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 clas-
sified 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 frag-
ment length polymorphism (RFLP) analysis in exon I and single-strand conformation poly-
orphism (SSCP) analyses in exon II and exon III.

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

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

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Oculocutaneous albinism (OCA) is a group of
severe genetic disorders of pigmentation charac-
terized by reduced or absent biosynthesis of the
melanin pigment in the melanocytes of the skin,
hair follicle, and eyes'. With the absence of pig-
ment protection, affected individuals are susceptible
to constant damage of sunlight and prone to cuta-
aneous 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 in-
volved, including enzyme (tyrosinase), melano-
some development, and the type of melanin pro-
duced (eumelanin versus pheomelanin)'. Among
these, tyrosinase-deficient albinism results from
mutations of the tyrosinase gene (TYR gene),
which cause deficient catalytic activity of tyrosi-

nase, a copper-containing enzyme that catalyzes
the steps of the melanin biosynthetic pathway'.
The majority of the TYR gene mutations are
Table 1. Primer pairs used to amplify TYR exon segment

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<th>Exon</th>
<th>sense</th>
<th>antisense</th>
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<td>I</td>
<td>5' TAAGATAAAGACTAAAAGTG 3'</td>
<td>5' TTATACCCGTGCCTGAAGAAG 3'</td>
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<tr>
<td>II</td>
<td>5' CTCAGGAGAAGTCTAAACAC 3'</td>
<td>5' AAATCGAAGATTCTGAATTC 3'</td>
</tr>
<tr>
<td>III</td>
<td>5' GAGTCTCAATACGGAATGAA 3'</td>
<td>5' TTTAAATCCATGACGACGT 3'</td>
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Table 2.

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<th>2</th>
<th>3</th>
<th>4*</th>
<th>5*</th>
<th>6*</th>
<th>7*</th>
<th>8</th>
<th>9</th>
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<tr>
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<tr>
<td>(R77Q)</td>
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<td>+</td>
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<td>Polymorphism in exon 2</td>
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<td></td>
<td>+</td>
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<tr>
<td>(P310insC)</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Polymorphism in exon 3</td>
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<tr>
<td>(D383N)</td>
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</table>

*; tyrosinase-positive cases

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

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.

Polymerase Chain Reaction (PCR)
The oligonucleotide primers were prepared according to the TYR gene sequences published previously 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. Thirty five cycles of PCR were performed in 50 microliter volume of 10mM Trisz-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.