INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 10, No. 4, p. 232-238, 2017

RESEARCH PAPER OPEN ACCESS

Identification of two Missense Mutations (p.Ile198Thr & p.Ser44Arg) in TYR gene in Pakistani families

Muhammad Luqman[*1], Asma Yousafzai[2], Nisar Ahmed[2], Asad Ullah[3], Riaz-ul-Amin[4], Abdul Hameed Baloch[5], Sara Naudhani[2], Shakeela Daud[2]

[1]*Department of Environmental Science, Faculty of Life Sciences, BUITEMS, Quetta, Pakistan*

[2]*Department of Biotechnology, Faculty of Life Sciences, BUITEMS, Quetta, Pakistan*

[3]*CASVAB, University of Balochistan, Quetta, Pakistan*

[4]*Department of Computer Science, FICT, BUITEMS, Quetta, Pakistan*

[5]*Lasbela University of Agriculture, Water and Marine Resources, Quetta, Pakistan*

Key words: Oculocutaneous albinism, Tyrosinase, Mutational analysis

http://dx.doi.org/10.12692/ijb/10.4.232-238 Article published on April 28, 2017

Abstract

Oculocutaneous albinism (OCA) is an autosomal recessive disorder. The most common type oculocutaneous albinism type 1 (OCA1) is caused by tyrosinase (*TYR*) gene. Biosynthesis of melanin in human are controlled by Tyrosinase enzyme, which is encoded by gene TYR present on 11q14.3 chromosome. TYR gene is expressed in melacocytes typically in the skin. A majority of TYR mutations deactivates tyrosinase to impair the capability of melanocytes to produce melanin life time. People with this form of albinism have white hair, light-colored eyes, and very pale skin. Some of the TYR mutations do not completely deactivate tyrosinase and melanocytes do produce some the melanin, cause OCA1B (MIM# 606952). In the current study a total of five families containing two or more affected individuals with oculocutaneous albinism (OCA) were enrolled. Clinical symptoms were recorded and pedigrees were drawn by visiting these families. Blood samples (5cc) were also collected from all affected individuals, their normal siblings and parents in EDTA containing falcon tubes. Genomic DNA was extracted using inorganic method. Linkage analysis was performed by three STR markers D11S1780, D11S1367 and D11S4175. Two families showed linkage to OCA1. Sequence analysis of exons and flanking intronic regions was performed for TYR gene. Two known missense mutations (p.Ile198Thr & p.Ser44Arg) causing oculocutaneous albinism type 1 were identified, whereas sequence analysis of normal individuals has shown no sequence change in respective codons. The current study is very important which confirms important mutations causing oculocutaneous albinism in Pakistani families.

* **Corresponding Author:** Muhammad Luqman ✉ hyphomycetes@yahoo.com

Introduction

Oculocutaneous albinism (OCA) is an autosomal recessive disorder. The most common type oculocutaneous albinism type 1 (OCA1) is caused by homozygous or heterozygous mutations in the tyrosinase (*TYR*) gene. In humans, tyrosinase is rate-limiting enzyme which catalyzes multiple steps involved in the synthesis of melanin pigment (Kumar *et al.,* 2011). Tyrosinase oxidizes the amino-acid tyrosine to dopaquinone, and via a series of additional chemical reactions, dopaquinone is converted to melanin, a substance which provides pigment in skin and hair (Kumar *et al.,* 2011). Melanin is also found in retina and plays an important role in normal vision (Ando *et al.,* 2007). So far more than 110 mutations have been reported in TYR genes associated with humans suffering from OCA1. These mutations impair normal functioning of tyrosinase enzyme, consequently preventing the production of melanin and causing problems with vision. Commonly, OCA is categorized into four types based on clinical & genetics outcomes. Oculocutaneous albinism type 1 (OCA1) is severest form of albinism and is caused by mutations in the tyrosinase (*TYR*) gene (MIM# 606933) present on chromosome 11q14.3 (Tripathi *et al.,* 1992; Chen *et al.,* 2002; Budisteanu *et al.,* 2010; Seven *et al.,* 2011). The *TYR* gene consists of five exons and spans approximately 65 kb of the genomic region. Most TYR mutations eliminate the activity of tyrosinase, preventing melanocytes from producing any melanin throughout life, Consequently, they are extremely photosensitive, are highly prone to sun damage, and at risk of developing skin cancer (Simeonov *et al.,* 2013). Furthermore, their visual acuity is reduced especially in those who have nystagmus, where eye movement is often involuntary (Simeonov *et al.,* 2013).

A second subtype of OCA is OCA2, which is triggered due to mutations in the *OCA2* gene. Third type is OCA3, which is prompted due to mutations in the tyrosinase-related protein gene *TYRP1* (MIM# 115501) and fourth type is OCA4, which is caused by mutations in transporter (membrane associated) gene *MATP* (MIM# 606202, a.k.a.MATP) (Passmore *et al.,* 1999; Puri *et al.,* 2000; Manga *et al.,* 2001; Suzuki *et al.,* 2003).

OCA1 type of albinism is most prevalent subtype in majority of populations especially Asian and Caucasian populations with about 1 per 40,000 of individuals (King *et al.,* 1995), while OCA2 and OCA3 are mor prevalent in African populations (Rooryck *et al.,* 2006). OCA4 subtype is most prevalent in fareast Asian populations like china and Japan with prevalence of 12.6% and 24-27% of patients respectively (Park *et al.,* 1996; Goto *et al.,* 2004; Inagaki *et al.,* 2004).

Earlier work on Oculocutaneous albinism in pakistan have reported a number of variations in TYR gene including c.344delGA, p.Ser315_a316del, p.Arg278*, p.Gln328Glu, p.Gly419Arg, p.Pro431Thr, p.Glu376*, p.Glu453*, p.Pro431Leu, p.Pro21Leu, p.Tyr41His, p.Cys35Arg, p.Asp486Tyr, p.Leu527Arg, c.1045-15 T > G, p.Pro743Leu, p.Ala787Thr of OCA2 p.Arg299His and p.Pro406Leu (Giebel *et al.,* 1991; Oetting *et al.,* 1992; Tripathi *et al.,* 1993; Spritz, 1993; Forshew *et al.,* 2005; Jaworek *et al.,* 2012; Kausar *et al.,* 2013). In the present study we analyzed 5 Pakistani OCA families and identified two different missense mutations (p.Ile198Thr & p.Ser44Arg) in TYR gene in two families. Although both were already reported, one of the missense mutation (p.Ile198Thr) is being reported only second time from Pakistan.

Materials and methods

Enrollment of subjects and clinical evaluations

A total of five families containing two or more OCA patients were enrolled. Pedigrees were also drawn for each family to trace the mode of inheritance. Family history was recorded of each patient. Other relatives affected with OCA were also included in the study. This study was approved by the institutional review board (IRB # 00007818) and conducted as per Helsinki Declaration guidelines. Informed consent of each subject was obtained. Complete ophthalmic examination including best-corrected visual acuity testing, slit-lamp examinations, dilated fundus examination and optical coherence tomography was performed. For the sake of comparison, sequence variants in genomic DNA of 20 normal nonrelated individuals with normal ocular findings and pigmentation were also included in the study.

Five cubic meter blood samples were collected from all the affected individuals, their normal siblings, their parents & normal controls in EDTA (anti-coagulant) containing falcon tubes.

DNA extraction and linkage analysis

Genomic DNA was extracted using non-organic method (Grimberg *et al.,* 1989) which involved RBCs lysis, protein digestion and precipitation followed by DNA isolation and purification. The DNA samples were dissolved in TE buffer (pH 8.0) and stored at −20 °C for further use. DNA was quantified by UV spectrophotometry and DNA dilutions were made for amplification and genotyping. Initially each of the family was screened for linkage to already reported region of OCA1 using three STR markers (D11S1780, D11S1367 and D11S4175). PCR amplifications were confirmed by running 2 µl of PCR product mixed with 1µl of 6X gel loading dye from each tube on 1.2 % agarose gel at a constant voltage 80 V for 30 min in 1X TBE buffer. The amplified product were visualized as a single compact fluorescent band of expected size under UV light and documented by gel documentation system (Syngene, Gene Genius Bio Imaging).

Mutational analysis

Five coding exons and adjacent intronic sequences of gene TRY (NM_000372.4) were amplified and sequenced.

PCR products were amplified using 100 ng of genomic DNA in a 25 µl reaction mixture containing 10 pmol of forward and reverse primers, 0.2 mM dNTPs, 10 mM Tris-HCl, 50 mM KCl, 1.5 Mm MgCl2, and 0.5 units of Taq polymerase (Invitrogen Corp., Carlsbad, CA). After initial denaturation at 95 °C for 4 min, 30 cycles were performed, which consisted of 95 °C for 1 min, 55 °C for 45 sec, and 72 °C for 2 min, with a final extension step of 72 °C for 10 min for all exons. PCR products were digested with exonuclease I and shrimp alkaline phosphatase (Fermentas Life Sciences, Glen Burnie, MD) and sequenced bi-directionally using BigDye Terminator v.3.1 kit (Applied Biosystems, Darmstadt, Germany). The sequencing data were analyzed using BioEdit v7.0.9 software (www.mbio.ncsu.edu/bioedit/page2.html) and NCBI 2 sequence BLAST (Tatusova *et al.,* 1999). Multiple sequence alignment was also performed to locate mutation in all sequence at once through CLUSTALW (www.ebi.ac.uk). Prediction of possible impacts of this mutation was carried out by using Polyphen-2 online program. (www.genetics.bwh.harvard.edu/pph2/).

Results and discussion

During the current study two families out of five has shown recessive mode of inheritance. Patients were aged between 8 to 45 years (Table 1). Clinical physiognomies of each case in both families are presented in Table 1.

Table 1. Clinical symptoms of affected patients of Pakistani OCA families.

Family No.	Patients age(s)	Hair color	Skin color	Nystagmus	Lens	Base Substitution	Mutation	References
1	8y, 17y, 23y	white	pink	yes	clear	c.593T>C	p.Ile198Thr	Shah *et al* 2014
4	30y, 31y, 35y, 45y	white	white	no	clear	c.132T>A	p.Ser44Arg	Opitz *et al* 2004

Screening for linkage was carried out for all families using three STR markers. Gene Mapper ID v3.2 software was used to analyze results. Two families were found linked with OCA1 and affected individuals were homozygous for the affected alleles. Both of these families were further analyzed and sequencing was carried out for possible mutations in TYR gene (NM_000372.4).

The patients in family one were all homozygous while the normal individuals were heterozygous (Fig 1). Sequence analysis has revealed a genetic change c.593 T>C (p.Ile198Thr), resulting in amino acid substitution of Isoleucine by Threonine, during inheritance from normal heterozygous mother to homozygous effected patient. This mutation is reported for the second time in Pakistan, first being in 2014 (shah *et al.,* 2014).

The multiple alignments indicated that isoleucine is extremely preserved amino acid at position 198 in normal individuals.

The patients in family four were all homozygous while the normal individuals were heterozygous (Fig 2). Sequence analysis has revealed already reported mutation c.132 T>A (p.Ser44Arg), resulting in amino acid substitution of Serine by Arginine, during inheritance from normal heterozygous mother to homozygous effected patient. No sequence change was found in 20 normal individuals at respective codons in tyrosinase gene.

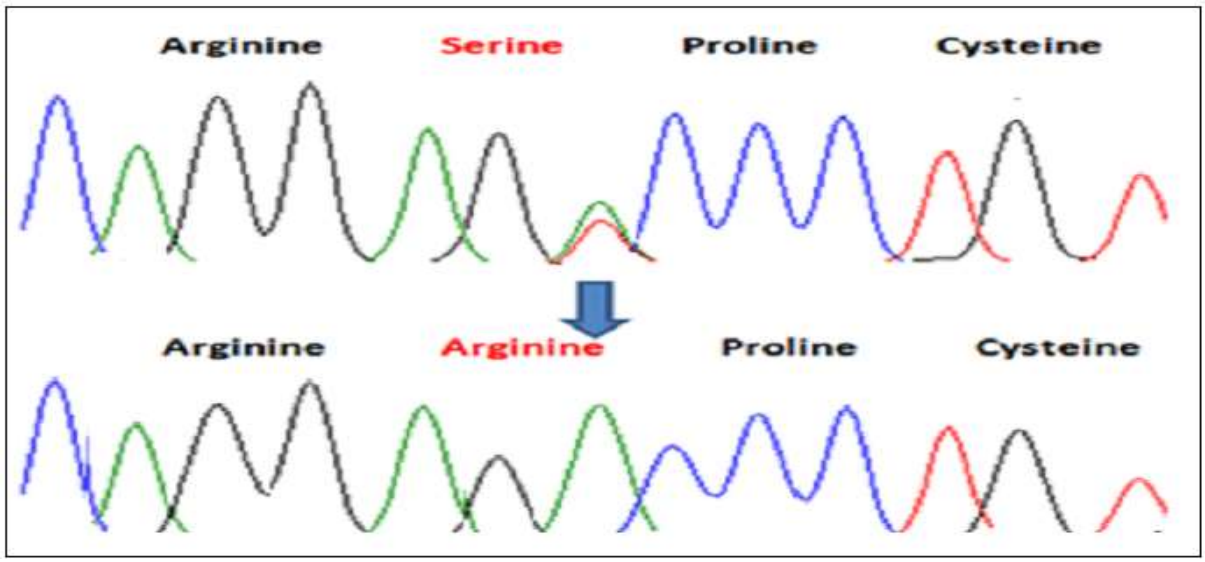

Fig. 1. Chromatogram of mutation c.593 T>C (p.Ile198Thr) in the TYR gene in a Pakistani family one.

Tyrosinase is a glycoprotein consisting of 529 amino acids encoded by TYR gene. It is ivolved in melanin production and many polyphenolic compounds. Tyrosinase oxidizes the amino-acid tyrosine to dopaquinone, and via a series of additional chemical reactions, dopaquinone is converted to melanin, a substance which provides pigment in skin and hair (Tripathi *et al.,* 1992; Chen *et al.,* 2002). During melanogenesis, tyrosinase may be released from the endoplasmic reticulum in the presence of a protonophore or proton pump inhibitors that increase the pH of intracellular organelles.

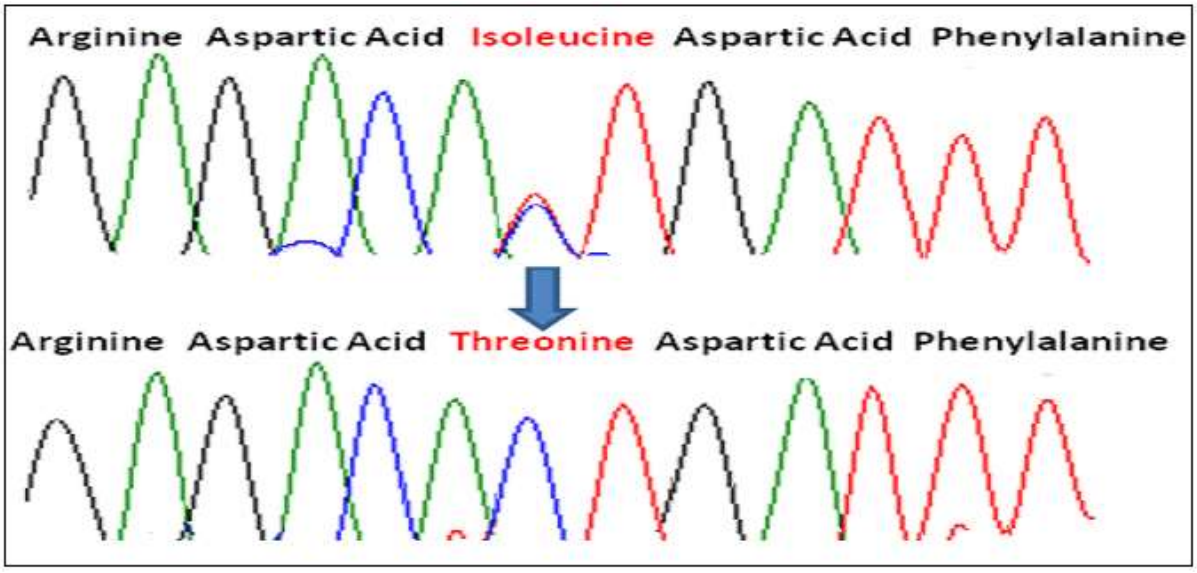

Fig. 2. Chromatogram of mutation c.230G>A (p.Arg77Gln) of family four.

Tyrosinase is then normally transported to the Golgi, and then to the melanosomes via the endosomal sorting system. Any alterations in TYR gene result in retention of tyrosinase enzyme in endoplasmic reticulum resulting in early degradation and impairment of tyrosinase activity (Manga *et al.,* 2001).

In current study two mutations has been detected i.e. p.Ile198Thr & p.Ser44Arg. First mutation (p.Ile198Thr) is detected in a Pakistani family whose effected individuals were having white hair, pale skin and light coloured eyes. Ages of effected individuals were between 9-21 years.

Prediction of possible impacts of this mutation suggested that this mutation is impairing protein structure and function. Tyrosinase is defined by five functional domains of the enzyme. Two of them are the copper binding sites. This mutation (p.Ile198Thr) is present in copper binding site A. So this missense mutations might have inactivated the tyrosinase due to the disruption in the catalytic site of the enzyme. By multiple sequence alignment it was evident that 198 Ile residue is highly conserved in vertebrates suggesting its importance in structure and function of tyrosinase enzyme. Further studies are needed to sort out role of this mutation in causing OCA.

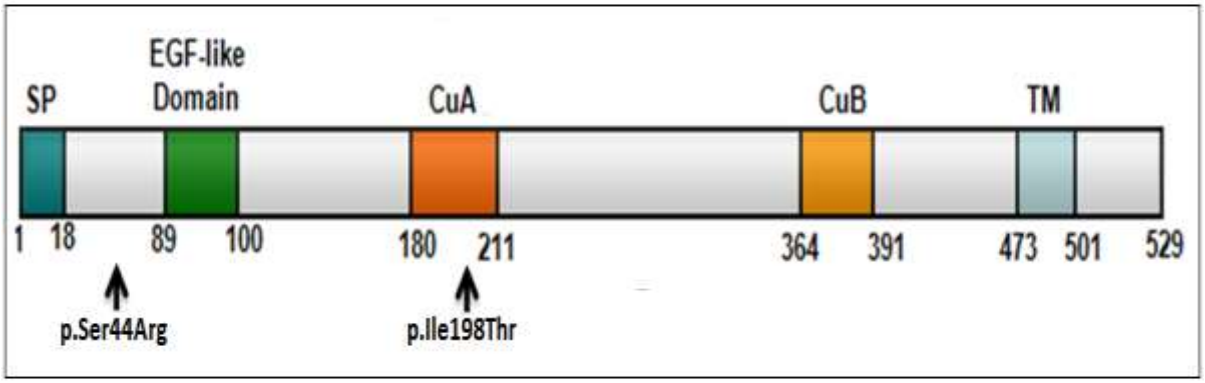

Fig. 3. Schematic representation of missense mutations in TYR. Black arrows showing the location of mutations.

During current study another missense mutations (p.Ser44Arg) is identified that was previously Asian populations (Kikuchi *et al.,* 1990; Nakamura *et al.,* 2002; Opitz *et al.,* 2004; Stokowski *et al.,* 2007). Previous research work on OCA in Pakistani families shows different pathogenic variants of TYR gene including c.344delGA, p.Arg278*, p.Ser315_a316del, p.Gln328Glu, p.Glu376*, p.Gly419Arg, p.Pro431Thr, p.Pro431Leu and p.Glu453*, p.Pro21Leu, p.Tyr411His, p.Cys35Arg, p.Arg299His, p.Pro406Leu, p.Asp486Tyr, p.Leu527Arg, c.1045-15 T > G, p.Pro743Leu, P.Ala787Thr of OCA2 (Giebel *et al.,* 1991; Oetting *et al.,* 1992; Tripathi *et al.,* 1993; Spritz, 1993; Forshew *et al.,* 2005; Jaworek *et al.,* 2012; Kausar *et al.,* 2013).

This study reconfirms some of the important mutations in the mutational spectrum of the TYR gene that would be helpful in assessment of prevalence of types genetic disorders causing OCA in Pakistani population.

Conclusion

Two known missense mutations (p.Ile198Thr & p.Ser44Arg) causing oculocutaneous albinism type 1 were identified, whereas sequence analysis of normal individuals has shown no sequence change in respective codons. The current study is very important which reconfirms some of the important mutations in the mutational spectrum of the TYR gene that would be helpful in assessment of prevalence of types genetic disorders causing OCA in Pakistani population.

References

Ando H, Kondoh H, Ichihashi M, Hearing VJ. 2007. Approaches to identify inhibitors of melanin biosynthesis via the quality control of tyrosinase. The Journal of Investigative Dermatology **127(4),** 751–61. http://dx.doi.org/10.1038/sj.jid.5700683

Budisteanu M, Arghir A, Chirieac SM, Cardos G, Lungeanu A. 2010. Oculocutaneous albinism associated with multiple malformations and psychomotor retardation. Pediatric Dermatology **27,** 212–214.

Chen K, Manga P, Orlow SJ. 2002. Pink-eyed dilution protein controlsthe processing of tyrosinase. Molecular Biology of the Cell **13,** 1953–64.

Forshew T, Khaliq S, Tee L, Smith U, Johnson CA, Mehdi SQ, Maher ER. 2005. Identification of novel TYR and TYRP1 mutations in oculocutaneous albinism. Clinical Genetics **68,** 182–184.

Giebel LB, Tripathi RK, King RA, Spritz RA. 1991. A tyrosinase gene missense mutation in temperature-sensitive type I oculocutaneous albinism. A human homologue to the Siamese cat and the Himalayan mouse. Journal of Clinical Investigation **87,** 1119–1122.

Goto M, Sato-Matsumura KC, Sawamura D, Yokota K, Nakamura H, Shimizu H. 2004. Tyrosinase gene analysis in Japanese patients with oculocutaneous albinism. Journal of Dermatoogical Science **35,** 215–220.

Grimberg J, Nawoschik S, Belluscio L, McKee R, Turck A, Eisenberg A. 1989. A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. Nucleic Acids Research **17(20),** 8390.

Inagaki K, Suzuki T, Shimizu H. 2004. Oculocutaneous albinism type 4 is one of the most common types of albinism in Japan. American Journal of Human Genetics **74,** 466–471.

Jaworek TJ, Kausar T, Bell SM, Tariq N, Maqsood MI, Sohail A, Ali M, Iqbal F, Rasool S, Riazuddin S, Shaikh RS, Ahmed ZM. 2012. Molecular genetic studies and delineation of the oculocutaneous albinism phenotype in the Pakistani population. Orphanet Journal of Rare Diseases **26(7),** 44.

Kausar T, Jaworek TJ, Tariq N, Sadia S, Ali M, Shaikh RS, Ahmed ZM. 2013. Genetic studies of TYRP1 and SLC45A2 in Pakistani patients with nonsyndromic oculocutaneous albinism. Journal of Investigational Dermatology **133,** 1099-1102.

Kikuchi H, Hara S, Ishiguro S, Tamai M, Watanabe M. 1990. Detection of point mutation in the tyrosinase gene of a Japanese albino patient by a direct sequencing of amplified DNA. Human Genetics **85,** 123-124.

Kumar CM, Sathisha UV, Dharmesh S, Rao AG, Singh SA. 2011. Interaction of sesamol (3,4-methylenedioxyphenol) with tyrosinase and its effect on melanin synthesis. Biochimie **93(3),** 562–9. http://dx.doi.org/10.1016/j.biochi.2010.11.014.PMID 21144881.

Manga P, Kromberg J, Turner A. 2001. In Southern Africa, brown oculocutaneous albinism (BOCA) maps to the OCA2 locus on chromosome 15q: P-gene mutations identified. American Journal of Human Genetics **68,** 782–787.

Nakamura E, Miyamura Y, Matsunaga J, Kano Y, Dakeishi-Hara M, Tanita M, Kono M, Tomita Y. 2002. A novel mutation of the tyrosinase gene causing oculocutaneous albinism type 1 (OCA1). Journal of Dermatoogicall Science **28,** 102-105.

Oetting WS, King RA. 1992. Molecular analysis of type I-A (tyrosinase negative) oculocutaneous albinism. Hum Genetics **90,** 258–262.

Opitz S, Käsmann-Kellner B, Kaufmann M, Schwinger E, Zühlke C. 2004. Detection of 53 novel DNA variations within the tyrosinase gene and accumulation of mutations in 17 patients with albinism. Human Mutation **23,** 630-631.

Park KC, Park SK, Lee YS. 1996. Mutations of the tyrosinase gene in three Korean patients with type I oculocutaneous albinism. Japanese Journal of Human Genetics **41,** 299–305.

Passmore LA, Kaesmann-Kellner B, Weber BH. 1999. Novel and recurrent mutations in the tyrosinase gene and the P gene in the German albino population. Human Genetics **105,** 200–210.

Puri N, Gardner JM, Brilliant MH. 2000. Aberrant pH of melanosomes in pink-eyed dilution (p) mutant melanocytes. Journal of Investigative Dermatology **115,** 607–613.

Rooryck C, Roudaut C, Robine E, Musebeck J, Arveiler B. 2006. Oculocutaneous albinism with TYRP1 gene mutations in a Caucasian patient. Pigment Cell Research **19,** 239–242.

Seven M, Yosunkaya E, Yilmaz SB, Karaca E, Guven G, Yuksel A. 2011. A new syndrome presenting with dysmorphic facies, oculocutaneous albinism, glaucoma, cryptorchidism and mental retardation. Genetic Counselling **22,** 25–34.

Shah SA, Din SU, Raheem N, Daud S, Mubeen J, Nadeem A, Tayyab M, Baloch DM, Babar MS, Ahmad J. 2014. Identification of a novel mutation (p.Ile198Thr) in gene TYR in a Pakistani family with nonsyndromic oculocutaneous albinism. Clinical and Experimental Dermatology **39,** 646–648.

Simeonov D, Wang X, Wang C, Sergeev Y, Dolinska M, Bower M, Fischer R, Winer D, Dubrovsky G, Balog J, Huizing M, Hart R, Zein W, Gahl W, Brooks B, Adams D. 2013. DNA Variations in oculocutaneous albinism: an updated mutation list and current outstanding issues in molecular diagnostics. Human Mutation **34,** 827-35.

Spritz RA. 1993. Molecular genetics of oculocutaneous albinism. Semin Dermatol **12,** 167-172.

Stokowski RP, Pant PV, Dadd T, Fereday A, Hinds DA, Jarman C, Filsell W, Ginger RS, Green MR, Van der Ouderaa FJ, Cox DR. 2007. A genomewide association study of skin pigmentation in a South Asian population. American Journal of Human Genetics **81,** 1119-1132.

Suzuki T, Miyamura Y, Matsunaga J. 2003. Six novel P gene mutations and oculocutaneous albinism type 2 frequency in Japanese albino patients. Journal of Investigative Dermatology **120,** 781–783.

Tatusova TA, Madden TL. 1999. BLAST 2 Sequences, a new tool for comparing protein and nucleotide sequences. FEMS Microbiology Letter **174,** 247-250.

Tripathi RK, Bundey S, Musarella MA, Droetto S, Strunk KM, Holmes SA, Spritz RA. 1993. Mutations of the tyrosinase gene in Indo-Pakistani patients with type I (tyrosinase-deficient) oculocutaneous albinism (OCA). American Journal of Human Genetics **53,** 1173–1179.

Tripathi RK, Hearing VJ, Urabe K, Aroca P, Spritz RA. 1992. Mutational mapping of the catalytic activities of human tyrosinase. Journal of Biological Chemistry **267,** 23707–23712.