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OCA1 in Different Ethnic Groups of India is Primarily Due to Founder Mutations in the Tyrosinase Gene

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Summary

Oculocutaneous albinism (OCA) is a heterogeneous group of autosomal recessive disorders characterized by an abnormally low amount of melanin in the eyes, skin and hair, and associated with common developmental abnormalities of the eye. Defects in the tyrosinase gene (*TYR*) cause a common type of OCA, known as oculocutaneous albinism type 1 (OCA1). The molecular basis of OCA has been studied extensively in different population groups, but very little information is available on Indian patients. Our investigation covering thirteen ethnic groups of India, some representing >20 million people, revealed that among 25 OCA families 12 were affected with OCA1, and that these cases were primarily due to founder mutations in *TYR*. We detected nine mutations and eight SNPs in *TYR*, of which six mutations (five point mutations & one gross deletion) were novel. In contrast to most reports describing compound heterozygotes, the presence of homozygotes in 10 out of the 12 pedigrees underscores the lack of intermixing between these ethnic groups in India. Haplotype analysis suggested a few founder chromosomes causing the disease in the majority of the patients. Direct detection of the mutations prevalent in specific ethnic groups could be used for carrier detection and genetic counselling.

Keywords: Albinism, Oculocutaneous albinism type 1, OCA1, Tyrosinase, TYR

Introduction

Oculocutaneous albinism (OCA) is a heterogeneous group of autosomal recessive disorders. It is characterized by abnormally low amounts of melanin in the eyes, skin and hair and also associated with common developmental abnormalities of the eye. In addition to poor visual acuity, the characteristic ocular features of OCA include nystagmus, strabismus, iris transillumination, photophobia, foveal hypoplasia and misrouting of the optic nerve fibres at the chiasm.

Although originally thought to be a Mendelian disorder caused by mutations in a single gene, subsequent research has shown that the genetics of albinism is complex (Oetting *et al*. 2003). The phenotypic heterogeneity of this condition is due to different gene mutations affecting various steps in the melanin biosynthetic pathway, resulting in varying degrees of decreased melanin pigmentation. At present at least 16 different genes have been identified which, when mutated, result in different types of albinism (Oetting *et al*. 2003; Tomita & Suzuki, 2004).

OCA1 results from mutations in the tyrosinase gene (*TYR*, MIM 606933) and is the second most common

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subtype, occurring in approximately 1 per 40,000 individuals in most populations (King *et al.* 2001). The human tyrosinase gene (*TYR*, 11q14–q21), consisting of 5 exons, spans ~65 kb of DNA and encodes a 58-kDa glycoprotein composed of 529 amino acids from a 1.964 kb transcript (NM_000372.3). Tyrosinase is a copper-containing enzyme expressed in the melanocytes, and catalyzes the oxidation of tyrosine to L-dihydroxy-phenylalanine (DOPA) and the subsequent dehydrogenation of DOPA to dopaquinone, the first two rate-limiting steps in the melanin biosynthetic pathway. Partial or complete lack of functional tyrosinase results in reduction or absence of melanin pigment in the skin, hair, and eyes throughout life from birth.

Despite impressive advancement in the genetics of albinism, and a large number of reported *TYR* mutations (http://www.cbc.umn.edu/tad/oca1mut.html), very little information is available on Indian patients (Sundaresan *et al.* 2004). To fill this void, we recruited OCA patients from eastern and southern parts of India, covering 13 distinct ethnic groups belonging to the Hindu caste system. The ethnic groups of these regions are reported to be genetically quite distinct, having different population histories (Basu *et al.* 2003), which provided us with an opportunity to study whether the sets of OCA mutations in these regions are also distinct or not. Negative results for the tyrosinase hair-bulb assay for most of the patients suggested that they might have mutations in the tyrosinase gene. Hence, we initially chose the tyrosinase gene for mutation screening.

Materials and Methods

Selection of the Study Subjects

Twenty-five OCA affected families were recruited from West Bengal (WB) and Andhra Pradesh (AP), representing eastern and southern states of India, respectively. Seven families representing six different ethnic groups (Garai, Brahmin, Dhibor, Tambuli, Gowala, Kayastha) were from WB; 14 affected individuals along with their family members were included in the study. The remaining eighteen families represented seven different ethnic groups (Vysya, Viswa Brahmin, Brahmin, Padmasali, Turupukapu, Perika, Reddy) from AP. From these southern Indian population groups we recruited 29 OCA patients and their family members. In total, the study group consisted of 180 individuals including both the OCA1 affected and unaffected family members. Along with ocular and cutaneous hypopigmentation, the diagnosis also involved other systemic and ocular examinations including photophobia, iris transillumination, abnormal ocular movements (nystagmus, strabismus), decreased visual acuity (usually diminished to as low as 6/60) and foveal hypoplasia. Patients were also tested for other ocular involvements such as cataract, glaucoma, retinal diseases etc. In addition, from each ethnic group 50 normal individuals were selected without any family history of ocular disease and albinism and evaluated for the presence of novel nucleotide changes. The study was conducted with the approval of the institutional review board (IRB), and followed the tenets of the Declaration of Helsinki. The tyrosinase hair-bulb assay was carried out by incubation of hair-bulbs with 0.1% L-DOPA solution, and indicated lack of enzyme activity in most of the patient samples when the hair bulbs did not darken in colour due to conversion of the substrate to melanin. Therefore, *TYR* was used as the first candidate gene to investigate the molecular defects among OCA affected individuals in our study group.

Collection of Blood Samples & Genomic DNA Preparation

Approximately 10 ml blood samples were collected with informed consent from OCA patients, their family members and normal individuals. Genomic DNA was prepared from fresh whole blood by the salting-out method using sodium-perchlorate, and DNA was dissolved in TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) (Johns & Paulus-Thomas, 1989).

Polymerase Chain Reaction (PCR), DNA Sequencing and Genotyping

All five exons as well as the splice junctions of *TYR* were screened for mutations in the patients as described below, and the suspect nucleotide change was tested for co-segregation with the OCA phenotype in the pedigrees. PCR was carried out in a total volume of 25.0 μl

containing 50-100 ng genomic DNA, 0.2 μM of each primer, 0.1 mM of each dNTP, $MgCl_2$ (as appropriate), and 0.5 unit of *Taq* polymerase (Invitrogen, Carlsbad, California) in a thermocycler (GeneAmp-9700, Applied Biosystems, Foster City, USA). PCR conditions and primer sequences for exons 4 and 5 were the same as described previously (Chaki *et al*. 2005) and those of exon 1 to 3 are available on request. The PCR products were examined for specificity by polyacrylamide (6%) or agarose (1.5%) gel electrophoresis as appropriate, and used for sequencing in an ABI Prism 3100 (Avant) DNA sequencer. Sequencing was done using nested primers to avoid background noise due to non-specific amplification products (if any) and compared with the RefSeq reference sequence NM_000372.3.

For genotyping with microsatellite markers, 3 CA-repeat markers were identified within (GDB: 11511691) and flanking (GDB: 11511689 & GDB: 11511690) the *TYR* locus. These repeat markers were amplified from the affected and immediate family members using fluorescently labelled primers. Amplified PCR products were subjected to Genescan analysis in an ABI Prism 3100 DNA Sequencing System using the 500 TAMRA Size Standard (Applied Biosystems, California, USA). The sizes of the alleles represented the length of the amplified DNA fragment for each marker locus (in bp). In each case, the haplotype was constructed using the genotype data, following the Mendelian pattern of inheritance of the markers in the family.

Restriction Enzyme Digestion

Those mutations that were predicted to alter a restriction site were assayed by digestion of the PCR products with appropriate restriction enzymes under the conditions described by the manufacturer (New England BioLabs, Beverly, MA). The DNA digests were analyzed by electrophoresis in 6% polyacrylamide gels.

Statistical Analysis

Maximum likelihood estimates of the haplotype frequencies were calculated using 50 normal ethnically matched chromosomes, taking 5 SNPs and 3 microsatellite markers linked to the TYR locus and computed via the EM algorithm using the program HAPLOPOP (Majumdar & Majumder, 1999).

Results and Discussion

Nine different *TYR* mutations were identified in 12 pedigrees representing six ethnic groups. These included one gross deletion, one small deletion, and two null and five missense mutations of which six represented novel changes. None of these mutations were detected in 100 alleles from ethnically matched controls. The genotypes of mutations in different ethnic groups, and the potential implications of the *TYR* defects, are described in Table 1, while detailed descriptions of the novel changes and the locations of all the identified mutations in the known domains of tyrosinase are illustrated in Fig 1.

In one of the Vysya pedigrees from southern India we detected a homozygous gross deletion in two patients (sibs) encompassing the 3′-region of *TYR* – we could amplify exons 1, 2 and 3 of *TYR* but not exons 4 and 5. In this context, it is worthwhile mentioning that the 3′ region (~68 kb) of *TYR,* encompassing exons 4 and 5, has 98.55% sequence identity with a pseudogene (*TYRL,* 11p11.2, MIM 191270) (Takeda *et al*. 1989; Giebel *et al*. 1991; Takeda *et al*. 1991) which makes it difficult to distinguish between these two homologous regions. Recently we devised a PCR strategy to amplify each locus separately (Chaki *et al*. 2005). Our results demonstrated that sequence corresponding to exons 4 and 5 only from *TYRL*, but not *TYR*, could be amplified from the affected sibs (Fig. 2). Next, we used another set of primer pairs which would coamplify exons 4 & 5 from both *TYR* and *TYRL*. On sequencing of these PCR products it was revealed that the variant bases between *TYR* and *TYRL* (Fig. 2, panel C) were heterozygous in the chromatograms for the control (Fig. 2, panel A), but contained the bases corresponding to *TYRL* only in the case of the patient (Fig. 2, panel B). This experiment allayed any suspicions regarding a PCR-related problem, and unequivocally proved the presence of a gross deletion encompassing exons 4 and 5 of *TYR*. Since exons 4 and 5 are flanked by a large intron (~57 kb) and the 3′-flanking region of the gene, any PCR based strategy could not be used to map the extent of the deletion. Even a successful, interpretable result from a southern blot would likely be complex

Table 1 TYR mutations detected in different ethnic groups of India

Ethnic Group (No. of pedigrees)	Mutation (aa change) Allele 1	Allele 2	Haplotypes (M1-S1-S2-S3-S4-S5-M2-M3)*	Remarks
Eastern India (West Bengal)				
Tambuli (1)	c.832 C > T (Arg278Stop)	c.1379delTT (frameshift)	Not determined	Reported mutations (Sundaresan *et al*. 2004), (Tripathi *et al*. 1993); nonsense mutation in exon 2 causes loss of CuB binding domain and the frameshift mutation in exon 5 results in aberrant protein formation.
Garai (1)	c.124 G > A (Asp42Asn)	c.124 G > A (Asp42Asn)	177-G-C-C-C-G-95-155 177-G-C-C-C-G-95-155	Novel change in exon 1. Substrate binding is likely to be affected.
Brahmin (1)	c. 1114 G > A (Gly372Arg)	Not found	Not determined	Novel change in exon 3. Sorting from ER and subsequent glycosylation are predicted to be affected.†
Kayastha (1)	c. 272 G > C (Cys91Ser)	c. 272 G > C (Cys91Ser)	179-G-C-C-C-G-95-161 179-G-C-C-C-G-95-161	Novel change in exon 1. Faulty disulfide bond formation resulting in ER mediated protein degradation is predicted as demonstrated by *in vitro* mutagenesis creating other mutations (Cys85Ser & Cys89Arg) (Svedine *et al*. 2004).
Southern India (Andhra Pradesh)				
Vysya (6)	Deletion of exons 4 & 5	Deletion of exons 4 & 5	179-G-C-C-C-G-95-157 179-G-C-C-C-G-95-157	Novel change. Deletion includes exons 4 & 5. Transmembrane domain along with the C-terminal region of the protein would be lost, which might affect maturation of the protein and lead to its mislocalization.
	c. 976 C > T (Gln326Stop)	c. 976 C > T (Gln326Stop)	177-G-C-C-C-G-95-161 177-G-C-C-C-G-95-161	Novel change in exon 2. CuB-binding domain would be lost.
	c.655 G > A (Glu219Lys)	Not found	Not determined	Novel change in the vicinity of the CuA domain.
Brahmin (2)	c. 1255 G > A (Gly419Arg)	c. 1255 G > A (Gly419Arg)	181-C-T-C-C-G-99-155 181-C-T-C-C-G-99-155	Reported mutation in Caucasians and individuals of Indo-Pakistani origin (King *et al*. 1991). The mutation is present in a cluster of other pathogenic mutations (codons 371–448) and is likely to represent a critically important region of the enzyme.

The GenBank RefSeq accession for *TYR* cDNA is NM_000372.3. Among the Vysya, a deletion mutation and Glu219Lys were detected in one pedigree each, while Gln326Stop was detected in four pedigrees. Haplotype analysis was done only for the homozygous mutations using microsatellite (M) and SNP (S) markers.

*__M1__ – Microsatellite marker no. 1, __S1__ – 1-533G > C, __S2__ – 1-301C > T, __S3__ – 1-199C > A, __S4__ – c.575C > A (S192Y), __S5__ – c1037-201G > A, __M2__ – Microsatellite marker no. 2, __M3__ – Microsatellite marker no. 3.

†Gly372Arg is located in the CuB-binding region of TYR and within the most important glycosylation site of the protein that is essential for its correct glycosylation, exit from ER and enzymatic activity (Olivares *et al*. 2003).

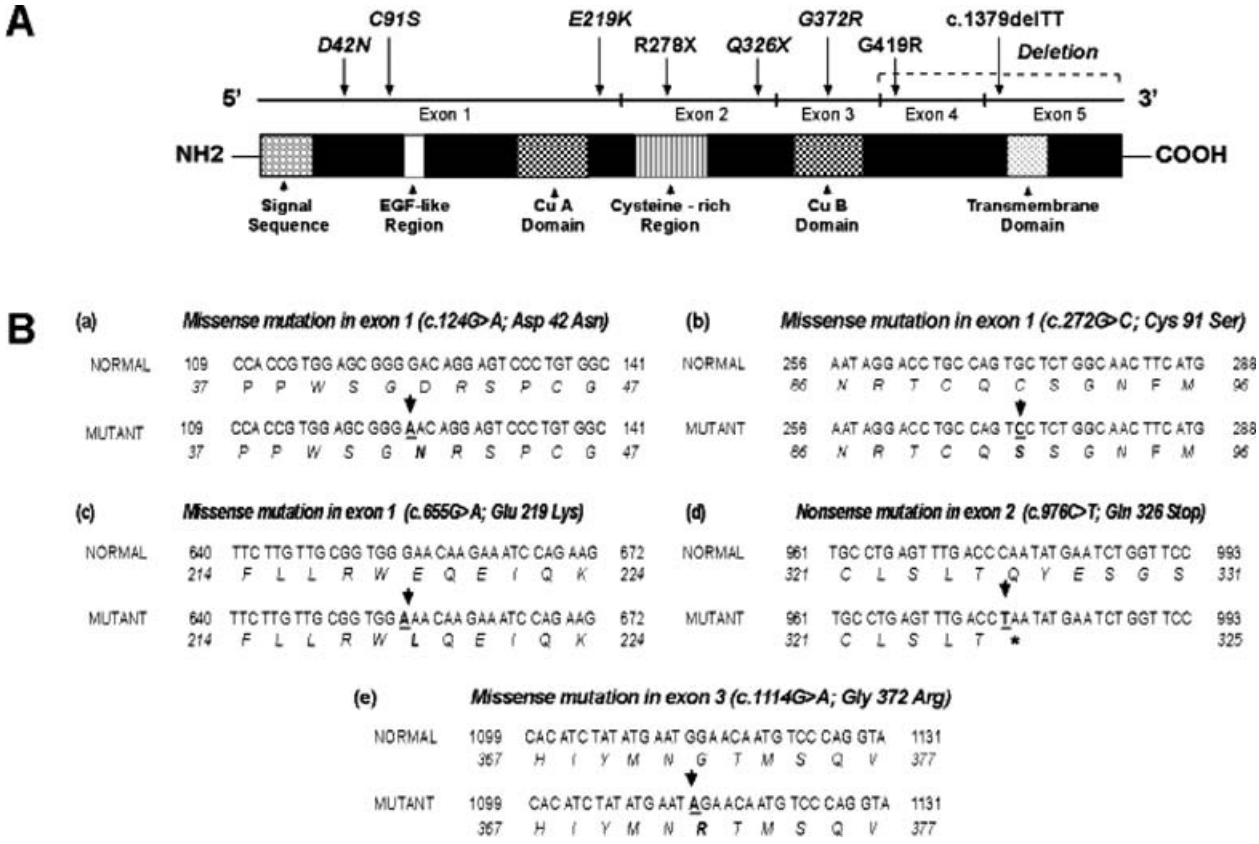

Figure 1 Tyrosinase mutations detected in eastern and southern Indian OCA1 patients. *Panel A*, The known protein domains of Tyrosinase (Protein RefSeq NP_000363.1) and the location of the mutations are shown. Italicized letters indicate novel mutations. *Panel B*, The novel point mutations (bolded and underlined) are indicated by arrowheads. Nucleotide sequences (Genbank accession no. NM_000372.3) are shown as triplet codons and italicized single letters underneath each codon indicate the corresponding amino acid. The mutant amino acids and the null mutations are shown by bold letters and asterisks, respectively.

and confusing, due to ~68 kb duplicated region of the genome with 98.55% sequence identity (Giebel *et al.* 1991).

We detected four novel missense mutations (Asp42Asn, c124G > A; Cys91Ser, c.272G > C; Glu219Lys, c.655 G > A and Gly372Arg, c.1114G > A) (Table 1). All four changes are non-conservative and the affected residues are conserved across different species; three (Cys91Ser, Glu219Lys and Gly372Arg) are even conserved within the TRP protein family (tyrosinase, tyrosinase related protein 1 and tyrosinase related protein 2). All these novel missense mutations were also observed to co-segregate with the disease phenotype in the affected pedigrees, and would be predicted to cause loss of the normal function of tyrosinase (Table 1). Loss of a *Psh*AI site due to one mutation (Asp42Asn), and gain of a *Drd*I site for another (Cys91Ser) were utilized to score these mutations in normal individuals and additional patients. The Tambuli patient was found to be a compound heterozygote for two previously reported mutations – a frameshift mutation, c.1379delTT, identified in Indian patients (Sundaresan *et al.* 2004) and Arg278Stop (R278X, c.832C > T), which represented a CpG change, and was previously reported among people of Asian ancestry (Tripathi *et al.* 1993).

OCA patients from two Brahmin pedigrees from southern India were detected as being homozygous for the missense mutation (c.1255G > A; Gly419Arg) which had been reported previously (King *et al.* 1991). Another novel nonsense mutation (Q326X, c.976C > T) was identified in a homozygous state among four out of seven Vysya-pedigrees from southern India. Although both these ethnic groups (Brahmin & Vysya) have large population sizes (currently >20 million), inbreeding has long been practiced in both groups. Thus, the finding of

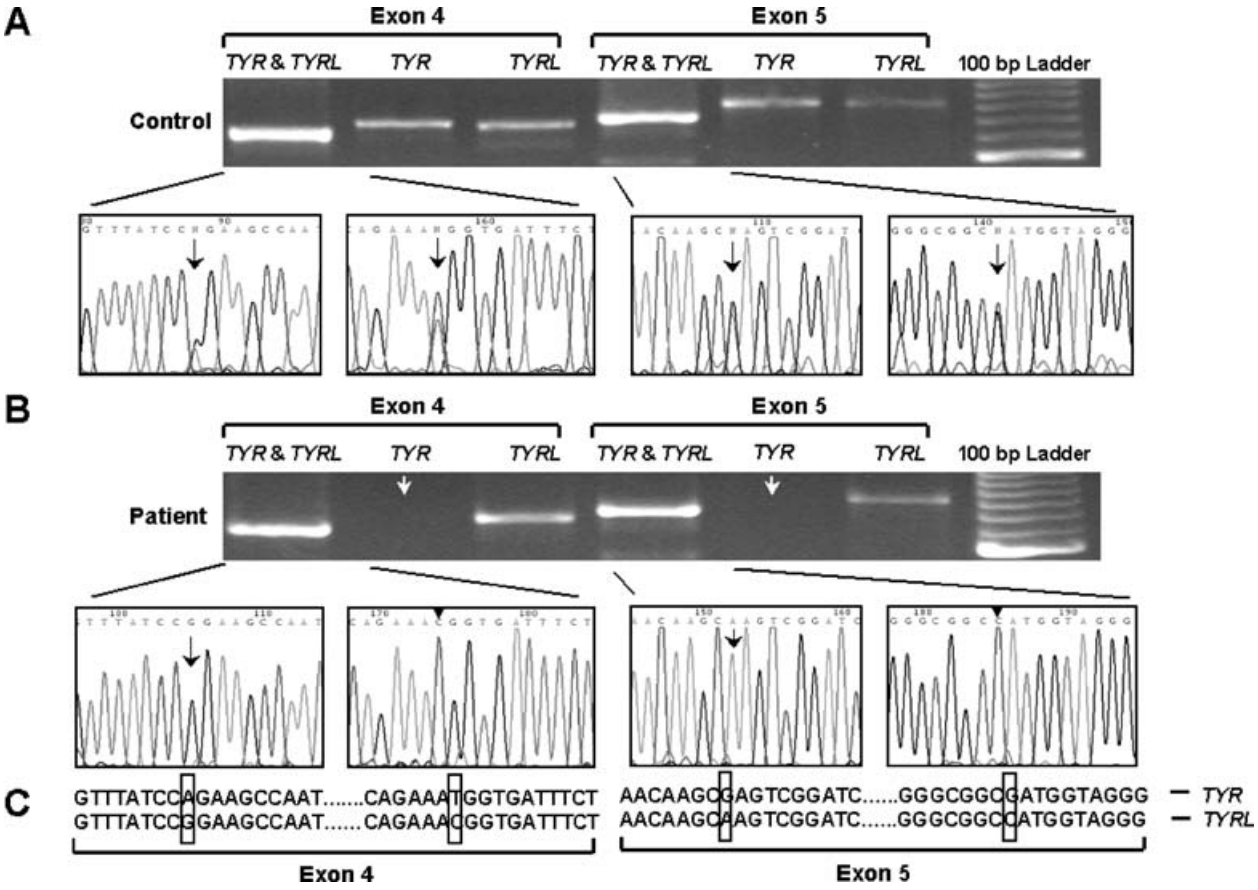

Figure 2 Detection of a gross deletion encompassing exons 4 and 5 of *TYR*. *Panel A*. Exons 4 and 5, along with their flanking sequences, were amplified from a control sample using different sets of primers, viz. common primer pairs that would amplify the homologous regions from both *TYR* and the pseudogene *TYRL* (exon 4 = 740 bp, & exon 5 = 823 bp for both loci); *TYR* specific primers (exon 4 = 790 bp, & exon 5 = 924 bp) and *TYRL* specific primers (exon 4 = 781 bp, & exon 5 = 917 bp). The PCR products were separated in an agarose gel (1.5%) as shown in the upper panel. The higher intensities of DNA-bands in the '*TYR* & *TYRL*' lanes are due to co-amplification from both loci. Chromatograms shown in the lower panel were obtained on sequencing the coamplified PCR products and show the variant bases between *TYR* and *TYRL* (e.g. c.1236A > G, c.1305T > C for exon 4 and c.1413G > A, c.1446G > C for exon 5; coordinates of bases given based on *TYR* sequence accession no. NM_000372.3), as indicated by the arrows. *Panel B*, The same set of experiments done with both Vysya sibs affected with OCA1; data corresponding to one patient is shown. No PCR product was obtained for *TYR* and the chromatograms show the presence of bases corresponding only to *TYRL*. *Panel C*. Variant bases between *TYR* (Contig Accession No. NT_008984.17) and *TYRL* (Contig Accession No. NT_009237.16) which are shown in the chromatograms are indicated by boxes.

the same mutation in multiple unrelated families in each ethnic group may be the consequence of a founder effect and inbreeding. This was also evidenced by haplotype analysis using microsatellite and SNP markers encompassing the *TYR* locus (Table 1).

The frequencies of each mutant haplotype were computed from analyzing 50 normal chromosomes from the same ethnic group using the HAPLOPOP program (Majumdar & Majumder, 1999). Among the control samples from eastern India (Garai and Kayastha ethnic groups) considerable diversity in haplotypes was observed. The total number of haplotypes was 44, with estimated frequencies ranging from 0.2% to 13.4%. The Asp42Asn mutation in the Garai, was associated with a single haplotype background (177-G-C-C-C-G-95-155); this haplotype was observed among controls with an estimated frequency of only 8.3%. A similar scenario was also observed among the Kayastha, where the Cys91Ser mutation occurred on a single haplotype background (179-G-C-C-C-G-95-161). This haplotype was observed in only 2.5% of the normal chromosomes. On the other hand, in

Table 2 TYR SNPs detected in different ethnic groups of India

Sl. No.	Nucleotide Change (amino acid change)	Location	Ethnic Groups	Frequency (Heterozygosity)	Remarks
1†	1–533 G > C	Promoter	Viswa Brahmin; Brahmin (S)	0.32	dbSNP no. rs5021654
2†	1–301 C > T	Promoter	Viswa Brahmin; Brahmin (S)	0.375	dbSNP no. rs4547091
3	1–199 C > A	Promoter (CAAT Box)	Viswa Brahmin; Perika	0.18	dbSNP no. rs1799989
4	c.575C > A (Ser192Tyr)	Exon 1	Padmasali; Vysya	0.095	dbSNP no. rs1042602
5*	c.1037 −201 G > A	Intron 2	Brahmin (E)	0.049	dbSNP no. rs12804012
6	T ins into a poly T sequence (−21 to −27 from exon 3)	Intron 2	Dhibor	0.047	Reported (Tanita *et al.* 2002)
7*	c.1184 +50 G > A	Intron 3	Brahmin (E)	0.095	dbSNP no. rs3793975
8*	c.1184 +383 A > T	Intron 3	Brahmin (E)	0.049	dbSNP no. rs3793974

S, South Indian; E, Eastern Indian.
*These three iSNPs in West Bengal (WB) Brahmins were in linkage disequilibrium with Gly372Arg mutation.
†These two pSNPs in Andhra Pradesh (AP) Brahmins were in linkage disequilibrium with Gly419Arg.

southern India the haplotype diversity among controls was lower (i.e. 25) with frequencies ranging from 1% to 16.6%. Each of the three distinct mutations found in southern India – the gross deletion (in Vysya), Gln326Stop (in Vysya) and Gly419Arg (in Brahmin) – was observed on a homogeneous but different haplotype background – 179-G-C-C-C-G-95-157, 177-G-C-C-C-G-95-161 and 181-C-T-C-C-G-99-155, respectively. None of these haplotypes was observed among the controls. However, in each case the closest haplotype differed from the mutant haplotype by one CA-repeat unit in a single microsatellite marker. Thus, in view of the high mutation rates known to prevail at microsatellite loci (Falush & Iwasa, 1999) and the small number of repeat size differences observed, it is likely that these three mutations originally occurred on the three different haplotype backgrounds found in normal chromosomes, and that haplotype divergence due to single allelic variation occurred subsequently.

In two pedigrees only one of the two mutations was identified in *TYR,* despite scanning the entire gene including the promoter region, which suggests that the second uncharacterized mutation may be located in the proposed Locus Controlling Region (LCR) almost 9 kb upstream of *TYR* (Fryer *et al.* 2003).

While screening the patient samples for *TYR* mutations we also detected several polymorphisms among the patients and unrelated normal individuals. A total of eight SNPs were detected – 3 in the promoter region (pSNP), one in the coding sequence (cSNP) and four in the introns (iSNP), including an in/del type polymorphism (Table 2). These SNPs, particularly those present in the promoter region, could modulate tyrosinase expression, and the involvement of such subtle variations in the complex mechanism of melanin biosynthesis resulting in the development of the varied OCA phenotype cannot be ruled out.

Our study shows that among ethnic groups of eastern and southern Indians OCA1 is caused primarily by a few founder mutations, and this underscores the lack of intermixing between these groups. Direct detection of these mutations prevalent in the specific ethnic groups could be used for carrier detection and genetic counselling to further contain the spread of this disorder.

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