Gene 511 (2012) 470–474

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short Communication

Molecular basis of albinism in India: Evaluation of seven potential candidate genes and some new findings

M. Mondal [a,1], M. Sengupta [a,1], S. Samanta [b], A. Sil [c], K. Ray [a,*]

[a] Molecular & Human Genetics Division, CSIR—Indian Institute of Chemical Biology, Kolkata-700032, India
[b] Department of Ophthalmology, Calcutta National Medical College and Hospital, Kolkata-700014, India
[c] Netra Niramay Niketan, Vivekananda Mission Ashram, Chaitanyapur, Purba Medinipur-721645, India

ARTICLE INFO

Article history:
Accepted 6 September 2012
Available online 23 September 2012

Keywords:
Albinism
TYR
OCA2
SLC24A5
SILV
TYRP2

ABSTRACT

Albinism represents a group of genetic disorders with a broad spectrum of hypopigmentary phenotypes dependent on the genetic background of the patients. Oculocutaneous albinism (OCA) patients have little or no pigment in their eyes, skin and hair, whereas ocular albinism (OA) primarily presents the ocular symptoms, and the skin and hair color may vary from near normal to very fair. Mutations in genes directly or indirectly regulating melanin production are responsible for different forms of albinism with overlapping clinical features. In this study, 27 albinistic individuals from 24 families were screened for causal variants by a PCR-sequencing based approach. *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, *SLC24A5*, *TYRP2* and *SILV* were selected as candidate genes. We identified 5 *TYR* and 3 *OCA2* mutations, majority in homozygous state, in 8 unrelated patients including a case of autosomal recessive ocular albinism (AROA). A homozygous 4-nucleotide novel insertion in *SLC24A5* was detected in a person showing with extreme cutaneous hypopigmentation. A potential causal variant was identified in the *TYRP2* gene in a single patient. Haplotype analyses in the patients carrying homozygous mutations in the classical OCA genes suggested founder effect. This is the first report of an Indian AROA patient harboring a mutation in *OCA2*. Our results also reveal for the first time that mutations in *SLC24A5* could contribute to extreme hypopigmentation in humans.

1. Introduction

Albinism represents a heterogeneous group of inherited disorders manifested by complete or partial lack of melanin in the skin, hair, and eyes. While oculocutaneous albinism (OCA) involves both ocular and cutaneous features, in ocular albinism (OA) the phenotype is confined primarily to the eyes.

Mutations in the tyrosinase (*TYR*), oculocutaneous albinism type 2 (*OCA2*/P gene), tyrosinase related protein 1 (*TYRP1*) and solute carrier family 45, member 2 (*SLC45A2*) genes cause the 4 classical types of OCA, viz. OCA type 1 (MIM: 203100 and MIM: 606952; Spritz et al., 1989), type 2 (MIM: 203200; Rinchik et al., 1993), type 3 (MIM: 203290; Boissy et al., 1996), and type 4 (MIM: 606574; Rundshagen et al., 2004), respectively. In all four cases the disease is transmitted

Abbreviations: A, adenosine; Ala, alanine; Arg, arginine; AROA, autosomal recessive ocular albinism; Asn, asparagine; C, cytidine; CSIR, Council of Scientific and Industrial Research; C-terminal, carboxy terminal; Cys, cysteine; DCT, DOPAchrome tautomerase; db SNP, The Single Nucleotide Polymorphism database; Del, deletion; DHICA, 5,6 dihydroxyindole carboxylic acid; DNA, deoxyribonucleic acid; DOPA, dihydroxyphenylalanine; *E. coli*, *Escherichia coli*; et al., et alia (and others); Exo–Sap, exonuclease–shrimp alkaline phosphatase; G, guanosine; Glu, glutamic acid; Gly, glycine; ID, identification/identity; Het, heterozygous; Hom, homozygous; i.e., id est, that is; Ins, insertion; Ile, isoleucine; Leu, leucine; Lys, lysine; MAF, minor allele frequency; *MATP*, membrane associated transporter protein gene; Met, methionine; MIM, Mendelian inheritance in man; ml, milliliter; MLP, Major Lab Project; mRNA, messenger ribonucleic acid; μl, microliter; NA, not applicable; ng, nanogram; OA, ocular albinism; OCA, oculocutaneous albinism; OCA2, oculocutaneous albinism type 2 (as well as the protein coded by *OCA2* gene); *OCA2*, oculocutaneous albinism type 2 gene; PCR, polymerase chain reaction; Phe, phenylalanine; PMEL17, premelanosome protein; PolyPhen, polymorphism phenotyping; Pro, proline; Prof, professor rs—refSNP; P gene, human homologue to the mouse pink-eyed dilution locus; Ser, serine; SIFT, sorting intolerant from tolerant; SILV, silver protein; *SILV*, silver gene; SIP, Supra Institutional Project; Slc24a5, mouse homologuous gene for *SLC25A5*; SLC45A2, solute carrier family 45, member 2 protein; *SLC45A2*, solute carrier family 45, member 2 protein gene; SLC24A5, solute carrier family 24, member 5; *SLC24A5*, solute carrier family 24, member 5 gene; Sl. no., serial number; SNPs, single nucleotide polymorphisms; Thr, threonine; T, thymidine; Tyr, tyrosine; TYR, tyrosinase protein; *TYR*, tyrosinase gene; TYRP1, tyrosinase related protein 1; *TYRP1*, tyrosinase related protein 1 gene; TYRP2, tyrosinase related protein 2; *TYRP2*, tyrosinase related protein 2 gene; Tyrp2, mouse homologuous protein for TYRP2; USA, United States of America; UTR, untranslated region; Val, valine; viz., videlicet (it is permitted to see).

* Corresponding author at: Molecular & Human Genetics Division, CSIR—Indian Institute of Chemical Biology, 4 Raja S. C. Mullick Road, Jadavpur, Kolkata-700032, India. Tel.: +91 33 2483 1984; fax: +91 33 2473 5197.

E-mail addresses: maitreyee.bonny@gmail.com (M. Mondal), sengupta.mainak@gmail.com (M. Sengupta), swapansamanta53@gmail.com (S. Samanta), asimsil@sify.com (A. Sil), kunalray@gmail.com, kray@iicb.res.in (K. Ray).

[1] The first two authors contributed equally to the work.

0378-1119/$ – see front matter
http://dx.doi.org/10.1016/j.gene.2012.09.012

as an autosomal recessive trait. Incidentally, the gene causing OCA type 2 is also known as *OCA2* and the other name, P gene, is not commonly used in the current literature related to albinism. Tyrosinase (TYR) acts as the key enzyme in melanin biosynthesis, catalyzing the rate limiting steps that convert L-tyrosine to L-DOPA and L-DOPA to DOPAquinone (Lerner et al., 1950). *TYRP1*, also a member of the tyrosinase-gene family, encodes TYRP1 protein that catalyzes the oxidation of 5,6-dihydroxyindole-2-carboxylic acid (DHICA) into indole-5,6-quinone-2-carboxylic acid in the melanin biosynthesis pathway. The OCA2 protein (homologous to the mouse pink-eyed dilution gene, *p*), present in the large granular fraction of melanocytes (Rinchik et al., 1993), appears to be an integral membrane protein of melanosomes and is thought to be a member of a transporter family that includes an *Escherichia coli* Na^+/H^+ antiporter. Considering its role in disease causation, it is likely that OCA2 might act as a tyrosine transporter (Lee et al., 1995). Human *SLC45A2* (also known as *MATP*, i.e. membrane associated transporter protein) has structural homology to plant sucrose-proton symporters. Presumably being located in the melanosomal membrane, it probably functions as a membrane transporter directing the traffic of melanosomal proteins and other substances to the melanosomes (Fukamachi et al., 2001). However, the precise role of neither OCA2 nor SLC45A2 proteins has yet been elucidated.

Two distinct forms of OA have also been recognized: the X-linked recessive Nettleship–Falls form (OA1; MIM: 300500; Bassi et al., 1995) and autosomal recessive ocular albinism (AROA) caused by mutations in *TYR*, *OCA2* and possibly *TYRP1*, and represent a phenotypically milder variant of OCA (Hutton and Spritz, 2008a). In many of the observed albinism cases, however, mutations remain unidentified, emphasizing the need to screen other potential candidate genes to better understand the molecular basis of the albinistic condition. In this context, *SLC24A5*, *TYRP2* and *SILV* have been regarded as important candidates for albinism.

SLC24A5 (solute carrier family 24, member 5), a putative sodium/potassium/calcium exchanger protein, has previously been implicated in hypopigmentation. In fact, Slc24a5-null mice have been reported to have albinistic features (Vogel et al., 2008). It has also been shown in a genome wide association study of skin pigmentation variation, using 1,620,742 SNPs in a population of 737 individuals of South Asian ancestry that *SLC24A5* SNP rs1426654 (Ala111Thr) is associated with skin pigmentation (Stokowski et al., 2007). The TYRP2 (tyrosinase related protein 2; orthologous to the murine slaty locus) functions as a DOPAchrome tautomerase (DCT), converting DOPAchrome to 5,6 dihydroxyindole carboxylic acid (DHICA) in the eumelanotic pathway. A defect in TYRP2 could result in a lack of eumelanin and could also lead to a decreased level of total melanin. Also, non-synonymous variants identified in mouse Tyrp2 have been reported to be responsible for a significant decrease in DOPAchrome tautomerase activity, as well as the slaty coat color in mice (Budd and Jackson, 1995; Jackson et al., 1992). *SILV* (also known as *PMEL17*) encodes a melanosomal matrix protein, whose expression is closely correlated with cellular melanin content (Bailin et al., 1996). It forms a fibrillar sheet structure in melanosomes on which precursors of melanin synthesis are deposited and concentrated; i.e., it might serve to accelerate melanin production (Leonhardt et al., 2011). SILV protein is also thought to be directly involved in the biogenesis of premelanosomes (Berson et al., 2001) and has been analyzed as a candidate OCA gene in multiple studies (Hutton and Spritz, 2008a, 2008b). However, to date, no pathogenic variant has been reported in any of these genes to explain ocular or cutaneous albinistic features.

OCA is one of the major causes of childhood blindness in India (Gothwal and Herse, 2000), which underscores the importance of identifying mutations causal to OCA in the different ethnic groups of the country followed by genetic counseling. Mutations identified in Indian patients so far are included in Indian Genetic Disease Database (Pradhan et al., 2011) with free accessibility (http://www.igdd.iicb.res.in/IGDD/home.aspx). In this study, we looked for genetic variants in *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, *SLC24A5*, *TYRP2* and *SILV* in Indians presenting with ocular and/or cutaneous albinistic features.

2. Materials and methods

2.1. Collection of blood samples and genomic DNA preparation from patients and controls

Twenty seven albinistic individuals from 24 families were enrolled in this study, mostly from the eastern and southern parts of India. Twelve of these families had been previously screened for defects in *TYR*, *OCA2*, *TYRP1*, *SLC45A2* and *SLC24A5* (Chaki et al., 2006; Sengupta et al., 2007, 2010), with no detectable mutations in any of these genes. In this study, the same families were screened for mutations in *SILV* and *TYRP2* only. One of the patients (*OCA109*) presented with albinistic eye features only, while his skin tone was very fair but certainly not albinistic, thereby representing an ocular albino (OA) patient. Another individual (*OCA97*) showed extreme hypopigmentation of the skin, but not the eyes and hair. However, detailed clinical data was unavailable to examine the ocular abnormalities, if any. The rest of the individuals enrolled in the study presented with both ocular and skin hypopigmentation. Detection of OCA and OA involved ophthalmologic examinations by our clinical collaborators (SS and AS), including testing of nystagmus, visual acuity and fundoscopy. Ethnically matched controls without any history of ocular disease were later selected from the general population, majorly from eastern India, based on the fact that barring one all of the potential novel mutations were identified in the eastern Indian patient cohort. Approximately 10 ml of peripheral blood was drawn with informed consent from the donors. Genomic DNA was prepared by the salting-out method, or by using the QIAGEN® PAXgene Blood DNA kit according to the manufacturer's protocol.

2.2. Polymerase chain reaction, DNA sequencing and in silico analysis of the variants

The exons, splice-sites and UTRs of the relevant genes were amplified by touchdown PCR. However, exon 19 of *OCA2* was not analyzed since it generates a small proportion of an alternatively spliced mRNA containing an in-frame stop codon resulting in a nonfunctional OCA2 protein (Duffy et al., 2007; Hutton and Spritz, 2008a; Lee et al., 1995). PCR was done for a total volume of 20 μl using 20–50 ng of DNA, 20 pmol of primers (the sequence of the primers used are available on request) and 10 μl Taq Premix (GeNet Bio). The amplicons were purified with Exo–Sap (USB®) and bidirectional-sequencing was performed in the ABI Prism 3130 DNA sequencer (ABI™). Haplotyping with SNPs and microsatellite markers was done following our previous reports (Chaki et al., 2006; Sengupta et al., 2010). For genotyping with microsatellite markers in case of *TYR* gene, 3 CA-repeat markers within (GDB: 11511691) and flanking (GDB: 11511689 and GDB: 11511690) the TYR locus were used. The markers were amplified from the affected and immediate family members using fluorescently labeled primers and subjected to Genescan analysis in an ABI Prism3100 DNA Sequencing System using the 500 TAMRA Size Standard (Applied Biosystems, California, USA) (Chaki et al., 2006). For haplotype analysis in *OCA2* locus, six polymorphic SNPs were genotyped in the members of the affected families (Sengupta et al., 2010). Relevant nucleotide changes were analyzed using SIFT (http://sift.jcvi.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and InterProScan (http://www.ebi.ac.uk/Tools/pfa/iprscan/).

3. Results and discussions

Among the 27 albinistic individuals from 24 unrelated families, 13 patients belonging to 12 different OCA-affected families, were screened for variants in *TYR*, *OCA2*, *TYRP1*, *SLC45A2* and *SLC24A5* in our previous studies (Chaki et al., 2006; Sengupta et al., 2007, 2010) but no potential