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Mutations of the Tyrosinase Gene in Indo-Pakistani Patients with Type I (Tyrosinase-deficient) Oculocutaneous Albinism (OCA)

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Summary

Oculocutaneous albinism (OCA) is a group of autosomal recessive disorders characterized by deficient synthesis of melanin pigment. Type I (tyrosinase-deficient) OCA results from mutations of the tyrosinase gene (*TYR* gene) encoding tyrosinase, the enzyme that catalyzes the first two steps of melanin biosynthesis. Mutations of the *TYR* gene have been identified in a large number of patients, most of Caucasian ethnic origin, with various forms of type I OCA. Here, we present an analysis of the *TYR* gene in eight Indo-Pakistani patients with type I OCA. We describe four novel *TYR* gene mutations and a fifth mutation previously observed in a Caucasian patient.

Introduction

Oculocutaneous albinism (OCA) is a heterogeneous group of autosomal recessive disorders in which biosynthesis of melanin pigment is reduced or absent (reviewed in Witkop et al. 1989; Spritz 1993). Lack of pigment in the skin results in severe photosensitivity and high risk of skin cancer; lack of pigment in the eye and consequent misrouting of the optic neural tracts results in photophobia, nystagmus, and greatly decreased visual acuity.

Type I (tyrosinase-deficient) OCA results from reduced or absent activity of tyrosinase (monophenol, L-dopa:oxygen oxidoreductase; E.C.1.14.18.1), a copper-containing enzyme that catalyzes the first two steps of melanin biosynthesis (Lerner and Fitzpatrick 1950) and at least one distal step in the pathway (Tripathi et al. 1992*a*). In classic type IA (tyrosinase-negative) OCA, tyrosinase activity and melanin biosynthesis are completely absent, and, in type IB ("yellow") OCA, tyrosinase activity and melanin biosynthesis are greatly reduced. To date, at least 33 different pathologic mutations of the tyrosinase gene (*TYR* gene) have been reported in patients with various forms of type I OCA (fig. 1), most of whom have been of Caucasian ethnic origin (reviewed in Spritz 1993; R. K. Tripathi, S. Droetto, S. A. Holmes, and R. A. Spritz, unpublished data).

In the present paper we present a molecular analysis of type I OCA in eight unrelated Indo-Pakistani patients, an ethnic group in which inbreeding is common and in which the psychosocial consequences of the albino phenotype can be especially profound. We identified mutations of the *TYR* gene in all six of these patients who had classic type IA OCA. These include four novel mutations and one mutation previously identified in only a single Caucasian individual. These results indicate that the Indo-Pakistani and Caucasian populations share at least some mutant tyrosinase alleles but that the total repertoire and prevalence of mutant alleles in these two populations is probably substantially different.

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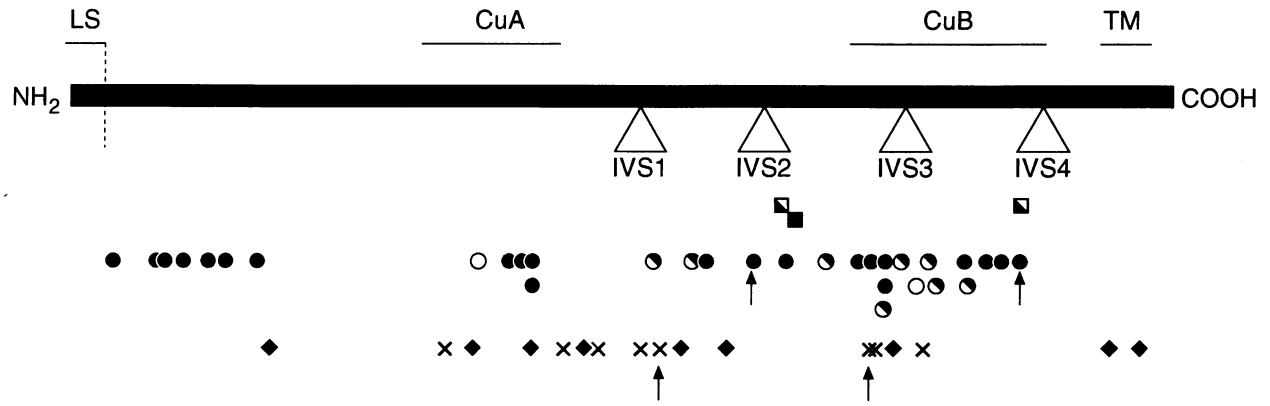


Figure 1 Locations of known *TYR* gene mutations associated with OCA. The blackened box denotes the 529-amino acid tyrosinase polypeptide; the broken line denotes the site of cleavage of the leader peptide. The positions of the four intervening sequences (IVSs) are indicated. Blackened circles indicate the sites of type IA OCA missense substitutions; half-blackened circles indicate the sites of type IB OCA missense substitutions; unblackened circles indicate the sites of nonpathological polymorphic amino acids 192 and 402. Blackened diamonds indicate the sites of frameshifts; ×'s indicate the sites of nonsense mutations; half-blackened squares indicate the sites of splice consensus mutations associated with type IA OCA; blackened squares indicate the sites of splice consensus mutations associated with type IB OCA. The arrows indicate the four novel mutations described here. The CuA and CuB regions of sequence homology with hemocyanins, and the tyrosinase leader sequence (LS) and transmembrane (TM) segments are indicated. Many of the indicated mutations represent our published data.

Subjects and Methods

Description of the Probands

Proband 1 was a 10-year-old Indian female with typical type IA OCA with no apparent pigmentation of the skin, hair, and eyes and greatly decreased visual acuity, nystagmus, and photophobia. Her parents were first cousins, as had been each of their parents. Her brother, the only sibling, was unaffected. Hairbulb tyrosinase assay was not performed.

Proband 2 was a 6-year-old Pakistani male with typical type IA OCA. His parents were first cousins. His sister, the only sibling, was unaffected. Hairbulb tyrosinase assay was not performed.

Proband 3 was a 7-year-old Bangladeshi male with typical type IA OCA. His parents were not consanguineous, and his five siblings were normally pigmented. Analysis of hairbulbs (Witkop et al. 1989) showed no detectable tyrosinase activity. Karyotype was normal.

Proband 4 was a 6½-mo-old girl with typical type IA OCA. Her parents were not consanguineous; her father was from Sri Lanka and her mother was from Guyana. She had no siblings. Hairbulb tyrosinase assay was not performed.

Proband 5 was a 15-year-old Pakistani male with typical type IA OCA. His parents were first cousins. His three siblings were unaffected. Analysis of hairbulbs showed no detectable tyrosinase activity.

Proband 6 was a 20-year-old Indian male with typical type IA OCA. His parents were not consanguineous. Two sibs were similarly affected, and three were unaffected. Analysis of hairbulbs showed no detectable tyrosinase activity.

Proband 7 was a 21-year old Pakistani male with type IB OCA. His hair was silver colored at birth, but subsequently darkened to golden. Irides were brown and transilluminated, and he had nystagmus and reduced visual acuity. His parents were first cousins, and many other members of this complex inbred kindred were affected. Analysis of hairbulbs showed slight tyrosinase activity in the proband and his similarly affected brother, although a sister had no detectable tyrosinase activity.

Proband 8 was an 11-year-old Pakistani male with type IB OCA. His hair was white at birth but subsequently darkened to pale golden. Irides were blue and transilluminated, and he had nystagmus and reduced visual acuity. His parents are first cousins, and a sister and cousin were similarly affected. Analysis of hairbulbs showed no detectable tyrosinase activity.

PCR Amplification of Human TYR *Gene Segments*

DNA segments containing the five *TYR* gene exons plus adjacent noncoding and flanking sequences (Giebel et al. 1991*b*) were amplified in duplicate from 0.1 μg of genomic DNA of each proband by 35 cycles of PCR

(Saiki et al. 1988) as described elsewhere (Giebel et al. 1991*a*).

Single-Strand Conformation Polymorphism (SSCP)/ Heteroduplex and DNA Sequence Analyses

For proband 1, the products of the duplicate PCR reactions of each exon were pooled, purified in 4% polyacrylamide gels, eluted, and cloned in M13mp19. The nucleotide sequences of at least six independent clones per exon were determined (Sanger et al. 1977).

For probands 2–8, the duplicate PCR products for each exon were pooled and were screened by combined SSCP/heteroduplex analysis using MDE gels (AT Biochemical) as described (Spritz et al. 1992); 14–17 unrelated, normally pigmented Indo-Pakistani individuals were analyzed simultaneously as controls. For tyrosinase exons 2, 3, 4, and 5, the PCR products were analyzed directly. For exon 1, the 1060-bp PCR product was first cleaved by *Taq*I and *Pst*I to 520-bp, 395-bp, and 130-bp fragments, which were then analyzed as a mixture. In some cases exon 5 was also analyzed as two overlapping fragments, 422 and 433 bp in size, amplified separately using primer pairs 5′-CTCCAAAGGACTGTGAAAGG-3′/5′-TGCAAATGGTCTTTACA-3′ and 5′-CACAGCTTGTATCAGAGCC-3′/5′-GGAGTCAGTTAATGTAGATT-3′, respectively (see Giebel et al. 1991*b*). *TYR* gene exon segments exhibiting aberrant SSCP/heteroduplex patterns were independently reamplified in duplicate and were purified, cloned, and sequenced as described above.

Restriction Enzyme Cleavage Analysis

TYR gene exon 3 PCR products amplified from DNA of proband 1, her parents, her normally pigmented brother, and four unrelated normally pigmented Indo-Pakistani individuals were tested for the codon 376 nonsense mutation by digestion with *Avr*II and polyacrylamide gel electrophoresis.

Results

Probands 1, 2, and 3 Have Novel Mutations of the TYR *Gene*

Cloning and DNA sequence analysis demonstrated that proband 1 is homozygous for a novel nonsense mutation within exon 3, codon 376CAG (Gln)→TAG (TER) (Q376TER; fig. 2). She was also homozygous for TCT (Ser) and CGA (Arg), respectively, at polymorphic codons 192 (Giebel and Spritz 1990) and 402 (Tripathi et al. 1991), which is consistent with the low frequencies of these polymorphisms in non-Caucasians. The Q376TER nonsense mutation creates a restriction site for *Avr*II (CCTAGG), whereas exon 3 normally contains no *Avr*II site. Therefore, we tested for the presence of the Q376TER mutation in the proband, her parents, her normally pigmented brother, and four unrelated, normally pigmented Indo-Pakistani controls, by *Avr*II cleavage of the exon 3 PCR product amplified from their DNA. This analysis confirmed that the proband was homozygous for the Q376TER mutation and showed that both her parents and her unaffected brother were heterozygotes, whereas the four controls were normal at this site (data not shown). Furthermore, we did not observe this mutation on SSCP/

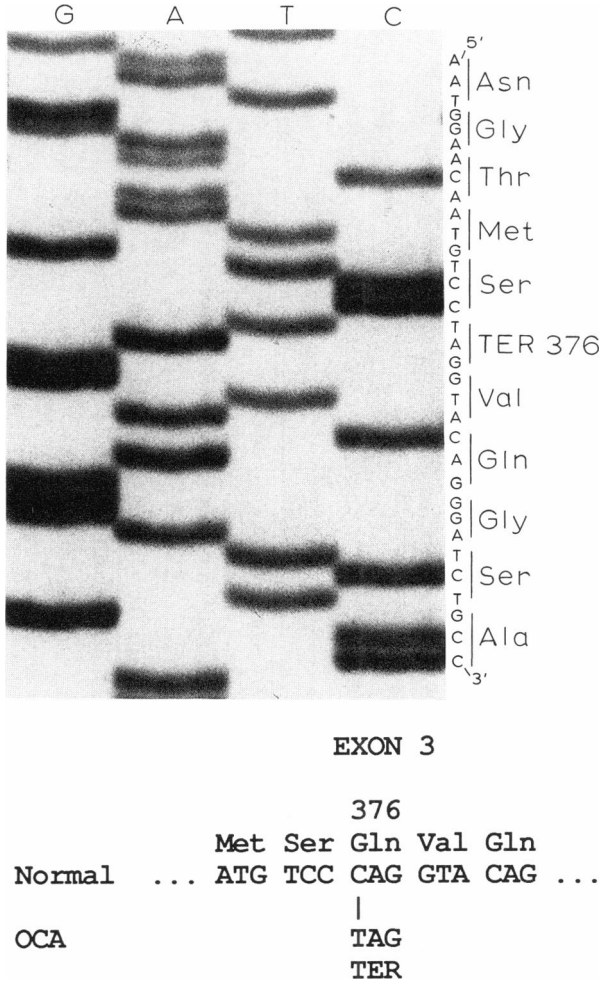


Figure 2 Sequences in region of the Q376TER nonsense mutation of proband 1. Only the abnormal allele is shown. The sequence indicated is that of the coding strand.

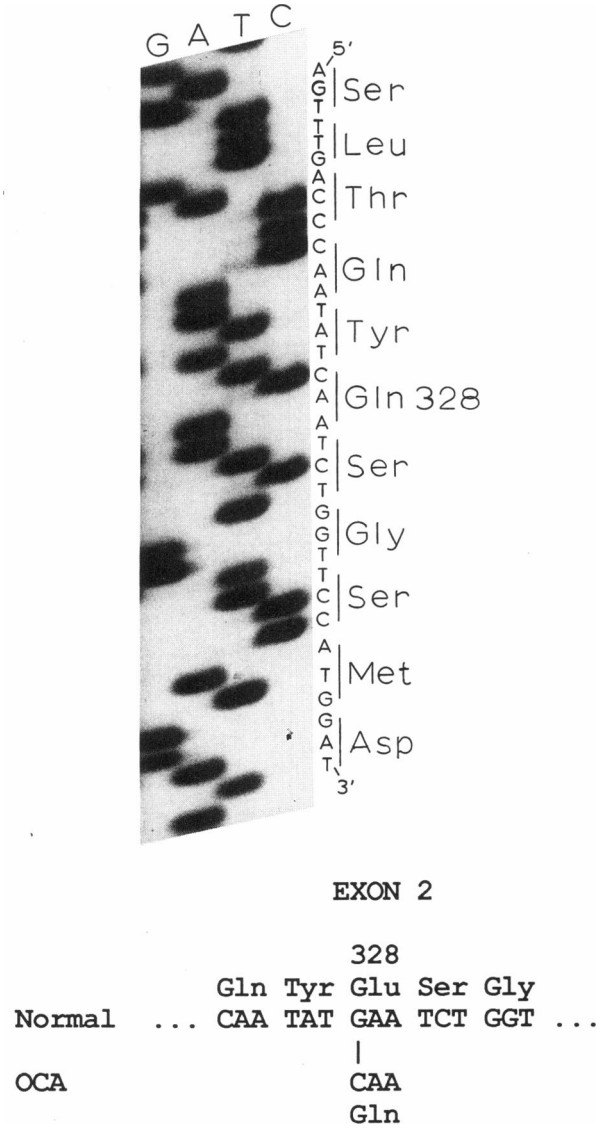


Figure 3 Sequences in region of the E328Q missense mutation of proband 2. The sequence indicated is that of the coding strand.

heteroduplex screening of the other seven OCA probands nor in a total of 17 unrelated Indo-Pakistani control individuals (data not shown).

SSCP/heteroduplex screening of the five *TYR* gene exons amplified from DNA of proband 2 demonstrated an abnormal pattern only for exon 2 (data not shown). Cloning and DNA sequence analysis of the exon 2 PCR product showed that he was homozygous for a novel missense mutation, codon 328GAA (Glu)→CAA (Gln) (E328Q; fig. 3). We did not observe this mutation on SSCP/heteroduplex screening of the seven other OCA probands and the 17 unrelated Indo-Pakistani controls.

SSCP/heteroduplex screening of proband 3 demonstrated an abnormal pattern only for *TYR* exon 4. Cloning and DNA sequence analysis of the exon 4 PCR product showed that he was heterozygous for another novel missense substitution, codon 431CCA (Pro)→CTA (Leu) (P431L; fig. 4). The other allele contained no apparent abnormality within exon 4. He was homozygous for CGA (Arg) at polymorphic codon 402. The proband's heterozygosity for the P431L mutation was confirmed by independently repeating the entire analysis of exon 4. Thus, proband 3 was a compound heterozygote, with one allele containing the P431L substitution and the other allele containing no detectable abnormal-

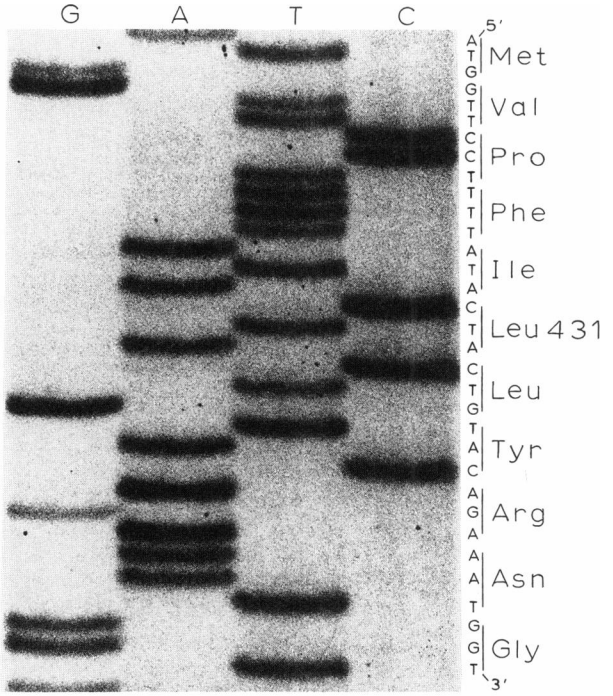
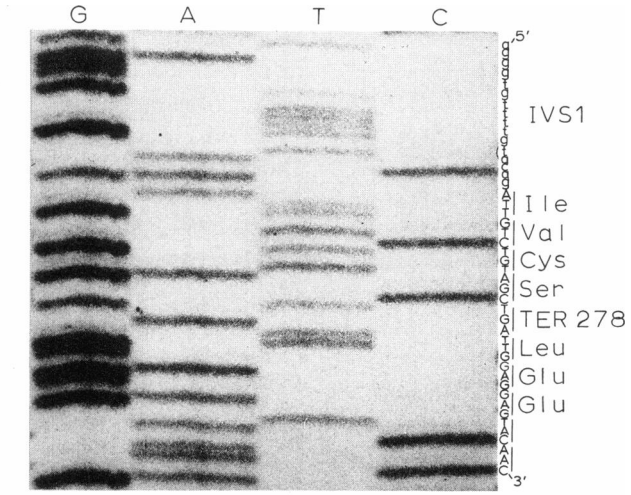


Figure 4 Sequences in region of the codon P431L missense mutation of proband 3. The sequence indicated is that of the coding strand.

ity within the regions analyzed. We did not observe the P431L mutation on SSCP/heteroduplex screening of the seven other OCA probands or the 17 Indo-Pakistani controls.

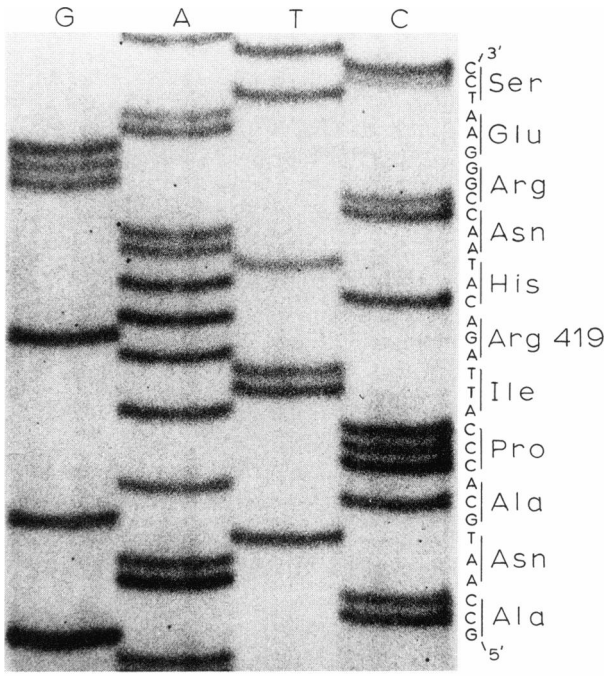
SSCP/heteroduplex screening of proband 4 demonstrated an abnormal pattern only for exon 2. Cloning and DNA sequence analysis of the exon 2 PCR product showed that she was heterozygous for another novel nonsense mutation, codon 278CGA (Arg)→TGA (TER) (R278TER; fig. 5), a possible result of deamination of 5-methylcytosine at a CpG site. The other allele contained no apparent abnormality within exon 2. Proband 4 was thus a compound heterozygote, with one allele containing the R278TER nonsense mutation and the other allele containing no detectable abnormality within the regions analyzed. SSCP/heteroduplex analysis of the exon 2 PCR product amplified from the DNA of her parents demonstrated that her mother, who was of Guyanan ancestry, was heterozygous for the R278TER mutant allele; the exon 2 pattern for her father, who was of Sri Lankan ancestry, was normal. Thus



EXON 2

				278			
		Cys	Ser	Arg	Leu	Glu	
Normal	...	TGT	AGC	CGA	TTG	GAG	...
				|			
OCA				TGA			
				TER			

Figure 5 Sequences in region of the R278TER nonsense mutation of proband 4. The sequence indicated is that of the coding strand.



EXON 4

					419			
		Pro	Ile	Gly	His	Asn		
Normal	...	CCC	ATT	GGA	CAT	AAC	...	
				|				
OCA				AGA				
				Arg				

Figure 6 Sequences in region of the G419R missense mutation of proband 5. The sequence indicated is that of the coding strand.

the patient's tyrosinase allele of paternal, Sri Lankan derivation was the one in which no mutation was identified.

Probands 5 and 6 Have a Previously Described Missense Substitution at Codon 419

SSCP/heteroduplex screening of proband 5 demonstrated an abnormal pattern only for tyrosinase exon 4. Cloning and DNA sequence analysis of the exon 4 PCR product showed that he was homozygous for a missense substitution, codon 419GGA (Gly)→AGA (Arg) (G419R; fig. 6) described elsewhere (King et al. 1991). Proband 5 was homozygous for CGA (Arg) at polymorphic codon 402. The proband's homozygosity for the G419R mutation was confirmed by independently re-

peating the entire analysis of exon 4. The codon 419 mutation was not observed on SSCP/heteroduplex screening of seven of the other eight OCA probands and 14 Indo-Pakistani controls.

However, SSCP/heteroduplex screening of proband 6 also demonstrated an abnormal pattern only for *TYR* exon 4 and similar to that of proband 5. Cloning and DNA sequence analysis of the exon 4 PCR product showed that he was heterozygous for the same G419R mutant allele (fig. 6) described above. The other allele contained no apparent abnormality within exon 4. Proband 6 was homozygous for CGA (Arg) at polymorphic codon 402. His heterozygosity for the G419R mutation was confirmed by independently repeating the entire analysis of exon 4. Thus, proband 6 was also a compound heterozygote, with one allele containing the G419R substitution and the other allele containing no detectable abnormality within the regions analyzed.

Probands 7 and 8 Have No Detectable Abnormalities of the TYR Gene

SSCP/heteroduplex screening of probands 7 and 8, both of whom have type IB OCA, demonstrated no apparent abnormalities.

Discussion

We have analyzed the *TYR* gene in eight Indo-Pakistani patients with type I OCA. We found mutations of the *TYR* gene in all six patients with type IA OCA but in neither of the two patients with type IB OCA. The Q376TER nonsense mutation and the E328Q and P431L missense substitutions have not been observed among any of the numerous OCA patients studied previously and thus may be specific to the Indo-Pakistani population. However, the R278TER mutant allele appears to be of Guyanan, rather than Indo-Pakistani, derivation.

The two nonsense mutations are clearly pathologic. The E328Q, G419R, and P431L missense substitutions are also almost certainly pathologic. First, none occurred among 14–17 normally pigmented Indo-Pakistani individuals (data not shown), and thus they are at least not frequent nonpathologic polymorphisms. Second, all three of these substitutions are nonconservative, and the G419R and P431L substitutions occur within the dense cluster of pathologic missense substitutions from tyrosinase codons 371–448 that we (Tripathi et al. 1992*b*) and others (King et al. 1991) have previously described (fig. 1). Third, all three of these amino acid residues are conserved between the human (Giebel et al. 1991*b*) and mouse (Yamamoto et al. 1987) *TYR* genes.

The G419R substitution has been observed once before (King et al. 1991), in a single compoundly heterozygous individual with type IA OCA, among the more than 200 patients of northern European Caucasian ethnic origin studied to date (reviewed in Spritz 1993; R. K. Tripathi, S. Droetto, S. A. Holmes, and R. A. Spritz, unpublished data). In contrast, we observed the G419R mutant allele in two of the eight Indo-Pakistani patients studied here. Proband 5 (whose parents were consanguineous) was homozygous for this substitution, and proband 6 (whose parents were unrelated) was heterozygous. These two patients are not related to each other; proband 5 was Pakistani, and proband 6 was Indian. Thus, the G419R substitution appears to be more frequent among Indo-Pakistani individuals than among Caucasians ($p \approx .004$ by Fisher's exact test). In addition, although the number of patients studied here is quite small, we did not observe either of the two mutations that are most frequent among Caucasians, which together account for approximately 25%–30% of mutant alleles in that group (Spritz 1993). It seems likely, therefore, that the repertoire and prevalence of mutant tyrosinase alleles is different in the Indo-Pakistani and Caucasian populations.

Probands 3, 4, and 6 were apparent compound heterozygotes for tyrosinase alleles in which we were not able to identify any abnormalities. These alleles might contain mutations located outside the regions of the *TYR* gene that we analyzed, they might contain mutations that are not detected by SSCP/heteroduplex screening, or they might contain partial gene deletions invisible to analyses dependent on PCR. Similarly, we were unable to detect any abnormalities in probands 7 and 8, both of whom had the phenotype of type IB OCA. Although the phenotypes of probands 7 and 8 resemble type II (tyrosinase-positive) OCA, which we recently showed to result from mutations of the *P* (pink-eyed dilution) gene (Rinchik et al. 1993; Lee et al., in press; R. A. Spritz, unpublished data), SSCP/heteroduplex analyses of their *P* genes appeared normal, and they were both heterozygous for several intragenic polymorphisms of *I* (data not shown). Among Caucasians, only about 10%–15% of obligate mutant tyrosinase alleles contain no detectable mutations (Spritz 1993). The possibly more frequent occurrence of tyrosinase alleles with occult mutations may complicate attempts at carrier detection and prenatal diagnosis of type I OCA in the Indo-Pakistani population, in which parental consanguinity is common and in which the psychosocial

consequences of the albino phenotype may be especially significant.

Acknowledgments

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