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# Prevalent and Novel Mutations of the Tyrosinase Gene in Korean Patients with Tyrosinase-deficient Oculocutaneous Albinism

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**We analyzed the tyrosinase (*TYR*) gene of 12 Korean patients with various types of oculocutaneous albinism (OCA). We identified five different mutations in the *TYR* gene in 4 patients with severe OCA and in 2 patients with mild OCA, but found no mutations in the 6 patients with mild OCA phenotypes. Among the 5 mutations, a frameshift mutation, P310insC, was detected most frequently (allele frequency=0.5), and the other mutations were found less frequently, two of which, L288delT and IVS2–7t→a,–10-–11deltt, are novel. This study may provide valuable information for the molecular diagnosis of and accurate genetic counseling for OCA1 in Koreans and perhaps other Asian groups.**

Oculocutaneous albinism (OCA) is a heterogeneous group of autosomal recessive disorders of melanin pigmentation found in all populations throughout the world (Spritz, 1994; Spritz and Hearing, 1994; Witkop *et al.*, 1989). The prevalence of OCA in Koreans is not known, but OCA occurs with a frequency of approximately 1/16,000-20,000 in the United States (Witkop *et al.*, 1989). OCA is characterized by reduced or absent biosynthesis of melanin pigment in melanocytes of the skin, hair follicle, and eye. As a result, affected individuals typically exhibit reduced visual acuity, nystagmus, strabismus, photophobia, and extremely fair complexion with a high risk of skin cancers due to actinic damage.

The two principal forms of OCA are OCA1 (tyrosinase-related OCA) and OCA2 (P-related OCA) (Spritz, 1994). OCA1 is associated with deficient activity of melanocyte tyrosinase, a copper-containing enzyme that catalyzes the rate-limiting step of melanin biosynthesis (Lerner and Fitzpatrick, 1950; Lerner *et al.*, 1949; Tripathi *et al.*, 1992a). In OCA1A (tyrosinase-negative OCA1), complete lack of melanin biosynthesis results from a complete absence of tyrosinase activity (Tomita *et al.*, 1989; Tripathi *et al.*, 1992a). In OCA1B (yellow OCA1), less severe hypopigmented phenotypes result from tyrosinase activity that is greatly reduced, but not absent (Giebel *et al.*, 1991a). OCA2 is typically less severe than OCA1, but it is difficult to distinguish OCA2 from OCA1B on clinical grounds (Spritz, 1994; Spritz and Hearing, 1994).

More than 50 different mutant alleles of the tyrosinase (*TYR*) gene have now been identified in patients with OCA1 (Spritz, 1994; Spritz and Hearing, 1994). However, only three OCA1 mutations, P310insC, R77Q, and D383N, have been identified in the three Korean patients studied to date (Park *et al.*, 1996). Here we report analysis of the *TYR* gene in 12 Korean patients with OCA. We identified five different pathologic *TYR* gene mutations in 4 patients with severe OCA and in 2 patients with mild OCA, but none in any of the 6 patients with mild OCA.

## Materials and Methods

### *PCR amplification of the human* TYR *gene*

Genomic DNA was isolated from peripheral-blood leukocytes from patients with OCA, available family members, and unrelated normal controls (Sambrook *et al.*, 1989). The PCR primers specific to the human *TYR* gene were designed to amplify each of the 3

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The abbreviations used are: OCA, oculocutaneous albinism; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism.

**Table 1.** Primer pairs used to amplify *TYR* exon segments by PCR

| Exon | Primer sequences | Nucleotide position[a] | Fragment size (bp) |
|---|---|---|---|
| 1A | 5'-TTTTCAGAGGATGAAAGCT-3' | 496-514 | 483 |
| | 5'-AGTTTCCACAGTTGAATCCC-3' | 978-959 | |
| 1B | 5'-CTTCACAGGGGTGGATGACC-3' | 881-900 | 436 |
| | 5'-CAAGAAGAGTCTATGCCAAG-3' | 1316-1297 | |
| 1C | 5'-TTGCCCATGAAGCACCAGC-3' | 1270-1288 | 293 |
| | 5'-TTATACCCTGCCTGAAGAAG-3' | 1562-1543 | |
| 2 | 5'-CCTCAGGAGAAGTCTAACAAC-3' | 1643-1663 | 445[b] |
| | 5'-ACAACACATATTCTTGGTC-3' | 2087-2059 | |
| 3 | 5'-TGGGTATCCAGAATGTAAA-3' | 2134-2152 | 356 |
| | 5'-TTTAAATCCAATGAGCACG-3' | 2489-2471 | |
| 4 | 5'-ATCTTTCCATGTCTCCAGA-3' | 2493-2511 | 369 |
| | 5'-TAAAGTTTTGTGTTATCTCA-3' | 2861-2842 | |
| 5 | 5'-CTCCAAAGGACTGTGAAAGG-3' | 2963-2982 | 415 |
| | 5'-GGTCTTTACAGAAAAATAC-3' | 3377-3359 | |

[a]The nucleotide position followed the numbering system of Giebel *et al.* (1991b).
[b]Since all of the Korean patients and normal controls studied here were found to have the allele consisting of 4 thymidines at nucleotides 2059-2061, the actual fragment size of exon 2 was 446 bp in the studied samples.

overlapping regions of large exon 1 with adjacent flanking sequences and each of the exons 2-5 plus adjacent flanking sequences (Table 1). Each exon segment was amplified from genomic DNA by 35 cycles of PCR using *Taq* DNA polymerase and heating at 94 ℃ for 1 min, followed by 35 cycles of reaction at 94 ℃ for 40 s, at 52 ℃ for 90 s, and at 72 ℃ for 2 min, and followed by incubation at 72 ℃ for 5 min (Saiki *et al.*, 1988).

*Identification of point mutations in the* TYR *gene*

The amplified exon segments were screened for point mutations by simultaneous analyses of single-strand conformation polymorphisms (SSCPs) and heteroduplexes (Lee *et al.*, 1995) using the MDE gel (AT Biochem, Malvern, PA, U.S.A.). Exon segments that showed aberrant patterns were independently re-amplified from genomic DNA in duplicate, cloned into pGEM-T (Promega, Madison, WI, U.S.A.), and the complete nucleotide sequences of at least six independent clones per re-amplified exon segment were determined (Sanger *et al.*, 1977).

## Results

The five exons of the *TYR* gene were amplified from genomic DNA of the OCA patients, available family members, and unrelated normal controls. The PCR products were screened for mutations by non-radioactive simultaneous SSCP/heteroduplex analyses as previously described (Lee *et al.*, 1995). Aberrant SSCP/heteroduplex band patterns were detected in 6 of the 12 Korean patients with OCA (Fig. 1). Specific mutations were determined by DNA sequencing of exon segments showing aberrant SSCP/heteroduplex band patterns.

Patient 1 was a 31 year old Korean female with typical OCA1A with white skin and hair, translucent irides, foveal hypoplasia, nystagmus, and strabismus. SSCP/heteroduplex screening and DNA sequence

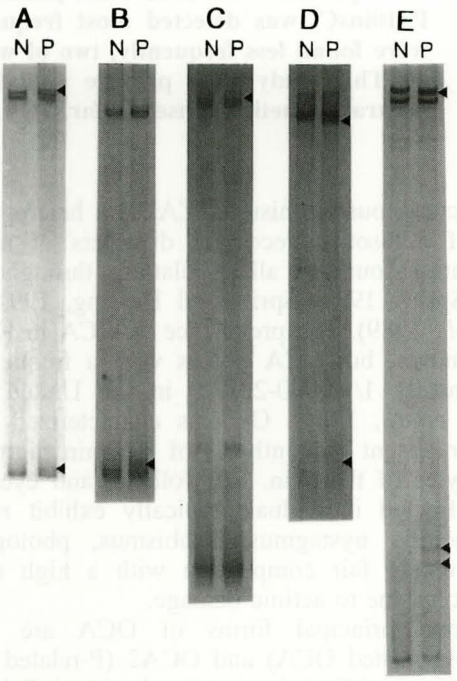


**Figure 1.** SSCP and heteroduplex patterns of the *TYR* exon PCR products from patients with OCA1 and normal controls. Slow-migrating bands and fast-migrating bands correspond to single-stranded DNAs and duplex DNAs, respectively. Abberrantly migrating SSCP and heteroduplex bands are marked with arrowheads. The exon segments showing aberrant SSCP/heteroduplex band patterns were cloned and sequenced to identify specific mutations. A-E, the band patterns of the *TYR* exon PCR products homozygous for normal alleles (N) and heterozygous for normal and mutant alleles (P); R77Q substitution, P310insC frameshift, R299H substitution, L288delT frameshift, and IVS2 −7t→a,−10-−11 deltt mutation, respectively.

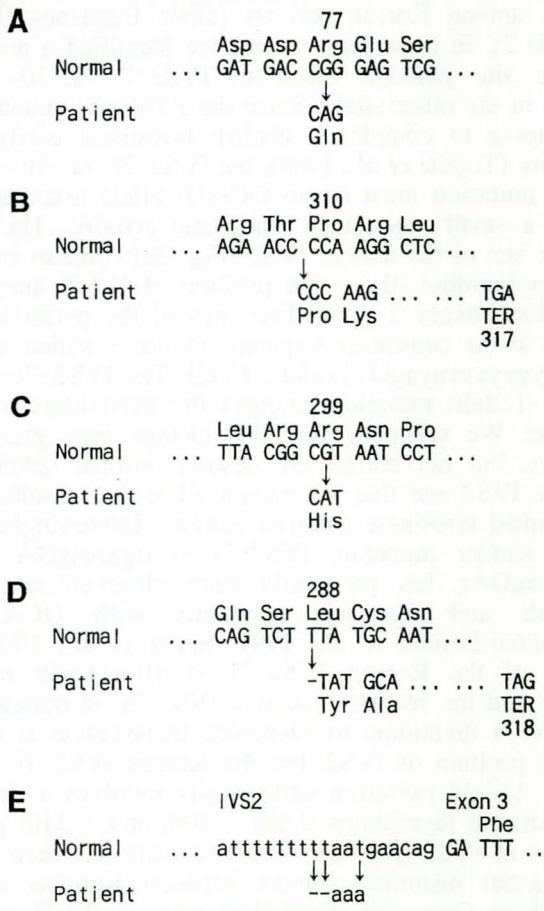


**Figure 2.** Mutations of the *TYR* gene found in Korean patients with OCA1. (A) R77Q substitution found in patient 1. (B) P310insC frameshift found in patients 1, 2, 3, 5, and 6. (C) R299H substitution found in patient 2. (D) L288delT frameshift found in patient 4. (E) IVS2−7t→a, −10- −11deltt mutation found in patients 5 and 6. The hyphen, "−", shown in D and E, represents a deleted nucleotide.

analyses demonstrated that she was a compound heterozygote for a missense substitution, R77Q (Figs. 1A and 2A), and a frameshift, P310insC (Figs. 1B and 2B). Both of these mutations had previously been identified in Japanese patients with OCA1A and completely abolish tyrosinase catalytic activity (Takeda *et al.*, 1990; Tomita *et al.*, 1989). In addition, we recently found these same two alleles in two additional Korean patients with OCA1A (Park *et al.*, 1996), suggesting that these two OCA1A alleles may be relatively common in Asian patients with OCA.

Patient 2 was a 5 year old Korean female with typical OCA1A, clinically similar to patient 1. SSCP/heteroduplex screening and DNA sequence analyses demonstrated that she was a compound heterozygote for the P310insC frameshift (Fig. 2B) described above and for another missense substitution, R299H (Figs. 1C and 2C). The R299H substitution has previously been found in Caucasian and Arab patients with OCA1A (Gershoni-Baruch *et al.*, 1994; Tripathi *et al.*, 1992b), but has not previously been found in Asians.

Patient 3 was a 3 year old Korean male with typical OCA1A. SSCP/heteroduplex screening and DNA sequence analyses demonstrated that he was heterozygous for the P310insC frameshift (Fig. 2B). No mutation could be identified in the other allele.

Patient 4 was a 3½ year old Korean female with typical OCA1A. SSCP/heteroduplex screening and DNA sequence analyses demonstrated that she was heterozygous for a novel frameshift, L288delT (Figs. 1D and 2D), resulting in a truncated and thus most likely nonfunctional polypeptide. No mutation could be identified in the other allele.

Patient 5 was a 4 year old male with mild OCA, with slight skin and iris pigmentation and yellow-brown hair. Visual acuity was reduced, but neither nystagmus nor strabismus was detected. SSCP/heteroduplex screening and DNA sequence analyses demonstrated that he was a compound heterozygote for the P310insC frameshift (Fig. 2B), and a novel splice site junction mutation, IVS2−7t→a,−1-−11deltt (Figs. 1E and 2E). Since the P310insC frameshift is known to be an OCA1A allele (Tomita *et al.*, 1989), the mild OCA phenotype of this patient is most likely associated with the IVS2−7t→a,−1-−11deltt mutation, which thus would be an OCA1B allele.

Patient 6 was a 6 year old Korean male with mild OCA, clinically similar to patient 5. SSCP/heteroduplex screening and DNA sequence analyses demonstrated that he was a compound heterozygote for the P310insC and the IVS2−7t→a,−1-−11deltt mutations described above (Figs. 2B and 2E), exactly as in patient 5.

Patients 7-12 all exhibited clinically mild OCA, with phenotypes similar to or milder than that of patient 5, except that patient 12 additionally showed mild mental retardation. SSCP/heteroduplex screening of the *TYR* genes of these patients demonstrated no apparent abnormalities. Therefore, these patients most likely have OCA2, rather than OCA1B.

In the course of this study we identified a novel silent polymorphism within IVS2, consisting of four versus three thymidines at nucleotides 2059-2061 of the published *TYR* genomic sequence (Giebel *et al.*, 1991b). Because all of the tested 37 Korean individuals were found to have only the allele with four thymidines, it appears that this allele is extremely predominant in Korean populations.

## Discussion

OCA is a rare autosomal recessive genetic disorder characterized by deficient biosynthesis of melanin pigment in the skin, hair, and eyes. OCA1 results from abnormalities of the *TYR* gene (Spritz, 1994; Tomita *et al.*, 1989), whereas OCA2 results from abnormalities of the *P* gene (Lee *et al.*, 1994; Rinchik

*et al.*, 1993). Although a large number of mutations have been described in the *TYR* gene in patients with OCA1 (Spritz, 1994; Spritz and Hearing, 1994), very few Asian patients have been studied previously (Park *et al.*, 1996; Takeda *et al.*, 1990; Tomita *et al.*, 1989).

We analyzed the *TYR* gene in 12 Korean patients with OCA. In four of these patients, all of whom were affected with apparent OCA1A, we identified 4 different pathologic mutations. Only one of these mutations was novel, a frameshift, L288delT. The L288delT frameshift results in a truncated and most likely completely nonfunctional tyrosinase polypeptide (Fig. 2D). The P310insC frameshift also results in a truncated tyrosinase polypeptide (Fig. 2B) and was reported to completely lack the tyrosinase catalytic activity (Tomita *et al.*, 1989). The R77Q substitution was demonstrated to have no detectable tyrosinase catalytic activity in transient expression assays (Takeda *et al.*, 1990). These latter two alleles, which had been identified only in Japanese patients (Takeda *et al.*, 1990; Tomita *et al.*, 1989), appear to be relatively frequent in Korean patients with OCA (Table 2). This result suggests that these mutant alleles may be specific to and prevalent in far-east Asian populations, although the total number of patients studied to date is still relatively small. The R299H substitution was first identified in a Caucasian patient and subsequently in an Arab patient both with OCA1A (Gershoni-Baruch *et al.*, 1994; Tripathi *et al.*, 1992b). Identification of the R299H substitution in patients with OCA1A demonstrates that it completely abolishes the tyrosinase catalytic activity. The presence of the R299H substitution in Korean populations suggests that it may have been introduced into the Korean population from these groups. However, there has been no substantial historical contact between Koreans and Caucasians or Arabs, and patient 2, in whom the R299H mutation was identified, has no known non-Korean ancestors. Thus, it is possible that the R299H allele may have arisen independently in Korea.

Eight of the Korean OCA patients studied here exhibited relatively mild OCA phenotypes, characteristic of either OCA1B or OCA2. Two of these patients were heterozygous for the P310insC frameshift, which appears to be the most prevalent OCA1 allele among Korean patients (allele frequency=0.5) (Table 2). In these two patients we identified a novel splice site junction mutation, IVS2−7t→a,−10−−11 deltt, in the other allele. Since the P310insC mutation is known to completely abolish tyrosinase catalytic activity (Tomita *et al.*, 1989), the IVS2−7t→a,−10−−11 deltt mutation must be an OCA1B allele associated with a small amount of enzymatic activity. The 3' splice site of the normal IVS2 (Fig. 2E) contains only one pyrimidine (the −7th position of IVS2) among the downstream 5 pyrimidine sites of the pyrimidine track in the consensus sequence of the 3' splice site, yyyyyyyyyyynyagG (Locker, 1992). The IVS2−7t→a, −10−−11deltt mutation changes the pyrimidine to a purine. We speculate that this change may greatly reduce, but not completely destroy, normal splicing of the IVS2 and that the mutant allele thus results in a residual tyrosinase catalytic activity. Interestingly, a very similar mutation, IVS2−7t→a (tgaacagGA → agaacagGA), has previously been observed among Jewish and Caucasian patients with OCA1B (Gershoni-Baruch *et al.*, 1994; Spritz *et al.*, 1997). Both of the Korean IVS2−7t→a,−10−−11deltt mutation and the Jewish/Caucasian IVS2−7t→a mutation involve a thymidine to adenosine transversion at the −7th position of IVS2, but the Korean IVS2−7t→a, −10−−11deltt mutation additionally involves a deletion of two thymidines at the −10th and −11th positions of IVS2 (Fig. 2E). Because there has been no substantial historical contact between Koreans and Jewish or Caucasian populations prior to the Korean war, the IVS2−7t→a,−10−−11deltt mutation may have been occurred in Korean populations, independently from the Jewish/Caucasian IVS2−7t→a mutation. Alternatively, the IVS2−7t→a OCA mutant allele may have been introduced into the Korean population, and was subsequently modified by the additional deletion of two thymidines.

In six of the patients with mild OCA we studied, we found no abnormalities of the *TYR* gene. As it is difficult or impossible to distinguish OCA1B from OCA2 on solely clinical grounds, it seems most likely that these six patients have OCA2.

Recently, there has been increased awareness of, and attention paid to, genetic diseases in Asian countries. Identification of prevalent and/or novel OCA alleles in Korea will greatly facilitate DNA-based

**Table 2.** Mutations of the *TYR* gene identified in Korean patients with OCA1

| Mutation | OCA type | Occurrence[a] | First identified in | Reference |
|---|---|---|---|---|
| R77Q | 1A | 1(2) | Japanese | Takeda *et al.*, 1990 |
| L288delT | 1A | 1 | Korean | This study |
| R299H | 1A | 1 | Caucasian | Gershoni-Baruch *et al.*, 1994 |
| P310insC | 1A | 5(3) | Japanese | Tomita *et al.*, 1989 |
| D383N | 1A | 0(1) | Caucasian | Spritz *et al.*, 1990 |
| IVS2−7t→a,−10−−11deltt | 1B | 2 | Korean | This study |

[a]The figures represent occurrences of each specific OCA allele in this study. The figures in parentheses represnent occureences in three Korean patients with OCA1A in the previous study (Park *et al.*, 1996).

diagnosis, prenatal diagnosis, and carrier screening of OCA in Korea and perhaps other Asian populations.

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## References

Gershoni-Baruch, R., Rosenmann, A., Droetto, S., Holmes, S., Tripathi, R. K., and Spritz, R. A. (1994) *Am. J. Hum. Genet.* **54**, 586-594.

Giebel, L. B., Tripathi, R. K., Strunk, K. M., Hanifin, J. M., Jackson, C. E., King, R. A., and Spritz, R. A. (1991a) *Am. J. Hum. Genet.* **48**, 1159-1167.

Giebel, L. B., Strunk, K. M., and Spritz, R. A. (1991b) *Genomics* **9**, 435-445.

Lee, S.-T., Strunk, K. M., Bundey, S., Laxova, R., Musarella, M., and Spritz, R. A. (1994) *New Eng. J. Med.* **330**, 529-534.

Lee, S.-T., Park, S.-K., Lee, K.-H., Holmes, S. A., and Spritz, R. A. (1995) *Mol. Cells* **5**, 668-672.

Lerner, A. B., and Fitzpatrick, T. B. (1950) *Physiol. Rev.* **30**, 91-126.

Lerner, A. B., Fitzpatrick, T. B., Calkins, E., and Summerson, W. H. (1949) *J. Biol. Chem.* **178**, 185-195.

Locker, J. (1992) *Gene Transcription: A Practical Approach* (Hames, B. D., and Higgins, S. J., eds) pp. 321-345, IRL, New York.

Park, K. C., Park, S. K., Lee, Y. S., Youn, S. W., Park, B. S., Kim, K. H., and Lee, S. T. (1996) *Jap. J. Hum. Genet.* **41**, 299-305.

Rinchik, E. M., Bultman, S. J., Horsthemke, B., Lee, S.-T., Strunk, K. M., Spritz, R. A., Avidano, K. M., Jong, M. T. C., and Nicholls, R. D. (1993) *Nature* **361**, 72-76.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Ehrlich, H. A. (1988) *Science* **239**, 487-491.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed, Cold Spring Harbor, Cold Spring Harbor, NY.

Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463-5467.

Spritz, R. A. (1994) *Hum. Mol. Genet.* **3**, 1469-1475.

Spritz, R. A., and Hearing, V. J., Jr. (1994) *Adv. Hum. Genet.* **22**, 1-45.

Spritz, R. A., Oh, J., Fukai, K., Holmes, S., Ho, L., Chitayat, D., France, T. D., Musarella, M. A., Orlow, S. J., Schnur, R. E., Weleber, R. G., and Levin, A. (1997) *Hum. Mutat.* **9**, in press.

Spritz, R. A., Strunk, K. M., Giebel, L. B., and King, R. A. (1990) *New Eng. J. Med.* **322**, 1724-1728.

Takeda, A., Tomita, Y., Matsunaga, J., Tagami, H., and Shibahara, S. (1990) *J. Biol. Chem.* **265**, 17792-17797.

Tomita, Y., Takeda, A., Okinaga, S., Tagami, H., and Shibahara, A. (1989) *Biochem. Biophys. Res. Commun.* **164**, 990-996.

Tripathi, R. K., Hearing, V. J., Urabe, K., Aroca, P., and Spritz, R. A. (1992a) *J. Biol. Chem.* **267**, 23707-23712.

Tripathi, R. K., Strunk, K. M., Giebel, L. B., Weleber, R. G., and Spritz, R. A. (1992b) *Am. J. Med. Genet.* **43**, 865-871.

Witkop, C. J., Jr., Quevedo, W. C., Jr., Fitzpatrick, T. B., and King, R. A. (1989) in *The Metabolic Basis of Inherited Disease* (Scriver, C. R., Beaudet, A. L., Sly, W. S., and Valle, D., eds) 6th Ed, pp. 2905-2947, McGraw-Hill, New York, NY.