



# Genetic analysis of oculocutaneous albinism type 1 (OCA1) in Indian families: two novel frameshift mutations in the *TYR* gene

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**Purpose:** Oculocutaneous albinism type 1 (OCA1) patients demonstrate a partial or total lack of melanin in the skin, hair and eye. OCA1 is an autosomal recessive genetic disorder caused by mutations in the *TYR* gene located at chromosome band 11q14-q25. The purpose of this study was to carry out genetic analysis of OCA1 in Indian families.

**Methods:** Genomic DNA was isolated from blood leukocytes of all the individuals in this study. Haplotype analysis was performed at the *TYR* locus using informative microsatellite markers. Eight sets of primers were used to amplify the entire coding region of the *TYR* gene for bidirectional direct sequencing mutation analysis.

**Results:** Two novel deletions (c.937del8, c.1379del2) and a previously known nonsense mutation (R278X) in the *TYR* gene were identified from a total of 8 oculocutaneous albinism patients in India.

**Conclusions:** Our study reports the distribution of two novel frameshift and a previously reported nonsense mutations in four OCA1 families from the Indian population. These findings will contribute to the development of a diagnostic method for OCA1 carrier status and genetic counseling for OCA1 affected families.

Oculocutaneous albinism (OCA) is a complex group of genetic disorders characterized by defects in the synthesis or transport of melanin. The result is a reduced or complete absence of melanin pigment in the skin, hair, and eyes. OCA is an autosomal recessive disorder classified into several types based on clinical and molecular categories. OCA1 is caused by mutations in the tyrosinase gene (*TYR* 11q14-q21), OCA2, or tyrosinase positive OCA is caused by mutations in the P protein gene (15q11.2-q12), OCA3, which is associated with mutations in the tyrosinase related protein gene (*TYRP1* 9p23), and OCA4, associated with mutations in the membrane associated transporter gene (*MATP* 5p). OCA1 can be further subdivided into two categories, OCA1A and OCA1B. OCA1A results from a complete lack of tyrosinase activity and produces a totally depigmented phenotype with affected individuals exhibiting white hair, white skin, and blue irides throughout life [1,2]. OCA1B, or yellow albinism is characterized by a reduced rate of tyrosinase activity, and, as a result, individuals are born with white hair that changes to blond or yellow with age [3,4]. OCA1A patients display visual acuities of 6/60 to 6/120. OCA1B patients exhibit blue eye color, but may be brown in darker groups with visual acuities from 6/18 to 6/120, whereas patients with ocular albinism show normal hair, skin, and eye color with abnormal visual acuities ranging from 6/15 to 6/120.

The *TYR* gene produces a 529 amino acid residue copper binding protein associated with the conversion of tyrosine to

DOPA and then to dopaquinone [5,6]. It consists of five exons spanning about 65 kb on chromosome 11q14-q21 [7,8].

The first pathological mutation in *TYR* gene was reported in an OCA patient in 1989 [9]. Since then, in excess of 100 mutations and polymorphisms in the tyrosinase gene have been reported (Albinism Database and Human Gene Mutation Database). Genetic analysis of OCA1 patients with different ethnic backgrounds revealed several mutations in the *TYR* gene [1,8]. Recently, Chaki et al. [10] noted the occurrence of a previously reported nonsense mutation (R278X) in a population in West Bengal. Here, we confirm the presence of the R278X mutation and also observe two novel frameshift mutations. Our findings will contribute to the molecular diagnosis of OCA1 and will aid genetic counseling in the Tili and other communities in West Bengal.

## METHODS

**Study subjects:** We selected four unrelated families (Figure 1) with oculocutaneous albinism from the state of West Bengal, India. Three of the four families studied have more than one family member affected by OCA. These four families live within 60 miles of one another (Bankura, Puruliya, and Hugli districts of West Bengal) and belong to either the Tamli or Tili community. All patients had typical features of OCA including hypopigmentation of the hair and skin, blue translucent irides, nystagmus, and reduced visual acuity (Table 1). We recruited a total of 22 individuals including 8 affected OCA patients for this study. The nature of the study was discussed and informed consent was obtained from all participants. The Institutional Review Board and Ethics Committee of the Aravind Eye Hospital, Aravind Medical Research Foundation, Vivekananda Mission Asram, Viveknagar, and West Bengal

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approved the study, and the tenets of the Declaration of Helsinki on Human trials were adhered to strictly.

Genomic DNA was isolated from peripheral blood leukocytes by a standard method [11]. Two informative microsatellite markers (D11S1887 and D11S1311) were selected to perform linkage analysis at the *TYR* gene at locus 11q14-q25. Mutation analysis was performed for all five coding exons of the *TYR* gene by PCR using primers that produced overlapping products (Table 2) for bidirectional sequencing (3730 DNA sequencer, Applied Biosystems, Applied Biosystems, Foster City, CA).

**Restriction digestion:** A robust, technically straightforward, and cost effective method of detecting known sequence variations is by using restriction enzymes. To test the efficacy of this method, we developed a restriction enzyme assay for each of the mutations detected by direct sequencing. The DNA samples were amplified using the primers 5'-ATT TCT GCC TTC TCC TAC TGA CTC-3', 5'-TGG GTC AAA CTC AGG CAA A-3' and 5'-CCA ACA TTT CTG CCT TCT CC-3', 5'-

**TABLE 2. SEQUENCES OF OLIGONUCLEOTIDE PRIMERS USED IN THE PRESENT STUDY ON *TYR* GENE MUTATIONS IN OCA1**

Exon	Forward primers (5'-3')	Reverse primer (5'-3')
1	CAAACCTGAAATTCATAACATATAAGG	GTGGACAGCATTCCCTCTCTCC
1	TTCAGAGGATGAAAGCTTAAGATAAA	CGTCTCTCTGTGCAGTTTGG
1	CTGGCCATTTCCTAGAGC	CCACCGCAACAAGAAGAGTC
1	CATCTTCGATTTGAGTGCCC	CCCTGCCTGAAGAAGTGATT
2	CCAACATTTCTGCCTTCTCC	TCAGCTAGGTCATTGTCTGAT
3	AGTTATAAATCAAATGGGATAATCA	ACATTTGATAGGCACCCTCT

The oligonucleotides used to assay for normal and variant allele of the *TYR* gene in each exon are illustrated. PCR amplification was performed in a 20 µl volume containing 1 U of Taq DNA polymerase, 200 µM dNTPs, 2 mM MgCl<sub>2</sub>, 50 pmol of primer. Thermocycling conditions were 94 °C for 13 min followed by 35 cycles of 94 °C for 1 min, 59 °C for 1 min, and 72 °C for 2 min, and finally 72 °C for 10 min.

**TABLE 1. CLINICAL FEATURES OF OCA1 PATIENTS**

OCA1 pedigrees	Individual	Visual acuity		Refraction		Nystagmus
		Right	Left	Right	Left	
Family I	1-1	6/60	6/60	0.00 - 1.50 x 180°	0.00 - 1.50 x 180°	+
	1-6	6/60	6/36	+1.75 + 2.00 x 75°	+1.75 + 2.00 x 75°	+
Family II	2-1	-	-	+2.00 + 2.00 x 180°	+2.00 + 2.00 x 180°	+
Family III	3-1	6/36	6/36	+2.00	+2.00	+
	3-2	6/60	6/60	+3.00	+3.00	+
	3-6	3/60	3/60	-6.00 - 2.00 x 180°	-2.50 - 1.50 x 180°	+
Family IV	4-1	4/60	4/60	0.00 - 1.50 x 180°	0.00 - 1.50 x 180°	+
	4-3	6/60	6/60	+5.00 + 1.00 x 90°	+4.00 + 1.50 x 90°	+

This table shows all the OCA1 affected individuals have defects in visual acuity, refractive errors, and have nystagmus in both eyes.

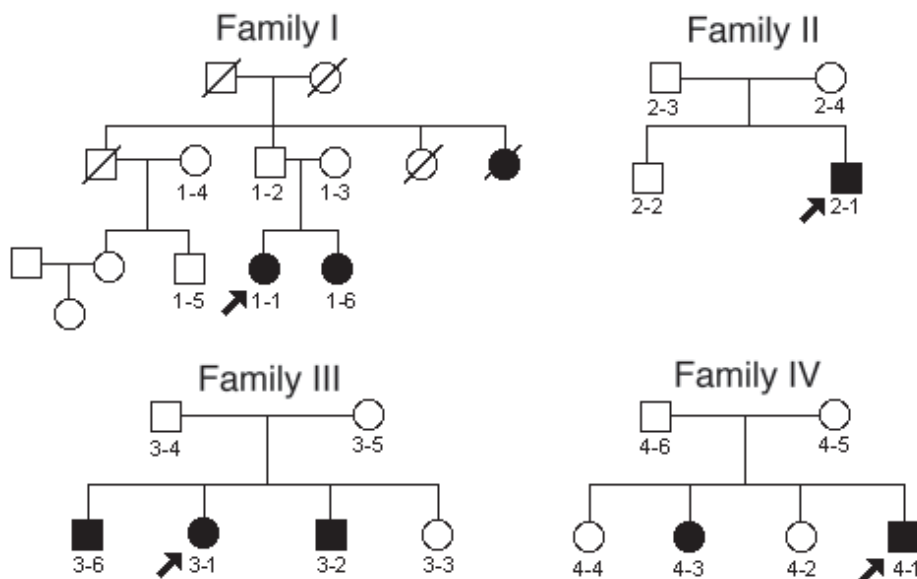


Figure 1. Pedigrees of Indian families with oculocutaneous albinism type 1. The filled circles represent affected females and the filled squares represents affected males. Open symbols are unaffected. The numbered individuals participated in this study.

TCA GCT AGG GTC ATT GTC GAT-3' to confirm the R278X and c.937del8 mutations in exon 2, respectively. The PCR products were digested with *AluI* (for the R278X lesion) and *EcoRI* (for the c.937del8 lesion) enzymes to differentiate between normal and mutant alleles and visualized on a 1.5% agarose gel. To detect the c.1379del2 mutation, the exon 5 primers (Table 2) were used to amplify a 634 bp fragment covering exon 5, which was subsequently digested using *EcoRI* and visualized on a 6% polyacrylamide gel.

### RESULTS

A total of four OCA1 families were enrolled in our study from three different districts of West Bengal, India. Among them eight individuals were clinically diagnosed as having typical features of OCA1, and 14 were normal without any sign of OCA (Table 1).

Haplotype analysis using microsatellite markers (D11S1887 and D11S1311) spanning the *TYR* locus revealed that the phenotype of families III, IV, and I were consistent

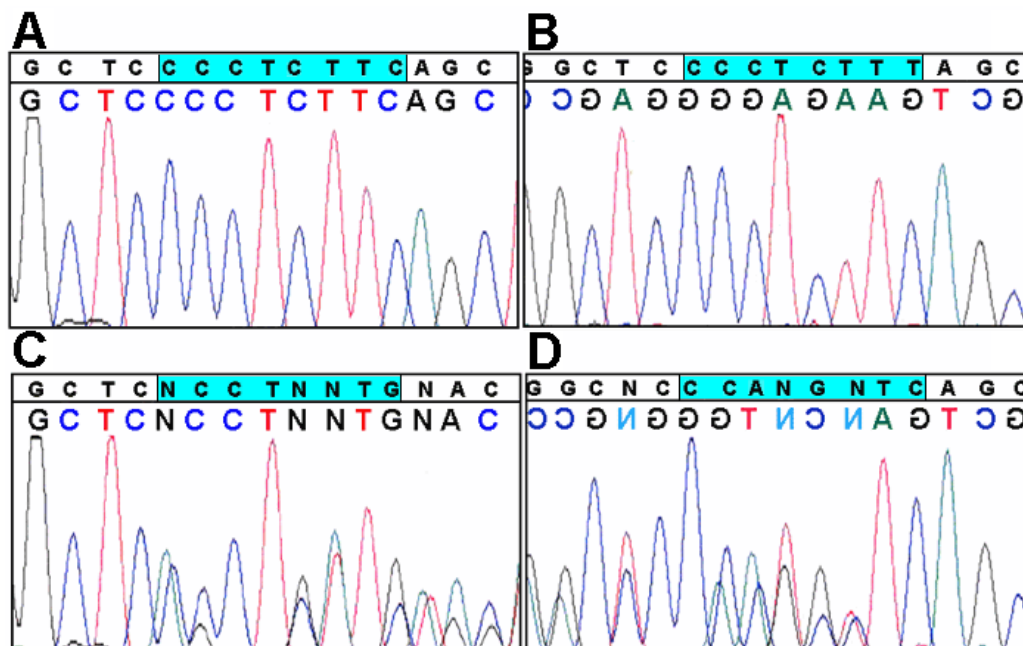


Figure 2. Chromatogram comparison between normal and mutant *TYR* gene. Chromatogram showing the sequence variations in the *TYR* gene in exon 2 are highlighted. Panels show the forward (A) and reverse (B) sequence derived from a normal individual and forward (C) and reverse (D) sequence derived from an OCA1 patient. An eight base pair deletion (c.937del8) in this OCA1 patient is highlighted.

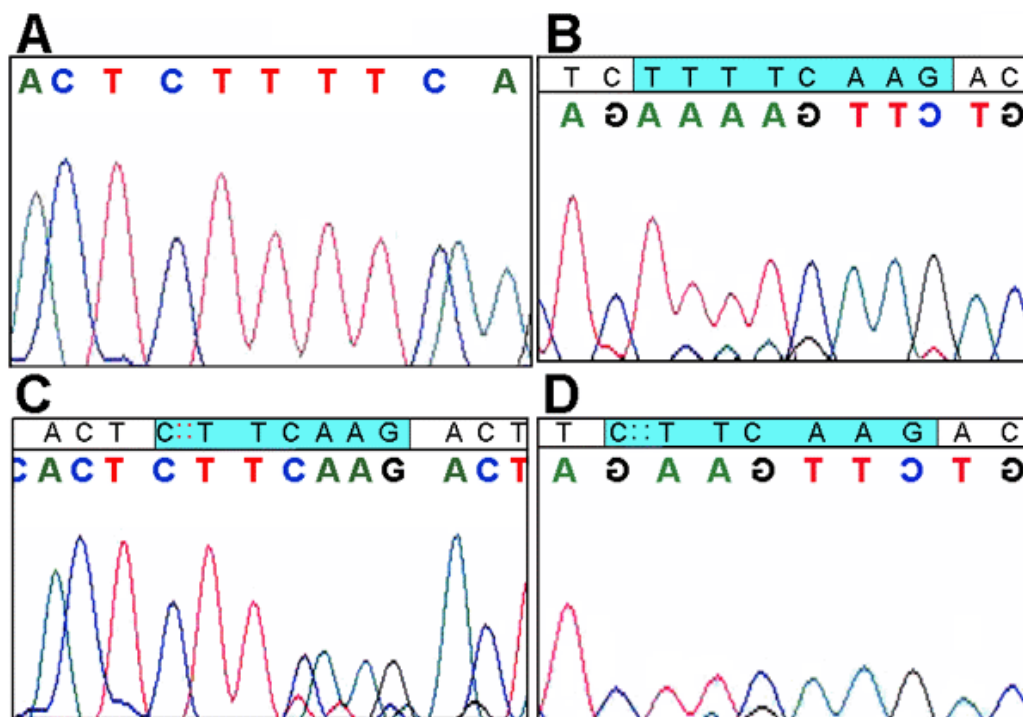


Figure 3. Chromatogram showing the homozygous deletion mutation in the *TYR* gene. Chromatogram showing the sequence in exon 5 of the *TYR* gene in mutant and the corresponding normal sequence. Panels show the forward (A) and reverse (B) sequence derived from a normal individual and forward (C) and reverse (D) sequence derived from an OCA1 patient. A two base pair deletion (c.1379del2) observed in this OCA1 patient is highlighted with double colons.

**TABLE 3. TYR GENE MUTATIONS IN PATIENTS WITH OCULOCUTANEOUS ALBINISM 1 (OCA1) IN INDIAN FAMILIES**

OCA1 Pedigrees	Individual	Disease status	Allele 1		Allele 2		Genotypic status
			Exon	Mutation Position (cDNA/bp)	Exon	Mutation Position (cDNA/bp)	
Family I	1-1	Affected	2	c.937del8	5	c.1379del2	Compound heterozygote
	1-2	Normal	5	c.1379del2		Wild type	Carrier heterozygote
	1-3	Normal	2	c.937del8		Wild type	Carrier heterozygote
	1-4	Normal	2	Wild type	5	Wild type	Normal homozygote
	1-5	Normal	2	Wild type	5	Wild type	Normal homozygote
	1-6	Affected	2	c.937del8	5	c.1379del2	Compound heterozygote
Family II	2-1	Affected	5	c.1379del2	5	c.1379del2	Compound homozygote
	2-2	Normal	2	Wild type	5	Wild type	Normal homozygote
	2-3	Normal	5	c.1379del2		Wild type	Carrier heterozygote
	2-4	Normal	5	c.1379del2		Wild type	Carrier heterozygote
Family III	3-1	Affected	5	c.1379del2	5	c.1379del2	Affected homozygote
	3-2	Affected	5	c.1379del2	5	c.1379del2	Affected homozygote
	3-3	Normal	5	c.1379del2		Wild type	Carrier heterozygote
	3-4	Normal	5	c.1379del2		Wild type	Carrier heterozygote
	3-5	Normal	5	c.1379del2		Wild type	Carrier heterozygote
	3-6	Affected	5	c.1379del2	5	c.1379del2	Affected homozygote
Family IV	4-1	Affected	2	Arg278STOP	5	c.1379del2	Compound heterozygote
	4-2	Normal	2	Wild type	5	Wild type	Normal homozygote
	4-3	Affected	2	Arg278STOP	5	c.1379del2	Compound heterozygote
	4-4	Normal	5	c.1379del2		Wild type	Carrier heterozygote
	4-5	Normal	2	Arg278STOP		Wild type	Carrier heterozygote
	4-6	Normal	5	c.1379del2		Wild type	Carrier heterozygote

This table describes two novel frameshift mutations and a known nonsense mutation in *TYR* gene in Indian OCA1 patients. The five exons of the *TYR* gene were sequenced for each individual using PCR followed by bi-directional sequencing, and the findings are shown.

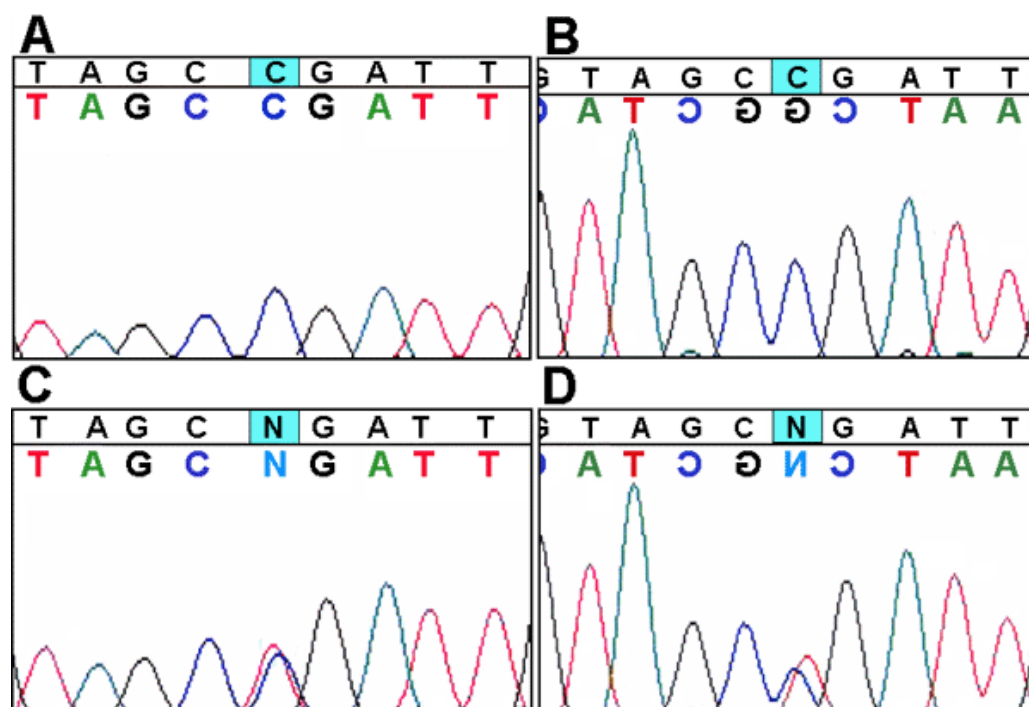


Figure 4. R278X mutation in exon 2 of the *TYR* gene. Chromatograms showing the sequence in exon 2 of the *TYR* gene. Panels show the forward (A) and reverse (B) sequence derived from a normal individual and forward (C) and reverse (D) sequence derived from an affected individual. The R278X nucleotide change is highlighted in all the chromatograms.

with disease segregation at the *TYR* gene (data not shown). Eleven additional markers were used (D5S426, D5S651, D6S280, D9S269, D9S1869, D15S165, D15S1002, DXS991, DXS1061, DXS8035, and DXS8051) to assess other candidate gene loci. No other locus was indicated as being associated with the phenotype in these four OCA1 families.

In the family I pedigree, the proband and her sister are affected by OCA1 (Figure 1). Direct sequencing analysis revealed that both affected individuals share two novel heterozygous deletions, one in exon 2 (c.937del8) and another in exon 5 (c.1379del2) of the *TYR* gene (Figure 2 and Figure 3). The unaffected father and mother are heterozygous for c.1379del2 in exon 5, and heterozygous for c.937del8 in exon 2, respectively (Table 3). Both deletions were further explored using the restriction enzyme *EaeI*. The heterozygous c.937del8 resulted in a loss of restriction site for the enzyme *EaeI* and produced three distinct fragments of 443, 245, and 198 bp. The homozygote normal produced two fragments of 245 bp and 198 bp (data not shown).

In the family III pedigree, two male patients and a female patient were diagnosed with OCA1 (Figure 1). In this family, the mutation c.1379del2 in exon 5 of the *TYR* gene was observed in homozygous state in all the affected members (Figure 3), whereas the unaffected members were heterozygous for this deletion (Table 3). The 2 bp deletion (c.1379del2) creates an *EaeI* site producing an 82 bp fragment and a 550 bp fragment whereas the wildtype sequence remained undigested (data not shown).

We observed the same pattern of inheritance of c.1379del2 in the case of family II pedigree with the proband and his parents (Table 3).

A previously reported heterozygous R278X mutation in exon 2 and a novel deletion c.1379del2 in exon 5 (Figure 4) were identified on separate alleles of two OCA1-patients from family IV (Figure 1). We observed that the proband's mother was heterozygous for the R278X mutation, while the father was heterozygous for the c.1379del2 mutation, both exhibited normal phenotype. The mutation R278X was previously observed in Indo-Pakistani patients and has been reported in the Tili community in West Bengal [10,12]. The heterozygous mutation R278X resulted in gain of restriction site for the enzyme *AluI*. Restriction digestion with *AluI* produced three dis-

tinct bands, 116 bp, 78 bp, and 32 bp for R278X mutation, whereas only two bands, 194 bp and 32 bp, were seen for wild type sequence (data not shown).

## DISCUSSION

A total of 111 mutations have been reported so far in the *TYR* gene from different ethnic groups, cited in Human Gene Mutation Database. Among the 111 mutations, 85 are nonsense/missense mutations, 4 are splicing, 15 are deletions, 6 are insertions, and one small indels. Tyrosinase is a membrane glycoprotein contains 529 amino acids, which include a signal peptide at the amino end (amino acid residues 1-18) and a transmembrane region (residues 474-499). The catalytic site of the enzyme contains two copper atoms ligated to six histidine residues (180, 202, 211, 363, 367, and 390) and possible glycosylation sites of asparagine residues (86, 111, 161, 230, 337, and 371). Halaban et al. [13] reported that the retention of misfolded tyrosinases in the endoplasmic reticulum due to the altered amino acid sequence, with subsequent degradation, may be a common reason for the absence of tyrosinase activity in albino melanocytes.

Most reported mutations and polymorphisms associated with albinism are in the tyrosinase gene associated with OCA1 [8]. Individuals with OCA1 are born with white hair and skin and blue irides. If their tyrosinase gene mutations produce an inactive enzyme or no enzyme, they are unable to make pigment throughout life. If either (compound heterozygous) or both (compound heterozygous or homozygous) of their mutations produce an enzyme with residual activity, they will form hair, skin, and iris pigment during the first decade of life. Hair color will turn golden blond and may eventually turn brown. Skin remains light but may tan on sun exposure. Iris color turns dark blue, gray, hazel-green, or light brown.

Haplotype analysis using two informative microsatellite markers showed consistent linkage between *TYR* gene and disease phenotype in families I, III, and IV. The additional markers did not indicate linkage with any of the other loci examined. Among these families there were no unexpected phenotypic characteristics [1,13] observed in OCA1 patients, and typical variations in visual acuity (Table 1).

West Bengal is the northern most province of India. The study area included three districts with an endogamous popu-

TABLE 4. *TYR* GENE MUTATIONS IN PATIENTS WITH OCULOCUTANEOUS ALBINISM 1 (OCA1) IN INDIAN FAMILIES

Base position	Codon/mutation	Exon	Nucleotide change	Amino acid substitution	Type of mutation	Reference
832	R278X	2	CGA -> TGA	Arg -> term	Nonsense	[12]
937- 944	-DCCCTCTTC	2	Delete CCCTCTTC	Term 312	Frame shift	
1379-1380	-D TT460	5	Delete TT	Term 460	Frame shift	

The table describes the variants identified in *TYR* gene with the predicted amino acid changes in Indian OCA1 families. The base position refers to positions relative to nucleotide position 1 of the first codon in the mRNA sequence. This is the first report of the two frame shift mutations.

lation of 9,300,000 in which approximately 200 people are affected. In our study, we were able to collect four pedigrees affected with OCA1, and the clinical features of affected OCA1 patients were given in Table 1. We have identified two novel frame shift mutations in the *TYR* gene (c.1379del2 and c.937del8) and a previously reported nonsense mutation (R278X) in OCA1 Indian patients (Table 4), which was observed in an Indo-Pakistani patient and patients in West Bengal [10,12]. In each of the four families, the deletion mutation c.1379del2 is commonly observed, and the mutation in the homozygous state segregates with the phenotype in families II and III. The phenotype is observed only when the heterozygous deletion mutation c.1379del2 is present either with c.937del8 or with R278X in families I and IV (Table 3). It has been reported that nonsense and frameshift mutations in OCA1 were randomly distributed throughout the *TYR* coding region, although frameshift mutations tend to occur in short repetitive sequences [14]. The frame shift and nonsense mutations produce truncated proteins and are associated with a complete lack of enzymatic activity. Digesting gene specific PCR products with restriction enzymes are a cost effective and robust method for the identification of previously characterized mutations within a population.

In summary, we have identified two novel deletions in the *TYR* gene, each of which results in a frameshift mutation, and a known nonsense mutation in Indian OCA pedigrees. Our investigation into mutations in the *TYR* gene in families affected with OCA1 will help early diagnosis of the disease and with genetic counselling to reduce the occurrence of this debilitating disease.

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