Molecular Analysis of Oculocutaneous Albinism Patients in Korea

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***Background*: Oculocutaneous albinism (OCA) is a genetic disorder of the melanin pigment system in which melanin synthesis is reduced or absent in the skin, hair, and eyes. OCA is classified into two major types, and tyrosinase-related OCA can be produced by mutations of the structural gene for tyrosinase enzyme (TYR gene).**

***Objective*: The purpose of this study was to analyze the segregation of mutant alleles of the TYR gene in tyrosinase-negative and tyrosinase-positive Korean OCA patients and families.**

***Methods*: We amplified exon I, II, and III of the TYR gene of Korean OCA patients and their families by polymerase chain reactions (PCR), and analyzed the mutations by restriction fragment length polymorphism (RFLP) analysis in exon I and single-strand conformation polymorphism (SSCP) analyses in exon II and exon III.**

***Results*: Two tyrosinase-negative cases showed mutations in exon I. Four tyrosinase-negative cases and one tyrosinase-positive case showed mutations in exon II, and one tyrosinase-negative case showed mutations in exon III. In summary, we found three kinds of mutation in four tyrosinase-negative OCA patients and one tyrsinase-positive OCA patient.**

***Conclusion*: RFLP and SSCP analysis can provide a basis for a rapid and sensitive screening system to detect TYR gene mutations of Korean OCA patients and their families.**

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Key Words : Oculocutaneous Albinism (OCA), Polymerase Chain Reaction (PCR), Restriction Fragment Length Polymorphism (RFLP),·Single-strand Conformation Polymorphism (SSCP)

Oculocutaneous albinism (OCA) is a group of severe genetic disorders of pigmentation characterized by reduced or absent biosynthesis of the melanin pigment in the melanocytes of the skin, hair follicle, and eyes[1]. With the absence of pigment protection, affected individuals are susceptible to constant damage of sunlight and prone to cutaneous malignancies.

OCA is classified into various types based on clinical findings and biochemical studies. In the different types of albinism, various defects in the production and distribution of melanin are involved, including enzyme (tyrosinase), melanosome developement, and the type of melanin produced (eumelanin versus pheomelanin)[2]. Among these, tyrosinase-deficient albinism results from mutations of the tyrosinase gene (TYR gene), which cause deficient catalytic activity of tyrosinase, a copper-containing enzyme that catalyzes the steps of the melanin biosynthetic pathway[3,4]. The majority of the TYR gene mutations are

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Table 1. Primer pairs used to amplify TYR exon segment

Exon I	sense	5' TAAGATAAAGACTAAAAGTG 3'
	antisense	5' TTATACCCTGCCTGAAGAAG 3'
Exon II	sense	5' CTCAGGAGAAGTCTAACAAC 3'
	antisense	5' AACTCAGAAATTCTGAATTC 3'
Exon III	sense	5' GAGTCTCAATACGGAATGAA 3'
	antisense	5' TTTAAATCCAATGAGCACGT 3'

Table 2.

Case	1	2	3	4*	5*	6*	7*	8	9
Polymorphism in exon 1 (R77Q)	+	+							
Polymorphism in exon 2 (P310insC)	+	+	+	+					+
Polymorphism in exon 3 (D383N)			+						

* ; tyrosinase-positive cases

known to be a single base pair point mutations[5]. Garrod included OCA in his original description of inborn errors of metabolism[6]. We have already shown the presence of three mutant alleles of tyrosinase gene (TYR gene) in three unrelated Korean albinism patients[7].

Here, we analyzed the TYR gene of nine Korean OCA patients and family members by restriction fragment length polymorphism (RFLP) and single-strand conformation polymorphism (SSCP) analyses.

MATERIALS AND METHODS

Patients

Nine unrelated Korean patients with features of tyrosinase-negative and tyrosinase-positive OCA and eight individuals of three family members were included in this study. Five of them were tyrosinase-negative OCA and the other four were tyrosinase-positive cases.

Extraction of genomic DNA

The genomic DNAs were extracted from peripheral blood leukocytes collected from each affected individuals and their family members according to the methods previously described by Park et al[8,9].

Polymerase Chain Reaction (PCR)

The oligonucleotide primers were prepared according to the TYR gene sequences published previously[10] and purchased from Korean Biotech, Inc(Taejeon, Korea) (Table 1). We have already shown that Korean OCA patients usually have mutations on exon I, exon II, and exon III of the TYR gene. Therefore, DNA segments corresponding to these exons of the TYR gene were amplified from genomic DNA according to the methods described previously[11]. Thirty five cycles of PCR were performed in 50 microliter volume of 10mM Tris-HCl(pH8.3), 50mM KCl, 1.5mM MgCl2, 0.2mM of each dNTP, 100 pmol of each primer and 1 unit of Taq DNA polymerase (Takara, Japan) using an automated thermal cycler . Each cycle consisted of 20 seconds at 94°C to denature the double-stranded DNA, 1 minute at 50°C for primers to anneal to their complementary sequences , and 1 minute at 74°C for the DNA strands to be extended. Amplified products were electrophoresed on 1% agarose gel and visualized by ethidium bromide staining.

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Fig. 1. HpaII RFLP analysis of PCR products of exon I from patient 1 to 8. The PCR products derived from patients 3 to 8(lane 3 to 8) contained the HpaII sites and PCR fragments of original size were digested by this enzyme. However, patients 1 and 2 were heterozygous for HpaII polymorphism and contained undigested PCR fragments of original size (arrows).

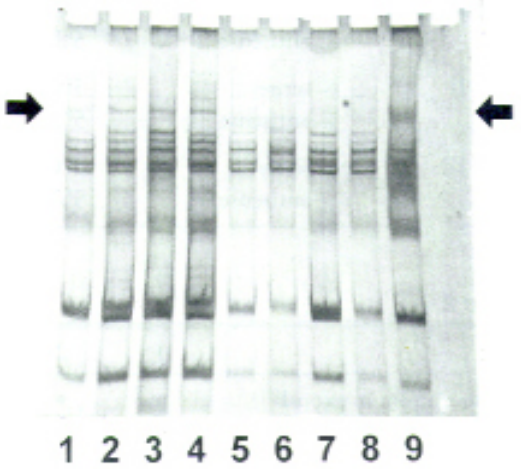

Fig. 2. SSCP analysis of the PCR products of exon II from patient 1 to 9. Mutant alleles appeared as shifted bands (arrows) in cases 1, 2, 3, 4, and 9.

Restriction Fragment Length Polymorphism Analysis

PCR products of exon I were digested with Hpa II restriction endonuclease and subjected to a 6% polyacrylamide gel electrophoresis. Since a single base substitution of G to A within exon I resulting in a missense mutation of codon 77 Arg (CGG) to Gln (CAG) (R77Q mutation) abolishes a HpaII restriction site (CCGG to CCAG), the HpaII digestion of PCR products could easily identify the R77Q mutation[12].

Single-strand Conformation Polymorphism Analysis

PCR products of exon II and exon III were screened for the presence of sequence changes by SSCP analyses[13,14] using MDE gels (AT Biochem). Gels comprising 0.5X MDE gel, in 0.6X TBE were prepared, and SSCP analysis was carried out on a vertical electrophoresis apparatus. Three to five microliter of PCR products were mixed with the same volume of loading buffer (95% formamide, 10 mM NaOH, 20 mM EDTA, 0.02% bromophenol blue), denatured at 95°C, cooled on ice immediately, and loaded onto the gel.

We have already reported that characteristic SSCP patterns are caused by C insertion mutation at codon 310 (CCA to CCCA) (P310insC) of exon II and a base substitution of G to A resulting in a missense mutation of Asp (GAT) to Asn (AAT) at codon 383 (D383N) of exon III by direct DNA sequencing[7].

Silver staining of electrophoresed gels

After electrophoresis, the gels were soaked in 0.1% silver nitrate solution for 10 min and rinsed with distilled water. After rinsing, the gels were soaked in 1.5% NaOH and 0.15% formaldehyde for 10 minute for visualization.

RESULTS

We could amplify three exons of the TYR gene in all individuals by PCR using each of the specific primers. The size of each PCR product was estimated by agarose gel electrophoresis.

Restriction Fragment Length Polymorphism Analysis

The PCR products of exon I derived from patients 3 to 8(lane 3 to 8) contained the HpaII sites and exon fragments of original size have diminished. However, patients 1 and 2 were heterozygous for HpaII polymorphism and contained undigested PCR fragments of original size (Fig. 1).

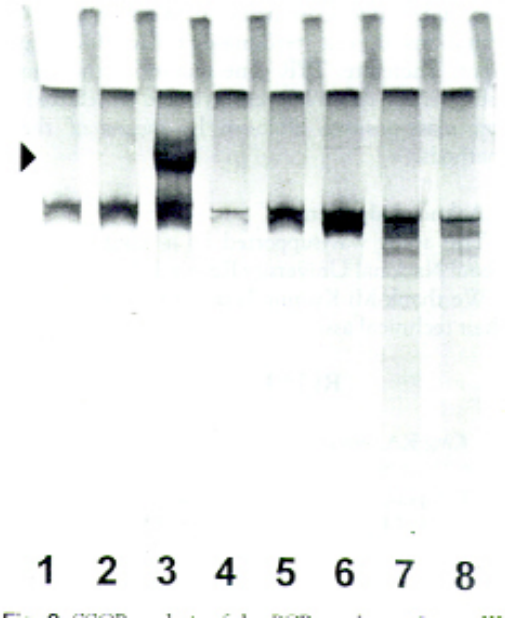

Fig. 3. SSCP analysis of the PCR products of exon III from patients 1 to 8. Mutant alleles appeared as shifted bands (arrows) in case 3.

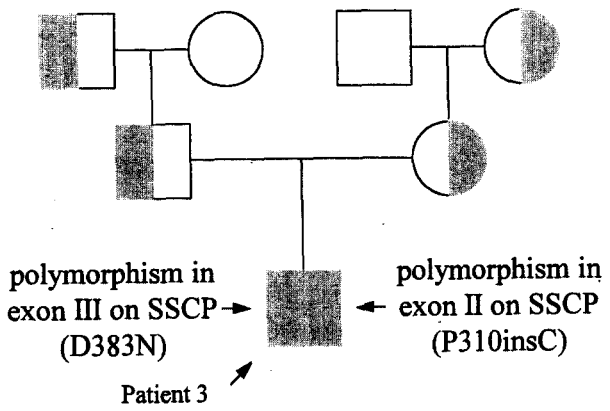

Fig. 5. Pedigree of patient 3. The arrow indicates the patient. Circles denote women, and squares denote men. The shaded symbols indicate alleles with the P310insC mutation in exon II and the blackened symbols indicate alleles with the D383N mutation in exon III. The mutant allele in exon III (D383N) was derived from the paternal grandfather and the mutant allele in exon II (P310insC) from the maternal grandmother.

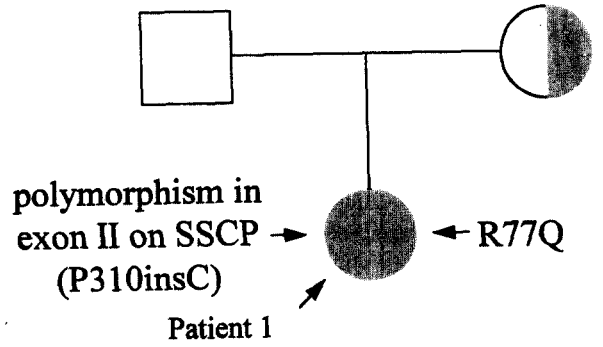

Fig. 4. HpaII RFLP analysis showed that the R77Q mutation was derived from her mother. The arrow indicates the patient. The father was not analyzed.

Single-strand Conformation Polymorphism Analysis

Additional bands were identified in exons II and III by SSCP analyses (Fig. 2 and Fig. 3).

Summary of mutations

Patient 1 and 2 had the mutations of exon I and exon II. By the HpaII digestion, we had already found that the R77Q mutation of patient 1 was inherited from her mother(Fig 4)[7]. Patient 3 had the mutations of exon II and exon III. To analyze the segregation of mutant alleles in the family of patient 3, the patient and members of his family were subjected to SSCP analysis with the PCR products of exons II and III[7]. The mutant allele of exon II was inherited from his maternal grandmother and the mutant allele of exon III from his paternal grandfather(Fig 5). The tyrosinase-positive case 4 has the mutation of exon II. In patient 9, we could find the mutation of exon II only (Table 2).

DISCUSSION

Oculocutaneous albinism (OCA), which causes physical or mental impairment, is basically inherited as an autosomal recessive trait. Recent advances in molecular studies has improved our understanding of the genetic heterogeneity of human albinism and their molecular pathogenesis.

According to the presence or the absence of tyrosinase activity, OCA is classified into tyrosinase-negative or tyrosinase-deficient OCA and tyrosinase-positive OCA. Tyrosinase-negative OCA is produced by mutations of the structural gene for the tyrosinase enzyme, and tyrosinase-positive OCA is known to be genetically mapped in chro-

mosome 15 where the so called P gene is located[15]. Tyrosinase-positive OCA includes Prader-Willi syndrome and Angelman syndrome[15].

Recently, cDNAs encoding human tyrosinase have been cloned[16], and the TYR gene mapped to the long arm of chromosome 11 at position q14-q21[17]. The authentic human TYR gene spans a region of more than 50 kbp and contains 5 exons[10,15]. The size of the exons varies from 819 nt in exon I to 148 nt in exon III. To date, more than 50 different mutations, mainly in the coding region of the TYR gene, have been reported in tyrosinase-related OCA patients[15].

It is very important to develop methods for improving carrier detection and prenatal diagnosis of oculocutaneous albinism. We have suggested the homogeneity of normal Korean TYR genes[18], and shown that the genetic markers of R77Q of exon I, P310insC of exon II, and D383N of exon III can be useful in the analyses of mutations of TYR genes in Koreans[7]. As specific mutations such as R77Q changes the HpaII restriction site, it is easy to identify this mutation by restriction endonuclease digestion. In the case of P310insC and D383N mutations, SSCP analyses can be applied to detect them. Moreover, exon I containing R77Q mutations is too large for SSCP analysis, while exon II with P310insC mutations and exon III with D383N mutations have suitable sizes for SSCP analysis. As direct sequencing requires more time and effort, RFLP analysis and SSCP analysis which were designed to detect point mutations, were done. We found that these techniques are very sensitive and rapid methods for detecting point mutations. Deletion mutation and even single base substitution were easily identified by these techniques. Thus, it is now possibe to analyze rapidly the coding portion of the TYR gene by RFLP and SSCP analyses in selected patients. These methods will be useful to screen the mutations of the TYR gene and can be applied to detect carriers in Korean OCA patients.

The hypothesis has been proposed that TYR gene mutations that modestly reduce tyrosinase activity could be detected in tyrosinase-positive albinism[19]. Tyrosinase-positive OCA is generally known to be related to the P gene located on chromosome 15. However, a few tyrosinase-positive cases have been reported to have TYR gene mutations[15]. These mutations are thought to cause incomplete tyrosinase function. We found exon II mutations in one tyrosinase-positive OCA patient. Therefore, TYR gene mutations that modestly reduce tyrosinase activity could be detected in tyrosinase-positive albinism by means of these methods.

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REFERENCES

1. King RA, Summers CG. Albinism. Dermatol Clin 6:217-228, 1988
2. Bolognia JL, Pawelek JM. Biology of hypopigmentation. J Am Acad Dermatol 19:217-247, 1988
3. Lerner AB, Fitzpatrick TB, Calkins E, Summerson WH. Mammalian tyrosinase: Preparation and properties. J Biol Chem 178:185-195, 1949
4. Kornwe AM, Pawelek JM. Mammalian tyrosinase catalyzed three reactions in the biosynthesis of melanin. Science 217:1163-1165, 1982
5. Tomita Y, Takada A, Okinaga S, Tagami H, Shibahara S. Human oculocutaneous albinism caused by single base insertion in the tyrosinase gene. Biochem Biophys Res Commun 164:990-996, 1989
6. Garrod AE. The Croonian lectures on inborn errors of metabolism: lecture I. Lancet 2:1-7, 1908
7. Park KC, Park SK, Lee YS, Youn SW, Park BS, Kim KH, Lee ST. Mutations of the Tyrosinase Gene in Three Korean Patients with Type I Oculocutaneous albinism. Jpn J Human Genet 41:299-305, 1996
8. Park KC, Chintamanei CD, Halaban R, Witkop CJ, Kwon BS. Molecular Analysis of a Tyrosinase-negative Albino Family. Am J Hum Genet 52:406-413, 1993.
9. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory manual. ed. New York: Cold Spring Harbor Laboratory, 1989
10. Giebel LB, Strunk KM, Spritz RA. Organization and nucleotide sequences of the human tyrosinase gene and a truncated tyrosinase-related segment. Genomics 9:1-11, 1991
11. Saikai RK, Gelfand DH, Stoffel S et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 238:487-491,

1988
12. Takeda A, Tomita Y, Matsunaga J, Tagami H, Shibahara S. Molecular Basis of Tyrosinase-negative Oculocutaneous Albinism. J Biol Chem 265:17792-17797, 1990
13. Orita M, Iwahana H, Kanazawa H et al. Detection of polymorphism of human DNA by gel electrophoresis as single-strand conformation polymorphism. Proc Natl Acad Sci USA 86:2766-2770, 1989
14. Park KC, Kim KH, Lee YS, Kwon BS. Single-strand Conformation Polymorphism Analysis of Point Mutation in a Tyrosinase-Negative Oculocutaneous Albinism. J Inher Metab Dis 17:123-126, 1994.
15. Spritz RA. Molecular genetics of oculocutaneous albinism. Hum Mol Genet 3:1469-1475, 1994
16. Kwon BS, Haq AK, Pomerantz SH, Halaban R. Isolation and sequence of a cDNA clone for human tyrosinase that maps at the mouse c-albino locus. Proc Natl Acad Sci USA 84:7473-7477, 1987
17. Barton DE, Kwon BS, Francke U. Human tyrosinase gene, mapped to chromosome 11 (q14 -> q21), defines second region of homology with mouse chromosome 7. Genomics 3:17-24, 1988
18. Park KC, Kim KH. Sequence Analysis of the Human Tyrosinase Gene in Korean. Ann Dermatol 7:34-38, 1995.
19. Oetting WS, King RA. Analysis of tyrosinase mutations associated with tyrosinase-related oculocutaneous albinism (OCA). Pigment Cell Res 7:285-290, 1994.