutations in the Korean population. Korean J Clin Pathol 1996; 16: 1-11.*
12. Furuumi H, Firdous N, Inoue T, Ohta H, Winichagoon P, Fucharoen S, Fukumaki Y. *Molecular basis of β-thalassemia in the Maldives. Hemoglobin 1998; 22: 141-51.*
13. Chakravarti A, Buetow KH, Antonarakis SE, Waber PG, Boehm CD, Kazazian HH. *Nonuniform recombination within the human β-globin gene cluster. Am J Hum Genet 1984; 36: 1239-58.*
14. Antonarakis SE, Orkin SH, Kazazian HH Jr, Goff SC, Boehm CD, Waber PG, Sexton JP, Ostrer H, Fairbanks VF, Chakravarti A. *Evidence of multiple origins of the β^E-globin gene in Southeast Asia. Proc Natl Acad Sci USA 1982; 79: 6608-11.*
15. Shimizu K, Park KS, Omoto K. *The DNA polymorphisms of the β-globin gene cluster and the arrangements of the α- and the γ-globin in Koreans. Hemoglobin 1989; 13: 137-46.*
16. Shimizu K, Park KS, Harihara S, Enoki Y. *Relationships between %Hb F or %G gamma and the haplotypes in the beta-globin gene cluster in the normal adult Japanese and Korean populations. Hum Hered 1992; 42: 216-21.*
17. Shimizu K, Hashimoto T, Harihara S, Tajima K, Sonoda S, Zaninovic V. *β-globin gene haplotype characteristics of Colombian Amerinds in South America. Hum Hered 2001; 51: 54-63.*
18. Chan V, Chan TK, Cheng MY, Leung NK, Kan YW, Todd D. *Characteristics and distribution of β-thalassemia haplotypes in South China. Hum Genet 1986; 73: 23-6.*
19. Huang S, Waber PG, Dowling CE, Wong C, Antonarakis SE, Cai RL, Wang MQ, Lo WH, Kazazian HH Jr. *β-thalassemia in China: a systematic molecular characterization of β-thalassemia mutations. Hemoglobin 1988; 12: 621-8.*
20. Shimizu K. *Characteristics of β^A chromosome haplotypes in Japanese. Biochem Genet 1987; 25: 197-203.*
21. Shimizu K. *DNA polymorphism patterns in the β-globin gene cluster in the Japanese population. Hemoglobin 1987; 11: 487-96.*
22. Flint J, Harding RM, Clegg JB, Boyce AJ. *Why are some genetic diseases common? Distinguishing selection from other processes by molecular analysis of globin gene variants. Hum Genet 1993; 91: 91-117.*
23. Matsuno Y, Yamashiro Y, Yamamoto K, Hattori Y, Yamamoto K, Ohba Y, Miyaji T. *A possible example of gene conversion with a common β-thalassemia mutation and Chi sequence present in the β-globin gene. Hum Genet 1992; 88: 357-8.*