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# *SLC45A2* variations in Indian oculocutaneous albinism patients

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**Purpose:** Oculocutaneous albinism (OCA) is an autosomal recessive disorder of melanin biosynthesis that results in congenital hypopigmentation of ocular and cutaneous tissues. It is also associated with common developmental abnormalities of the eye. Mutations in the *solute carrier family 45, member 2* gene (*SLC45A2*, also called *MATP*) cause oculocutaneous albinism type 4 (OCA4), which is the second most prevalent type of OCA in Japan. So far, 24 pathological mutations have been reported in *SLC45A2*, but there is no report from India. Interestingly, in almost 31% of the cases, the second mutation has never been found. The purpose of this study was to investigate the molecular basis of OCA among Indians using *SLC45A2* as the candidate gene.

**Methods:** From our patient pool, consisting of 50 unrelated OCA pedigrees covering 17 ethnic groups of eastern and southern India, 20 patients (from 19 affected families) lacking any mutation in the *tyrosinase* gene (*TYR*) were screened further for nucleotide variants in *SLC45A2*. All seven exons and splice-site junctions of *SLC45A2* were amplified and sequenced from the OCA patients and from 50 ethnically matched healthy controls. Nucleotide changes were detected by identifying 'double peaks' in the chromatogram due to heterozygosity as well as by pairwise BLAST analysis of the sequence output data with a normal copy of *SLC45A2*. Haplotype analysis was done among the affected sibs using three newly identified microsatellite markers placed within and in flanking regions of the *SLC45A2* locus.

**Results:** Four novel mutations (c.126G>A [Met42Ile], c.190G>A [Gly64Ser], c.904A>T [Thr302Ser], and c.1042C>T [Arg348Cys]) and one reported mutation (c.469G>A [Asp157Asn]) were identified in *SLC45A2*. All the novel changes cosegregated with the disease and none were present in control samples. Consistent with previous reports, we did not find the second mutant allele in three unrelated patients. Haplotype analysis using microsatellite markers in the family of one such proband suggested that the affected sibs inherited the mutant allele (Arg348Cys) from their father but different *SLC45A2* alleles from the mother. In addition, five single nucleotide variants were identified which included E272K and L374F polymorphisms that have been reported to be associated with human ethnicities.

**Conclusions:** Our study reveals that 10% of the total OCA cases from eastern and southern Indian ethnic groups carry mutations in *SLC45A2*. Among 10 variants found in the gene, five are pathogenic changes. Our data, based on haplotype analysis on a single family, suggest that the disease is caused in the affected sibs either by a single mutation in *SLC45A2* and a defect in another locus, or *SLC45A2* is not responsible for the disorder in the family, but the pathogenesis is caused by a mutation in another gene not yet characterized in these patients.

Oculocutaneous albinism (OCA) is a heterogeneous group of autosomal recessive disorders characterized by cutaneous and ocular hypopigmentation along with developmental abnormalities of the eyes. Some of the ocular features of OCA include poor visual acuity, nystagmus, strabismus, iris transillumination, foveal hypoplasia, and misrouting of the optic nerve at the optic chiasma. OCA patients are also at a high risk of skin cancer due to the lack of melanin pigment, which plays a protective role against ultraviolet (UV) rays. To date, 16 genes have been identified in which a mutation can cause different types of albinism [1]. OCA type 4 (OCA4, OMIM 606574), caused by mutations in the *solute carrier family 45, member 2* gene (*SLC45A2*, also known as *MATP*, 5p13.3, OMIM 606202), is one of the four major subtypes of OCA. The phenotype of OCA4 patients is generally similar to those having OCA2, i.e. yellow hair, white skin with localized pigmented regions, and irises that are partially or completely pigmented with a tan colored melanin.

Human *SLC45A2* (GenBank NM_016180) with seven exons spans about 40 kb of the genome and encodes a protein containing 530 amino acids (GenBank NP_057264). The mature SLC45A2 protein is predicted to contain 12 putative transmembrane domains and exhibits structural homology to plant sucrose-proton symporters [2]. Previously, Fukamachi et al. [3] reported that a *MATP* homolog in medaka fish 'b' encodes a transporter, which mediates melanin synthesis. Human SLC45A2, presumably located in the melanosomal membrane, probably functions as a membrane transporter directing the traffic of melanosomal proteins and other substances to melanosomes [2]. However, the precise role of the protein is yet to be elucidated.

To date only a few reports are available describing a total of 24 *SLC45A2* defects in OCA patients, but there is no report from India though OCA is one of the four major causes of

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childhood blindness in the country [4]. Interestingly, among the total number of unrelated OCA4 patients described so far in the literature, the second mutation could not be identified in *SLC45A2* for about 31% (12/39) of the cases. It is thus possible that the uncharacterized mutations are located in an unexplored region of the gene (e.g. distal promoter or locus controlling region, if any) or that the precipitation of the disease results from an additional defect in another locus. In this study, we investigated the molecular basis of OCA among Indians using *SLC45A2* as the candidate gene.

## METHODS

*Selection of the study subjects:* Out of a total 50 OCA pedigrees recruited for the study from eastern and southern India, 20 OCA patients representing 19 unrelated OCA-affected families were found to carry no mutation in the *tyrosinase* gene (*TYR*). All 20 of these patients from the remaining OCA pedigrees were enrolled in the study. Apart from hypopigmentation, the diagnosis involved ophthalmologic examinations including the testing of abnormal ocular movement (nystagmus and strabismus), a visual acuity test, fundoscopy, and tests for other ocular involvement such as cataract, glaucoma, or retinal diseases. Controls without any history of ocular disease were selected from the general population. The study protocols adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board.

*Collection of blood samples and genomic DNA preparation:* Approximately 10 ml peripheral blood samples were collected with informed consent from the OCA patients with the help of the collaborating doctors. Similarly, blood samples were also collected from normal individuals with no personal or family history of OCA. EDTA was used as an anticoagulant. The Internal Review Committee on Research using Human Subjects cleared the project. Genomic DNA was prepared from fresh whole blood by the salting-out method using sodium-perchlorate, and the DNA was dissolved in 10 mM Tris-HCl, 0.1 mM EDTA (TE; pH 8.0) [5].

*Polymerase chain reaction:* Polymerase chain reaction (PCR) was carried out in a total reaction volume of 25 µl containing 50-100 ng genomic DNA, standard buffer, $MgCl_2$ (as appropriate), 0.1 mM of each dNTP, 0.2 µM of each primer, and 0.1 units of *Taq* DNA polymerase (Invitrogen, Carlsbad, CA) in a MJ Research PTL-225 thermocycler (GeneAmp-9700, Applied Biosystems, Foster City, CA). The exons were amplified using the primer sequences and PCR conditions described in Table 1. The PCR products were analyzed by elec-

**Table 1. Primers and PCR conditions for amplification of *SLCA5A2* exons**

| Primer Name | Primer Sequence (5' to 3') | Amplified Region/ Region to be Sequenced | Amplicon (bp) | PCR Condition |
|---|---|---|---|---|
| | | ***Primers for PCR*** | | |
| MATP 1aF<br>MATP 1cR | AGGCTCCACGTCAAATCCAG<br>GGTCAAACACATGAACATCCTC | Exon 1 and flanking region | 559 | 94°C 30s, 52°C 30s, 72°C 1 min for 35 cycles using 1.0 mM $MgCl_2$ |
| MATP 2F<br>MATP 2R | AACGCGGATGATTCTAAAACAGGA<br>CTCATTGTCTGGGGAGCTGA | Exon 2 and flanking region | 280 | 94°C 30s, 54°C 30s, 72°C 45s for 30 cycles using 1.0 mM $MgCl_2$ |
| MATP 3aF<br>MATP 3bR | GGGAGTGTCTATGCATGAGG<br>CAACAAAGAGCAAGAATATTTTCCCTTG | Exon 3 and flanking region | 452 | 94°C 30s, 52°C 30s, 72°C 1 min for 35 cycles using 1 mM $MgCl_2$ |
| MATP 4F<br>MATP 4R | AGCTGGCTGAGTTTCTGCAG<br>CCTCAACAGGTGTTAATGGAGG | Exon 4 and flanking region | 265 | 94°C 30s, 54°C 30s, 72°C 30s for 30 cycles using 2.0 mM $MgCl_2$ |
| MATP 5F<br>MATP 5R | AGAGGTGGAGAAGCAGAGTG<br>GAAGACATCCTTAGGAGAGAG | Exon 5 and flanking region | 236 | 94°C 30s, 54°C 30s, 72°C 30s for 30 cycles using 2.0 mM $MgCl_2$ |
| MATP 6F<br>MATP 6R | ATGAGGCACTGCCAGCTGTA<br>CCCAAGGCAGAGGTTCAATG | Exon 6 and flanking region | 286 | 94°C 30s, 54°C 30s, 72°C 30s for 30 cycles using 2.0 mM $MgCl_2$ |
| MATP 7F<br>MATP 7R | GCCCTAAATGACAGTTCCTTG<br>TGTGCTTCACTGTCTCTGAG | Exon 7 and flanking region | 326 | 94°C 30s, 52°C 30s, 72°C 30s for 30 cycles using 1.0 mM $MgCl_2$ |
| | | ***Nested Primers for Sequencing*** | | |
| MATP 1aR<br>MATP 1bF<br>MATP 1bR<br>MATP 1cF | GGTCACATACGCTGCCTCCA<br>CAGACTCATCATGCACAGCA<br>ATGCCCACGAGCATCATGAC<br>CAGCATTGTGTGGTTCCTCA | Exon 1 and flanking region | *NA | *NA |
| MATP 3bF<br>MATP 3aR | GCCCCACTTACAGAGGTTGC<br>GATAGAACCATACTCGTACATTCC | Exon 3 and flanking region | *NA | *NA |

The primers were selected such that the amplicons contain coding sequence (exons) and the flanking splice junctions [10]. Any variation in the sequence identified by DNA sequencing was evaluated for its potential deleterious effect on the gene product (mRNA and/or protein). In case of exons 1 and 3, nested primers were used for sequencing the amplicons for better fidelity of the data generated. The asterisk and "NA" denotes not applicable.

trophoresis in 6% polyacrylamide gels and visualized under UV light. Only those PCR products that had a single amplification product with no evidence of nonspecific amplification were used for DNA sequencing as described below.

*DNA sequencing:* The PCR products free of contaminating bands due to nonspecific amplification were column-purified using a Qiagen PCR-purification kit (Qiagen, Hilden, Germany), and bidirectional sequencing was performed in an ABI Prism 3130 DNA sequencer (Applied Biosystems, Foster City, CA) using dye-termination chemistry. Nucleotide changes were promptly detected by identifying 'double peaks' in the chromatogram due to heterozygosity of the DNA sample analyzed and confirmed by sequencing from the opposite direction. Additionally, the sequences were analyzed using pairwise BLAST to examine if there were any changes from the normal sequence available in the database.

*Restriction enzyme digestion:* Mutations identified by DNA sequencing were screened in additional OCA patients and control samples by amplifying the suspected region of genomic DNA by PCR and the products were digested with appropriate restriction enzymes that distinguished between the mutant and normal alleles under the conditions described by the manufacturer (New England BioLabs, Beverly, MA) in a total reaction volume of 20 µl. The DNA fragments in the digest were separated by electrophoresis in 6% polyacrylamide gels, stained with ethidium bromide, and visualized using a UV transilluminator. Alleles were scored based on the DNA band patterns in the gel.

*Genotyping:* For this purpose, three highly heterozygous dinucleotide repeat markers ($TC_{24}TA_{13}CA_{17}$, $TG_{23}$, and $AC_8AG_{23}$) were identified within (GDB:11525101) and flanking (GDB:11525100 and GDB:11525102) the *SLC45A2* locus and submitted to GDB. Genotyping at these loci was done by genescan analysis in an ABI Prism 3100 DNA Sequencing System using 500 ROX Size Standard (Applied Biosystems).

## RESULTS & DISCUSSION

Our initial investigation revealed that in a pool of 50 OCA pedigrees collected from eastern and southern India, 31 harbored tyrosinase gene (*TYR*) defects, thus those patients represented OCA1 cases [6,7]. In 5 of the remaining 19 pedigrees, we identified five nonsynonymous changes in *SLC45A2* that were absent in 100 alleles of ethnically matched controls (Table 2). All the patients representing these five pedigrees were identified with yellowish-brown hair, white skin, and their irises were partially pigmented (hazel brown in most cases) with a tan colored melanin. All the patients were diagnosed with low visual acuity as well as with nystagmus, strabismus, and iris transillumination. However, none of them were detected with any other retinal diseases. Four of these changes are novel and were detected in four different pedigrees representing three different ethnic groups - c.126G>A (Met42Ile, M42I) and c.190G>A (Gly64Ser, G64S) in exon 1; c.904A>T (Thr302Ser, T302S) in exon 4; and c.1042C>T (Arg348Cys, R348C) in exon 5. Two of these encoded residues for two variants (M42I and G64S) are conserved in five other species (dog,

**Table 2. Nucleotide variants of *SLC45A2* in Indian oculocutaneous albinism patients representing different ethnic groups of India**

| Sl No. | Nucleotide change | Genomic location | Amino acid change | Location in the protein | Ethnic Group# | Geographical location# | Mutation / SNP | Novel / Reported |
|---|---|---|---|---|---|---|---|---|
| 1 | c.126G>A | Exon 1 | Met42Ile | TM1 domain | Viswa Brahmins | South India | Mutation | *Novel* |
| 2 | c.190G>A | Exon 1 | †Gly64Ser | TM1 domain | Turupukapu | South India | Mutation | *Novel* |
| 3 | c.469G>A | Exon 2 | ‡Asp157Asn | Near TM4 domain | Viswa Brahmins, Vysya | South India | Mutation | Inagaki et al, 2004 |
| 4 | c.904A>T | Exon 4 | †Thr302Ser | Between TM6 and TM7 domains | Turupukapu | South India | Mutation | *Novel* |
| 5 | c.1042C>T | Exon 5 | †Arg348Cys | 1st residue after TM7 domain | Dhibor | East India | Mutation | *Novel* |
| 6 | c.814G>A | Exon 3 | Glu272Lys | Between TM6 and TM7 domains | - | - | SNP | rs26722 |
| 7 | c.987G>A | Exon 4 | Thr329Thr | TM7 domain | - | - | SNP | rs2287949 |
| 8 | c.1033-44C>A | Intron 4 | *NA | *NA | - | - | SNP | rs35398 |
| 9 | c.1122G>C | Exon 5 | Leu374Phe | TM8 domain | - | - | SNP | rs16891982 |
| 10 | c.1593+24A>C | 3' UTR | *NA | *NA | - | - | SNP | *Novel* |

In five of the nineteen pedigrees lacking the TYR mutation, we identified five nonsynonymous changes in *SLC45A2* that were absent in 100 alleles of ethnically matched controls and could represent potential mutations in the gene. The allelic and genotype frequencies of the coding SNPs (furnished in this table) in Indian and the HAPMAP populations have been illustrated in Figure 4. The "cross" denotes that the mutation was identified in heterozygous conditions in one pedigree only. The "double cross" indicates that the mutation is prevalent in German and Korean populations, identified in homozygous conditions within one pedigree and in compound heterozygous state with Met42Ile in another pedigree. The asterisk and "NA" means that it was not applicable and the hashmark means that the ethnic group and geographical location are mentioned for mutations only.

pig, mouse, medaka fish, and frog) for which the sequence of the homologous protein is known (Figure 1). In two other cases (T302S and R348C), the residue in the human protein is replaced by conservative amino acids in other species. Since the mouse protein also contains an 'S' at a homologous position for the T302S variant, in humans, it is possible to deduce that this change found in one OCA patient might be a rare innocuous variant, but its absence in controls is intriguing. The fifth nonsynonymous variant, a reported missense mutation c.469G>A (Asp157Asn, D157N), was identified in a homozygous condition as well as in a heterozygous condition with M42I in two south Indian pedigrees.

Interestingly, among the five affected pedigrees, three were identified with single *SLC45A2* mutations (i.e. G64S, T302S, and R348C). In one of these pedigrees, there were two affected sibs both having the R348C mutation in exon 5 creating a loss of the *Bst*U1 restriction site (Figure 2). The *Bst*U1-RFLP assay proved that the mutant allele was inherited from the father and was absent in the mother. To test whether the affected sibs also shared the second *SLC45A2* allele, which is a prerequisite for an autosomal recessive mode

**Mutation in Exon 1: M42I**

| | | | | | |
|---|---|---|---|---|---|
| *Homo sapiens* | 32 - | TSRLIMHSMA | **M** | FGREFCYAVE | -52 |
| *Canis familiaris* | 32 - | TSNLIMHSMA | **M** | LGREFCYAVE | -52 |
| *Sus scrofa* | 32 - | TGSLVMHSMA | **M** | FGREFCYAVE | -52 |
| *Mus musculus* | 32 - | TGRLVMHSMA | **M** | FGREFCYAVE | -52 |
| *Oryzias latipes* | 59 - | RGRLILHSMV | **M** | FGREFCYAVE | -79 |
| *Xenopus laevis* | 52 - | TGRLIMHSMA | **M** | FGREFCYAVE | -72 |

**Mutation in Exon 1: G64S**

| | | | | | |
|---|---|---|---|---|---|
| *Homo sapiens* | 54 - | AYVTPVLLSV | **G** | LPSSLYSIVW | -74 |
| *Canis familiaris* | 54 - | AYVTPVLLSV | **G** | LPKSLYSTVW | -74 |
| *Sus scrofa* | 54 - | AYVTPVLLSV | **G** | LPKSLYSVVW | -74 |
| *Mus musculus* | 54 - | AYVTPVLLSV | **G** | LPKSLYSMVW | -74 |
| *Oryzias latipes* | 81 - | AFVTPVLLSV | **G** | LPRSLYSLVW | -91 |
| *Xenopus laevis* | 74 - | AFVTPVLLSV | **G** | LPRSLYSLVW | -94 |

**Mutation in Exon 4: T302S**

| | | | | | |
|---|---|---|---|---|---|
| *Homo sapiens* | 292 - | NHAEQTRRAM | **T** | LKSLLRALVN | -312 |
| *Canis familiaris* | 292 - | NPSKQISKTM | **T** | MTSLLRVLMN | -312 |
| *Sus scrofa* | 293 - | PPAEQTQRTM | **T** | IKSLLRALMS | -313 |
| *Mus musculus* | 292 - | KPSGQSQRTM | **S** | MKSLLRALVN | -312 |
| *Oryzias latipes* | 341 - | QPIKEDQKKM | **T** | FRSLMKAIFN | -361 |
| *Xenopus laevis* | 311 - | DREEQVQKRM | **T** | VKSLISALFT | -331 |

**Mutation in Exon 5: R348C**

| | | | | | |
|---|---|---|---|---|---|
| *Homo sapiens* | 338 - | FTDFMGQIVY | **R** | GDPYSAHNST | -358 |
| *Canis familiaris* | 338 - | FTDFMGQIVY | **H** | GDPYSAHNST | -358 |
| *Sus scrofa* | 339 - | FTDFMGQIVY | **H** | GDPYSAHNST | -359 |
| *Mus musculus* | 338 - | FTDFMGQIVY | **H** | GDPYGAHNST | -358 |
| *Oryzias latipes* | 397 - | ETDEMGQIVY | **R** | GNPYAEHNST | -407 |
| *Xenopus laevis* | 357 - | FTDFMGQIVY | **H** | GNPYAEHNST | -377 |

Figure 1. Conservation status of the wild-type amino acids of SLC45A2 across different species. Regions of human SLC45A2 (GenBank NP_057264) harboring the newly characterized mutations were compared with the homologous proteins available for other species by multiple sequence alignment. The bold letters indicate the wild-type amino acids at the location of mutations, and the coordinates of the segments of the peptides selected are also shown.

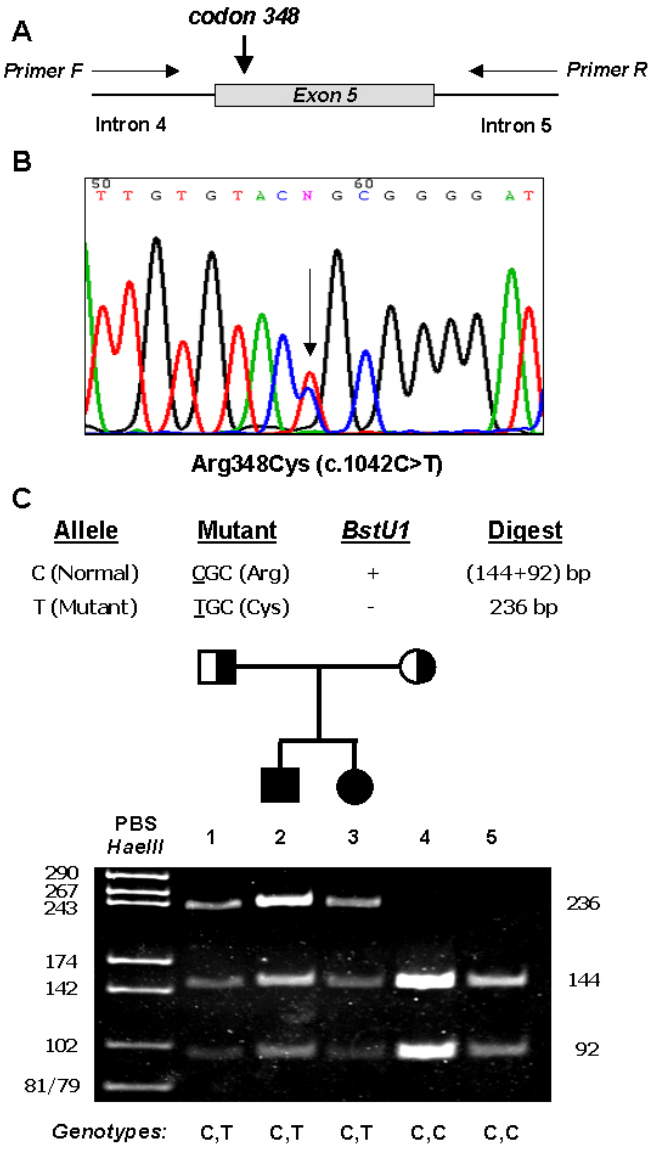


Figure 2. Detection of Arg348Cys mutation in the affected pedigree by direct sequencing and restriction fragment length polymorphism analysis. **A**: This panel shows the location of the 348th codon within *SLC45A2*. **B**: A representative chromatogram showing the location of the Arg348Cys mutation in heterozygous condition as indicated by the double peak (arrowhead). **C**: The allelic difference resulting from the Arg348Cys mutation was determined by *Bst*U1 digestion of the PCR product obtained from exon 5 of the gene and the DNA digests of the five representative samples (four members of an affected family and a healthy control) were analyzed in a 6% polyacrylamide gel. The genotype of each individual is shown below the lane. The sizes of *Bst*U1 uncut (236 bp) and digested (144 bp and 92 bp) DNA fragments are shown on the right side of the gel while the sizes (in bp) of the molecular weight marker (PBS X *Hae*III) are shown on the left. Lanes 2 and 3 represent the DNA digests of two sibs affected with the mutation whereas lanes 1, 4, and 5 represent the father, mother. and healthy control, respectively.

of transmission, microsatellite markers encompassing *SLC45A2* were genotyped, and haplotypes were constructed to follow the segregation pattern. As shown in Figure 3, the second allele is not shared between the two sibs, clearly demonstrating that the albino phenotype in the family is not caused exclusively by defects in the *SLC45A2* gene. Therefore, the data suggest two possibilities: (1) As demonstrated in other genetic diseases, the disease in this family is caused by a digenic mode of inheritance and may explain the basis for some of the *SLC45A2* linked OCA cases (31%) in which the second mutation has not been identified in the gene; or (2) Despite the presence of the mutant *SLC45A2* allele in both the sibs, the disorder is caused by a defect in another locus not yet characterized in these patients. However, lack of additional samples in the other two pedigrees harboring the other two mutations (G64S, T302S) prevented us from conducting similar experiments to further explore the molecular basis of the pathogenesis in families with a single mutant allele in *SLC45A2*.

Mutation screening by DNA sequencing of *SLC45A2* revealed five single nucleotide innocuous changes, which include one synonymous (c.987G>A, Thr329Thr; rs2287949) and two nonsynonymous (c.814G>A, Glu272Lys; rs26722 and c.1122G>C, Leu374Phe; rs16891982) variations, one change in intron 4 (c.1033-44C>A; rs35398), and the other in 3'-UTR (c.1593+24A>C). Among the coding single nucleotide polymorphisms (SNPs), E272K and L374F have been previously reported to be associated with human ethnicities and 272K and 374L variants have been reported to be associated with

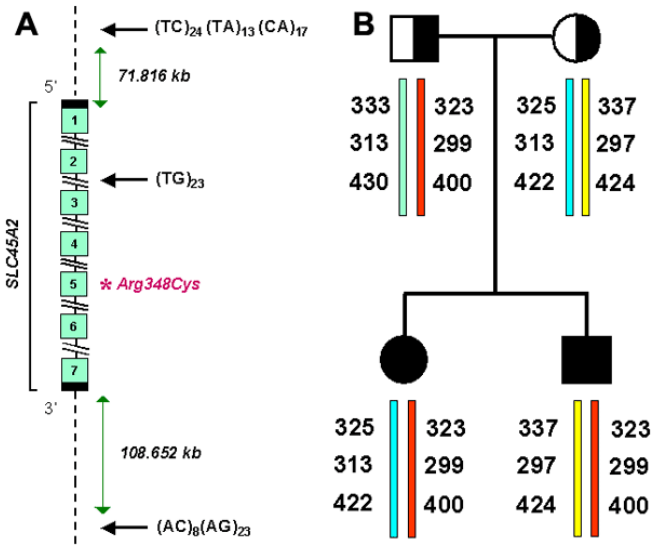


Figure 3. An oculocutaneous albinism-affected pedigree showing a potential digenic mode of inheritance involving *SLC45A2*. **A** illustrates the locations of the three microsatellite-markers identified in the *SLC45A2* locus for cosegregation studies and the position of the Arg348Cys mutation in *SLC45A2*. **B**: Haplotype analysis in a pedigree demonstrates that the two affected sibs share one allele carrying the Arg348Cys mutation in *SLC45A2* that has been transmitted from their father whereas they harbor different alleles from their mother.

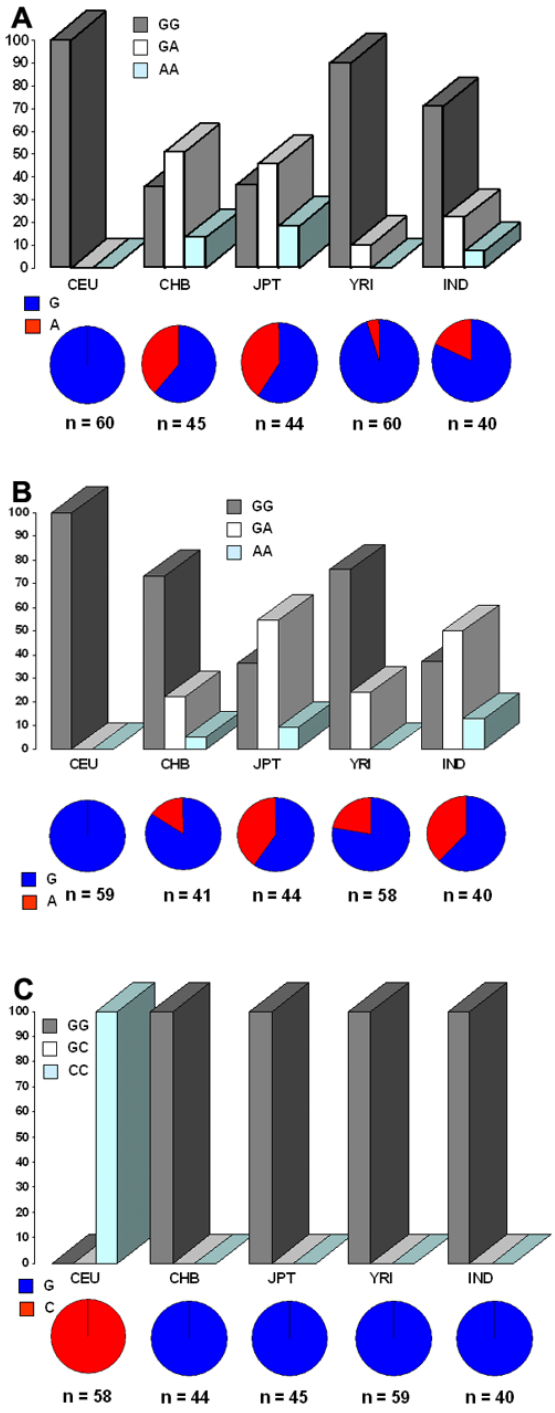


Figure 4. Comparison of three coding single nucleotide polymorphisms (Glu272Lys, Thr329Thr, and Leu374Phe) in SLC45A2 between Indian and other population groups. In each panel, the bar diagram shows the genotype frequencies while the pie chart shows the allele frequencies. For Glu272Lys (**A**), IND differed in genotype frequency from the rest of the populations. For Thr329Thr (**B**), the genotype frequency of IND was found to be similar to the JPT population whereas in the case of Leu374Phe (**C**), IND matches the rest of the world populations except CEU. CEU, Utah residents with ancestry from northern and western Europe collected by CEPH (Centre d'Etude du Polymorphisme Humain); CHB, Han Chinese in Beijing, China; JPT, Japanese in Tokyo, Japan; YRI, Yoruba in Ibadan, Nigeria; and IND, India.

dark hair, skin, and eye color in Caucasians [8,9]. The allelic variants of all the three coding SNPs were determined in 40 normal individuals of eastern and southern India. These showed a remarkable difference in genotype frequencies of E272K with respect to the HAPMAP-studied Asian populations while L374F was found to be monomorphic for the 374L variant (Figure 4), similar to all noncaucasian populations. Considering the population history, a study with a larger sample pool containing different ethnic groups may shed light on the functional role of these *SLC45A2* variants, if any.

This is the first report of *SLC45A2* mutations among Indian OCA patients. We identified 10 variants in the *SLC45A2* gene among which five were pathogenic changes. Our study shows that among ethnic groups of eastern and southern India, defects in *SLC45A2* cause OCA in up to 10% of the cases.

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