



Higher prevalence of OCA1 in an ethnic group of eastern India is due to a founder mutation in the tyrosinase gene

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Purpose: Oculocutaneous albinism (OCA) is a group of autosomal recessive disorders characterized by deficient synthesis of melanin pigment and associated with common developmental abnormalities of the eye. It is one of the major causes of childhood blindness in India. The disease is common among an ethnic group (Tili) of Eastern India, which represents about 12.56% of the Bankura district population (approximately 0.4 million) of West Bengal. The purpose of the study was to investigate the molecular lesions causing OCA within this ethnic group for the unequivocal diagnosis of the carriers and attempt to decipher the cause for the high prevalence of OCA.

Methods: Fourteen OCA-affected Tili families consisting a total of 161 individuals, including 26 patients, were recruited for the study. A lack of tyrosinase (TYR) activity among all the patients was ascertained by the tyrosinase hair bulb assay. Mutation screening in the tyrosinase gene (*TYR*) was done by single strand conformational polymorphism (SSCP) and DNA sequencing. The restriction fragment length polymorphism (RFLP) assay was carried out to determine the frequency of the pathogenic changes among the normal individuals. Haplotype analysis was performed at the *TYR* locus using a set of informative microsatellite and SNP markers.

Results: All the patients were homozygous for a null mutation (c.832C>T, Arg278stop) in *TYR* exon 2, which might cause a complete loss of enzyme activity. The mutation occurred in the same haplotype background. The frequency of the disease in this ethnic group was estimated to be significantly higher than the world average.

Conclusions: OCA1 in the Tili population is due to the occurrence of a founder mutation in the *TYR* as indicated by haplotype analysis. Higher prevalence of the mutation in the population group is due to marriage within the same community. The diagnostic RFLP assay can be utilized for genetic counseling and thereby will help to reduce the disease load on the population.

Oculocutaneous albinism (OCA) is a heterogeneous group of autosomal recessive disorders that often results in the reduction or complete absence of melanin in the skin, hair, and eyes. It is also associated with common developmental abnormalities of the eye. At least 16 different genes have been identified that, when mutated, result in different types of albinism [1]. All forms of OCA are represented with photophobia, strabismus, moderate to severe visual impairment, and nystagmus.

Oculocutaneous albinism type 1 (OCA1; OMIM 203100), results from mutations in the tyrosinase gene (*TYR*, 11q14-q21; OMIM 606933). The clinical phenotype resembling OCA1 is caused by the absence of (OCA1A) or residual catalytic activity of (OCA1B) tyrosinase [2]. *TYR* with 5 exons spanning more than 65 kb of DNA [3] encodes a 58 kDa glycoprotein of 529 amino acids [4].

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Despite a large number of reported *TYR* mutations (Albinism database), very little is known about the molecular basis of OCA in Indian patients although the disease is quite prevalent in some of the geographical locations. In the Bankura district of West Bengal, OCA is common among the Tili ethnic group. We initially reported the presence of a *TYR* mutation (R278X) among the Tili [5]. A subsequent study confirmed the presence of the same mutation and reported two frameshift mutations in four affected families representing an ethnic group, which was termed “either Tili or Tamli” [6]. These family samples were collected from the same region of India. It is important to note that Tili and Tamli are two distinct ethnic groups and hence may not have the same genetic basis for occurrence of OCA. Tamli, also known as Tamuli or Tambuli, mostly live in the Burdwan and Hooghly districts of West Bengal. The available literature regarding their origin suggests that they are an offshoot of one of the trading castes [7]. However, Tili or Teli, is a community whose name derives from the Sanskrit word talika or taila, referring to the oil extracted from sesame and mustard. Risley [7] suggested that they must have been recruited from a different class of Hindu society.

In contrast to the recent report [6] on “Tili or Tamli” our study based on 188 Tili individuals suggested a lack of any mutation other than R278X among Tili, the ethnic group which represents about 12.56% of the Bankura population (0.4 mil-

lion). Therefore, we argue that the genetic basis of the disease in the population group should be investigated thoroughly so that advice regarding genetic screening and subsequent counseling is based on clearly established facts for the relevant ethnic or population group. Here we report results of our investigation based on OCA affected families, carefully selected by interviewing the local Tili population group and using experiments designed to provide unequivocal answers. The mutation occurred in the same haplotype background suggesting a founder effect. Our data suggest that R278X is the major, if not exclusive, mutation within the Tili ethnic group.

METHODS

Fourteen OCA affected Tili families, consisting of a total of 161 individuals including 26 patients, were recruited for the study. Approximately 10 ml of peripheral blood samples were collected in EDTA, from 48 Tili individuals including 19 patients, 29 family members with the normal phenotype, and an OCA-affected boy and his normal brother belonging to the Tamli (also known as Tambuli) ethnic group, with their in-

formed consent. The study protocols adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board. Apart from albinism, the diagnosis involved ophthalmologic examinations including the testing of abnormal ocular movement (nystagmus and strabismus), a visual acuity test, funduscopy, and tests for other ocular involvement such as cataract, glaucoma, or retinal diseases. Controls were selected from the general Tili population without any family history of ocular disease or albinism to determine the frequency of the carriers for the *TYR* mutation. Prior to collection of blood samples, a government-registered local organization for the welfare of the Tili community was contacted for information on the ethnic group. Next, one of us (SS), a clinician working in the Bankura district for last 25 years and having good knowledge about the community, visited individual households of Tili families, interviewed household members to ensure their ethnicity along with other relevant information regarding OCA, and collected the blood samples with the consent of the donors.

TABLE 1. PRIMERS AND PCR CONDITIONS FOR AMPLIFICATION OF *TYR* EXONS 1, 2, AND 3

Primer	Primer sequence (5'-3')	Amplified region	Amplicon (bp)	PCR condition
Primers for SSCP				
Tyr1bF	GTCCTGCAGACCTTGTGAGG	Exon 1	490	94 °C 30 s, 60 °C 30 s, 72 °C 30 s for 30 cycles
Tyr1bR	GATGACATAGTCTGAGCTGATGG			
Tyr1cF	CTTCATGGGATTCAACTGTGG	Exon 1	596	94 °C 30 s, 58 °C 30 s, 72 °C 30 s for 30 cycles
Tyr1cR	GAAGTGATTGTTAAGGTTCCCTCC			
Tyr2bF	CTACTGACTCAGTGGTGGTGAC	Exon 2	346	94 °C 30 s, 60 °C 30 s, 72 °C 30 s for 30 cycles
Tyr2aR	CTCCTAGGACTTTGGATAAGAG			
Tyr3bF	GGGATAATCACATAGGTTTTTCAGTC	Exon 3	268	94 °C 30 s, 64 °C 30 s, 72 °C 30 s for 30 cycles
Tyr3aR	CCTCTATTTAAATCCAATGAGCACG			
Primers for sequencing				
Tyr1aF	GTGAGCTATCCTTAGGAGTTGTC	Exon 1 & flanking region	1539	94 °C 30 s, 62 °C 30 s, 72 °C 90 s for 30 cycles
Tyr1cR	GAAGTGATTGTTAAGGTTCCCTCC			
Tyr2aF	GCCATTATCTTACAATTGCC	Exon 2 & flanking region	730	94 °C 30 s, 50 °C 30 s, 72 °C 1 min for 30 cycles
Tyr2bR	TGAATTATAACGTGCTGACC			
Tyr3aF	GGCTCAACCTCTTTTACCTGG	Exon 3 & flanking region	938	94 °C 30 s, 56 °C 30 s, 72 °C 1 min for 30 cycles
Tyr3bR	CCTATAGCAGTTCTGTGTGTCC			

The 490 bp fragment amplicon of Tyr1bF and Tyr1bR was digested with the *Mla*IV (New England Biolabs, Beverly, MA) to generate 3 smaller fragments (127 bp, 167 bp, and 192 bp) and subjected to SSCP. The 596 bp fragment amplicon of Tyr1cF and Tyr1cR was digested with the *Xcm*I (New England Biolabs, Beverly, MA) to generate 3 smaller fragments (168 bp, 197 bp, and 229 bp) and subjected to SSCP. The three amplicons of *TYR* were sequenced using nested primers. For exon 1, Tyr1aR (CTCTAGGGAAATGGCCAGCGG), Tyr1bF, and Tyr1cF were used. For exon 2, Tyr2bF and Tyr2aR were used. For exon 3, Tyr3bF and Tyr3aR were used. The PCR conditions used 2.0 mM MgCl₂ except for Tyr3aF and Tyr3bR which used using 1.5 mM MgCl₂.

A tyrosinase hair bulb assay was performed to identify potential OCA1 cases [8]. The coding sequence and splice site junctions of *TYR* were amplified from the genomic DNA of the patients and controls, and the mutation was identified by direct DNA sequencing of the samples showing band-shift in SSCP. While exons 1-3 were amplified following standard protocol (Table 1), exons 4 and 5 were amplified using gene specific primers to avoid co-amplification of the pseudogene *TYRL* (OMIM 191270) based on our recently described method [9]. Three CA-repeat markers (GDB:11511689, GDB:11511691, and GDB:11511690), identified within the *TYR* locus and its flanking regions, were used to determine the genotypes of the patients and their parents. Genescan analysis was done in the ABI Prism 3100 DNA Sequencing System using 500 ROX Size Standard (Applied Biosystems, Foster City, CA). The mutation identified by DNA sequencing was analyzed in the additional control Tili samples with *BsI* restriction enzyme (New England BioLabs, Beverly, MA). The mutant allele was indicated by the loss of the *BsI* site.

RESULTS & DISCUSSION

In our sample pool, all the patients seemed to be affected with OCA1 due to mutations in the *TYR*, as indicated by the tyrosi-

nase hair bulb assay. On clinical examination, these patients were found to have the pigment deficiency in the peripheral retina along with other associated changes to the visual system including decreased visual acuity (usually diminished to as low as 6/60) secondary to foveal hypoplasia, photophobia, iris transillumination, nystagmus, strabismus, and loss of binocular vision.

SSCP analysis revealed that all the Tili patients had the same characteristic band shift (Figure 1A) of the amplicon containing the sequences for the second exon of *TYR*. The DNA sequence analysis (Figure 1B) of multiple patient samples revealed the same homozygous change (c.832C>T) that would create a premature stop codon (Arg278stop) resulting in a truncated and completely inactive enzyme lacking one potential copper binding region (OCA1A). The mutation occurred in a CpG context and reported previously in different population groups, for example, Japanese [10], Guayanan [11], Moroccan Jewish [12], and Indo-Pakistani [13] populations. To examine whether the prevalence of this mutation in the Tili population was due to a founder effect, we did haplotype analysis using the newly identified microsatellite markers (GDB:11511689, GDB:11511691, and GDB:11511690) within the *TYR* locus and its flanking regions. All 19 patients were found to have

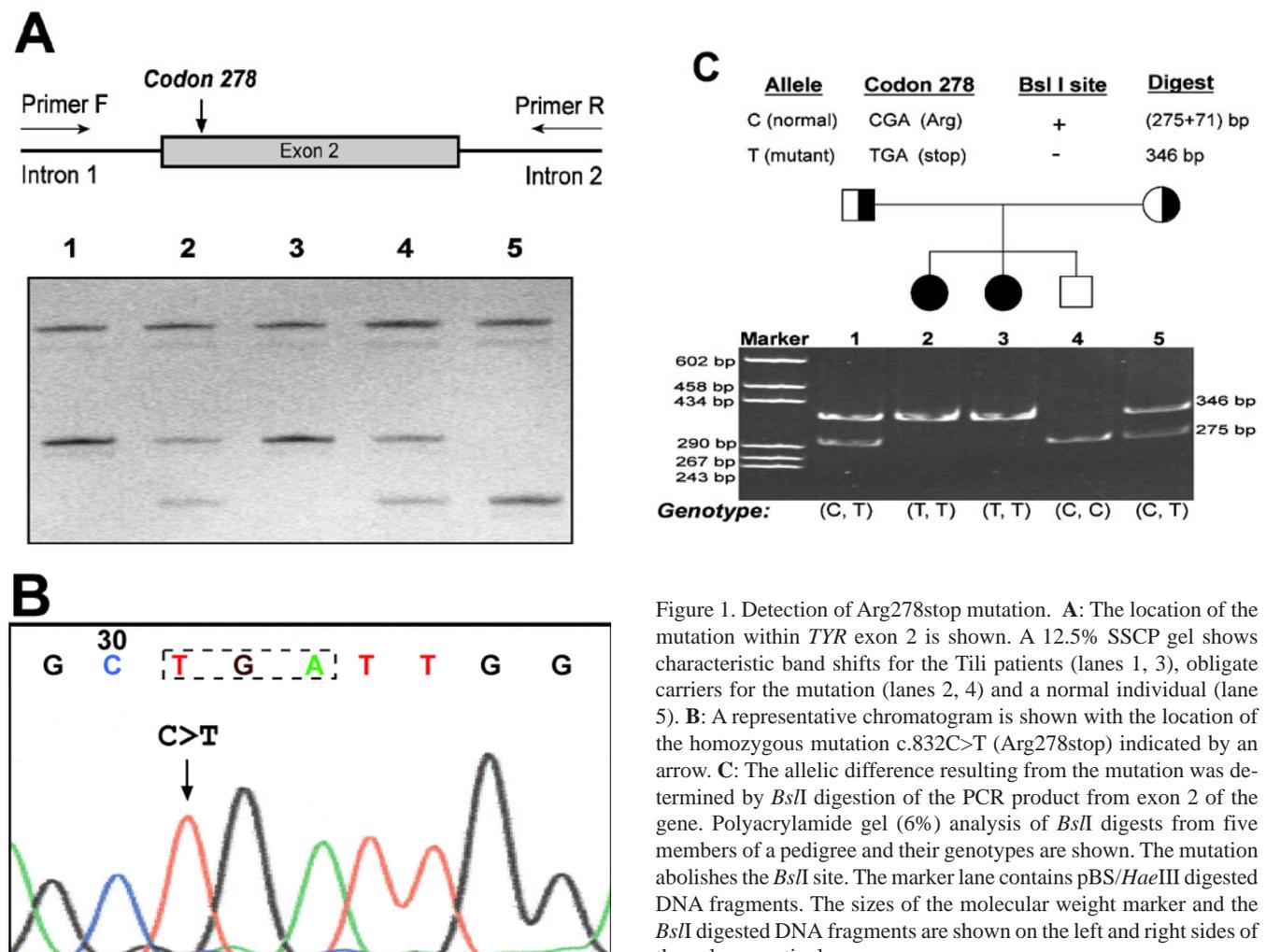


Figure 1. Detection of Arg278stop mutation. **A:** The location of the mutation within *TYR* exon 2 is shown. A 12.5% SSCP gel shows characteristic band shifts for the Tili patients (lanes 1, 3), obligate carriers for the mutation (lanes 2, 4) and a normal individual (lane 5). **B:** A representative chromatogram is shown with the location of the homozygous mutation c.832C>T (Arg278stop) indicated by an arrow. **C:** The allelic difference resulting from the mutation was determined by *BsI* digestion of the PCR product from exon 2 of the gene. Polyacrylamide gel (6%) analysis of *BsI* digests from five members of a pedigree and their genotypes are shown. The mutation abolishes the *BsI* site. The marker lane contains pBS/*HaeIII* digested DNA fragments. The sizes of the molecular weight marker and the *BsI* digested DNA fragments are shown on the left and right sides of the gel, respectively.

the same haplotype (179-101-157). The same result was obtained using a set of SNP markers: 1-533G->C, 1-301C->T, 1-199C->A, c.575C->A (S192Y), c1037-201G->A (data not shown).

To determine the carrier frequency of this mutation within this ethnic group, an RFLP-based diagnostic assay was performed (Figure 1C) on the DNA samples from 27 unrelated normal individuals of the same population group, who on specific inquiry informed us that they did not have any OCA affected members in their families. As a larger number of such donors were difficult to obtain, based on the limited samples, the initial carrier frequency of the mutation was found to be 11.11% (3/54 alleles) among the Tili and the disease frequency was estimated to be significantly higher than the world average [14].

In a recent paper [6], in addition to R278X, two frameshift mutations have also been reported in exons 2 and 5, to cause OCA in the "Tili or Tamli" ethnic groups of West Bengal, India. To investigate the occurrence of these two mutations in the Tili and Tamli populations (these are not the same groups as mentioned earlier), we directly sequenced exons 2 and 5 from OCA patients of both these ethnic groups. We amplified *TYR* exon 5 (avoiding co-amplification of the pseudogene *TYRL*) as we recently described [9]. Our investigation clearly showed that while the Tamli patient was a compound heterozygote for Arg278stop and c.1379delTT (data not shown), none of the Tili individuals (patients, their family members, or unrelated controls) contained the deletion mutation in exon 5 of *TYR*. We did not detect a deletion mutation in Exon 2 of any individual of our cohort, consisting of 14 large Tili families and one Tamli family. The R278X mutant chromosome in the Tamli family has the same haplotype as in the Tili mutation suggesting a common origin for both. It is likely that the mutation was introduced into the Tamli lineage by the marriage of a person of this community to a Tili individual harboring the mutation. However, such marriages are relatively uncommon.

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