DOI: https://doi.org/10.53350/pjmhs2115102546

ORIGINAL ARTICLE

Identification of Genetic Mutations in *TYR* and *OCA2* Genes in Congenital Families with Oculocutaneous Albinism (OCA)

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ABSTRACT

Aim: The objective of the present study was to recruit congenital families of oculocutaneous albinism (OCA) and mutations in *TYR* and *OCA2* genes are identified, which is further expanding the mutation spectrum in this population.
Methods: Two consanguineous families with OCA were recruited and whole blood was collected. Clinical examination was carried out to determine the visual acuity and related eye, skin and hair examinations. Genomic DNA was extracted by standard phenol-chloroform method. Targeted exome sequencing by TruSight one sequencing panel sequencing was carried out. Sanger sequencing was performed for mutation detection in tyrosinase (*TYR*) and the *OCA2* genes and co-segregation in OCA families.
Results: Clinically, the affected individuals of two OCA families showed clinical characteristics including white to pale skin, white or blonde hairs, irritant to light, nystagmus and reduced vision. DNA sequencing showed the genetic mutation of *TYR* and *OCA2* genes in two OCA families. In family 1, the nucleotide variant (c.1255G>A; p.Gly419Arg) was detected in*TYR* gene, while in another family, the splice-site variant c.1045-15T>G was identified in *OCA2*.
Conclusion: This study concluded that identification of *TYR* and *OCA2* mutations in OCA disease are commonly associated with the population where the consanguinity is persistent. These findings expanded the molecular basis of oculocutaneous albinism in Pakistani families and established the mode of genetic counselling and for diagnostic outcome.
Keywords: Consanguineous families; Oculocutaneous albinism (OCA); mutations; tyrosinase (*TYR*); *OCA2* gene.

INTRODUCTION

Oculocutaneous albinism (OCA) is an autosomal recessive anomaly consequences due to decreased or diminished melanin pigment synthesis in hair, eyes and skin. Genetic mutation of respective genes impede different types of albinism due to directly or indirectly associated with melanin abnormalities[1]. Oculocutaneous albinism can be evaluated for differential diagnosis from less common syndromic types with accompanying additional clinical presentation like enhanced infectious susceptibility and neurological associated features along with partial albinism[2]. OCA is mainly categorized into two types syndromic and non-syndromic OCA. The autosomal recessive non-syndromic OCA is characterized into the seven different sub-types on molecular basis (OCA1 to OCA7) with the causative allele. The prevalence rate of albinism is projected 1/17,000 worldwide, demonstrating that approximately 1 in 70 individuals are carrying OCA alleles as carriers[3].

The subtype oculocutaneous albinism type-1(OCA1) is the most prevalent OCA that affects about 50% of OCA associated defects[4]. OCA showed wide-ranging clinical characteristic, with OCA1A documented as the most damaging due to absolute deficiency of melanin resulting completely white hairs and skin. On other hand, variable range of pigmentations are found in hair, skin and eyes with other types of albinism other OCA subtypes (OCA1B, OCA2-OCA7) due to variable concertation of melanin producing growth, while coloration remains usually reduced as compared to unaffected individuals[5].

Received on 24-04-2021
Accepted on 14-08-2021

Till present, causative genetic mutations are linked with six identified genes including *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, *SLC24A5* and *C10orf11* which are associated with nsOCA phenotype[6]. Tyrosinase (*TYR*) gen (MIM# 606933) is the responsible molecular factor for type 1 OCA, localized on chromosome 11q14.3, having five coding exons. Tyrosinase catalyzed two critical chemical reaction in the biosynthesis of melanin, and defects in enzymatic catalysis due to *TYR* mutation can lead to deficient or reduced pigmentation of the hairs, skin and eyes[7]. More than 500 mutations in *TYR* gene has been reported in different ethnic population.

The *OCA2* gene (MIM# 203200) localized on chromosome 15q11.2-q12, responsible to cause OCA type 2. This gene comprises of 25 exons (23 coding and two non-coding) covers 345 kb of nucleotide sequence This gene encodes OCA2 protein which regulates the arrangement and transferring the tyrosinase and its related protein; tyrosinase-related protein-1 (*TYRP1*) to the cell membrane[8,9]. At the present, more than 300 *OCA2* nucleotide variants have been recognized in OCA associated disorders[10,11].

The nucleotide changes linked to *TYR* and *OCA2* are responsible in congenital families of OCA in Pakistani ethnic region, which is approximately one third of OCA related variations; reported in this community[12]. Several studies investigated the genetic variations of the *TYR* and *OCA2* linked with OCA families of Pakistani population, and

prospectively denoted the regional founder mutations. The most common mutation of *TYR* is c.1255G>A; p.Gly419Argwhich accounts 12.9% of all *TYR* mutations. On the other hand, *OCA2* splice-site variant c.1045-15T>G is 4.3% in Pakistani population[4,12,13]. In present study, two families were recruited and mutations in *TYR* and *OCA2* genes are identified, which is further expanding the mutation spectrum in this population.

METHODS

Before the start of the study, ethical approval was obtained from University of Health Sciences, Lahore, Pakistan. This was observation descriptive study conducted during 2018-2020. Two oculocutaneous albinism (OCA) families having two or more affected individuals were ascertained. The probands with OCA were clinically examined and diagnosis was established (the basis of phenotypical presentations of OCA which includes absence/ or scarcity of pigmentation in skin, hairs and eyes in comparison to the unaffected family members) from the teaching hospital in the Punjab province of Pakistan.

A pedigree of each family was drawn according to standard methods. Families with autosomal recessive inheritance were selected. Detailed clinical and family history were taken. Families having two or more affected individuals in the extended family with OCA were included. Patients with any syndromic forms of albinism and Ocular albinism were excluded. About 5 ml of whole blood was collected in EDTA vacutainers for molecular studies. DNA was extracted from whole blood following a ReliaPrep™ Blood gDNA Kit of Promega /Phenol-chloroform standard protocol. DNA was quantified by nano-drop method, and PCR was run to amplify the desired fragment. Illumina TruSight one sequencing panel according to the protocol described earlier, performed next generation sequencing (NGS) on one affected case in each family. By use of targeted sequencing panel which covers >4800 different genes associated with eye clinical phenotypes/characteristics and the Sanger sequencing was performed for 17 known genes of OCA[14]. DNA sequencing was carried out to identify the causative mutation and co-segregation was done by sequencing the samples of carrier and normal individuals as described earlier[5].

In silico studies were carried out by using different computer based tools to predict the patho-gentic effects of the mutated variants. To determine the effects of *TYR* and *OCA2* mutant sequences, the commonly used online software including Mutation Assessor2 (www.mutationassessor.org/), POLYPHEN-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/) were applied. The *in silico* modalities were devised on a variable algorithm to classify the detected mutations/variations into the pathogenic and neutral effects.

RESULTS

Description of Families: Figure 1 represents the pedigree, affected individual and sequencing results. Family 1 was recruited form the province of Punajb. The ethnic caste was Arain Chaudhary and pedigree was drawn till fourth generation. In family 1, three affected and two unaffected individuals were ascertained from the consanguineous family with albinism in two sib-ships (Figure 1a). The family 2 was recruited from Lahore and it was consanguineous family of caste “Jutt”. The pedigree was sketched till fifth generation and three individuals were affected with albinism. In this family, seven individuals participated including the parents (Figure 2a).

Clinical Description: In family 1, the color of hair in affected individual (IV:1) was white to light golden and the color of skin was reddish-white (Figure 1b). It was also noted the scars due to sun burn scars on face and hands. On clinical eye examination, variable features including nystagmus, photophobia, transparent grey iris, foveal hypoplasia and albiniotic fundus were observed (Table I).

In family 2, the hair color of OCA affected individuals was brown to dark brown. At birth, the color was light brown but darkness was increased with the passage of time. The skin color was reddish-white and eyes were brown in color (Figure 2b). On clinical examination, photophobia and nystagmus were the main clinical features observed in the affected family members (Table I).

Table I: Vision of affected individual of family 1 and family 2 of OCA.

Eye examination	Family 1 (IV:1)		Family 2 (V:2)	
	Right eye	Left eye	Right eye	Left eye
SPH	+8	+8	+2.5	+2.5
CYL	+1	+2	+1.0	+1.5
AXIS	95	95	100	85
Corrected V.A	1.0	0.9	0.6	0.6

VA; *visual acuity*

Mutation analysis of families: In family 1, the combination of targeted and Sanger sequencing identified a mutation, c.1255G>A; p.Gly419Arg in exon 4 of *TYR* gene which is a missense and previously reported in OCA families. The affected individuals were homozygous showing A/A allele pattern at nucleotide position c.1255G>A, the heterozygous carrier carried the allele G/A pattern and the unaffected/normal individuals showed the wild-type homozygous G/G allele (Figure 1c). In family 2, the sequencing techniques combinely detected a previously known splice-site mutation c.1045-15T>G in *OCA2* gene. In sequencing chromatogram, the affected individual was homozygous showing allele G/G pattern at c.1045-15T nucleotide position, while heterozygous carrier (parents or the carrier siblings) demonstrated the allele T/G pattern (Figure 2c).

In-silco analysis of TYR and OCA2 mutations are described for damaging and pathogenic effect, which is presented in table II.

Table II: Impact of *TYR* and *OCA2* mutations by *in silico* analyses.

Features	Family 1	Family 2
Causative Gene/s	*TYR*	*OCA2*
Nucleotide variant	c.1255G>A; p.Gly419Arg	c.1045-15T>G
Mutation type	Homozygous; Missense	Homozygous; Splice-site
Novel or Known mutation	Known mutation	Known mutation
SIFT	Predicted the damaging effect	----------
PolyPhen-2	Probably damaging impact	----------
Mutation Assessor	Pathogenic	----------
gnomAD MAF	0.00006155	0.00002445

Fig. 1: a. The pedigree of OCA family 1b. Image of an affected individual IV: 1 showing clinical characteristics of OCA1, c. Sequencing chromatograms of *TYR* showing mutation c.1255G>A; p.Gly419Arg

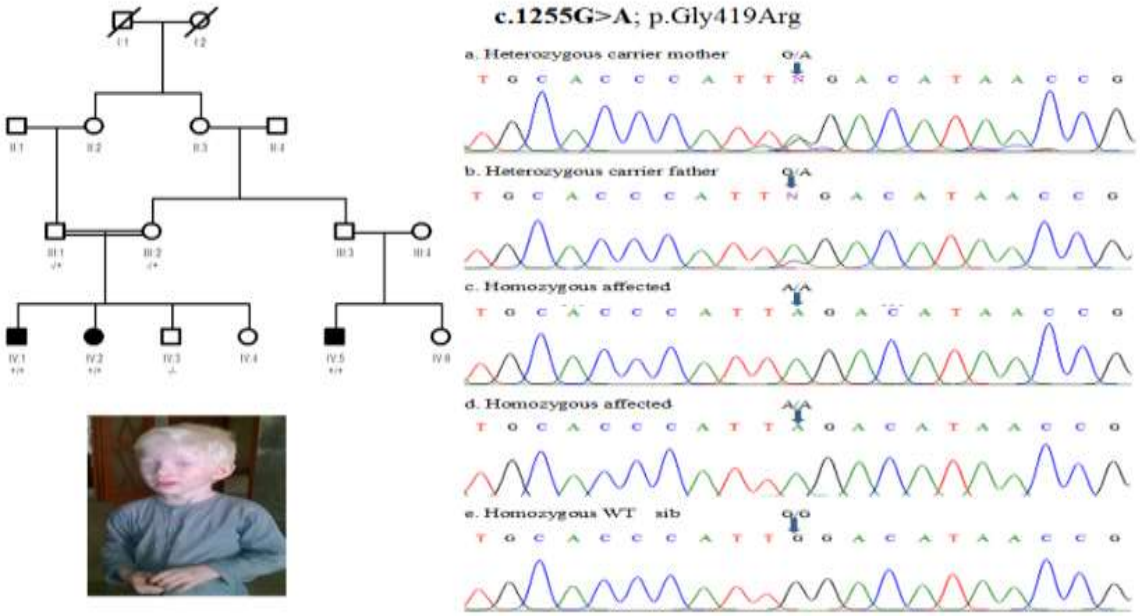
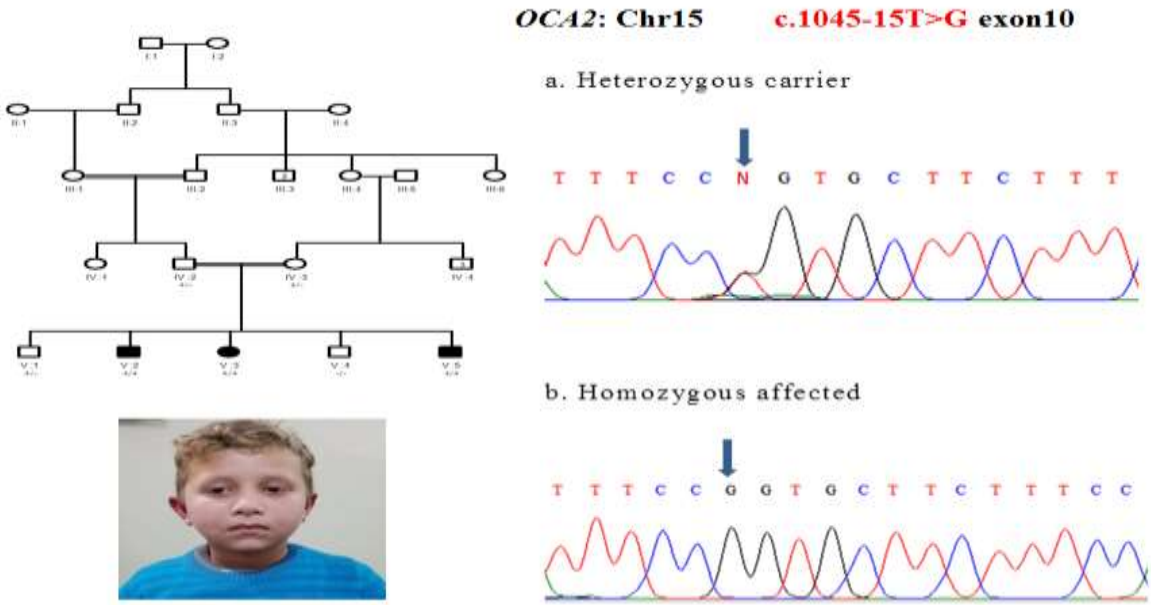


Fig. 2: a. The pedigree of family 2 showing different generations and sib-ship); b. An affected individual of the family 2; c. DNA sequencing chromatogram represent the position of *OCA2* c.1045-15T>G mutation in carrier (heterozygous) and mutant (homozygous).



DISCUSSION

Oculocutaneous albinism (OCA) is a rare autosomal genetic anomaly causing due to complete absence or deficiency of melanin pigment which normally imparts the color in skin, eyes and hair. It is characterized as a heterogeneous defect due to involvement of diverse causative genes associated with variable clinical characteristics and types of non-syndromic oculocutaneous albinism (nsOCA). Till present, seven types of OCA have been explored and the OCA1 type is the furthermost condition which is reported in Pakistani families and also in other ethnic groups due to genetic alteration in *TYR*[4,5,11,12,15-17].

In family 1, a previously known mutation c.1255G>A; p.Gly419Arg of *TYR* gene has also been detected in the present study. In Pakistani population, the overall frequency is 22.2% for this mutation is considered as prevalent mutation because it has been reported in variable OCA Pakistani families mostly speaking the Punjabi language. This mutation of *TYR* gene is described in further Pakistani language and

groups comprising Siraiki, Sindhi, Balochi and Kashmiri ancestry and depicting the role of founder mutation[4,5,11,12,18]. The frequency of this common mutation is comparatively documented in neibourhoods of subcontinent where it is 20% in mixed population of India and 17% in South Indian. On the other hand, the frequency of this common variant of *TYR* is very low in Caucasian population (only 0.83% of families reported with this variant)[19]. The mechanism for this variation in occurrence between the populations designates the impact of founder-mutation effect in the subcontinent mainly in the Indo-Pakistan era

The second most prevalent type is OCA2 which is originated due to *OCA2* mutations, consequentially lead to changes in the p protein, and hence, it impact the normal biosynthesis of melanin pigment. The transmembrane protein OCA2 is found in the melano-somal membrane. Various reports propose that this protein may play a role in the transportation of tyrosine amino acid which is the precursor for melanin synthesis and also regulate the melanosome maturation[19].

In present study, a previously reported splice-site nucleotide variation c.1045-15T>G of *OCA2* was detected and co-segregated in OCA family precisely. An earlier study on OCA Pakistani families by Jaworek *et al.*, 2012 reported this novel mutation first time[20]. Exon-trapping assay was applied to detect the nucleotide sequence in OCA family and the splice-site nucleotide variant c.1045-15 T>G involves the type of mRNA which causes the skipping of exon 10 with loss of twenty four amino acids and third domain scarcity in OCA2 transmembrane protein. The c.1045-15T>G splice-site nucleotide variant of *OCA2* has been investigated in large number of families, has showed the incidence of about 7.2% of *OCA2* variants and 30.6% of all known OCA alterations in Pakistani families[4,12,20]. In according to gnomAD database, the splice-site *OCA2* variant is only documented from South Asia, the most possibly from the Pakistani origin, presenting the 0.00002394 allele frequency, so it may have founder role of spreading mutation in this region.

CONCLUSION

The result of this study concluded that *TYR* mutations are the most predisposing factor for the development of OCA in Pakistan families. On the other hand, the second most common gene responsible for the development of OCA in Pakistan is the *OCA2.* In the present study, one each mutations has been determined in *TYR* and *OCA2* genes respectively, which is explaining the heterogeneity of this disorder commonly impeding in Pakistani population.

Acknowledgement: I am grateful to the families who participated in this study. I am also thankful to University of Health Sciences for providing the equipment facilities and thankful to Pakistan Science Foundation (PSF) for providing research funds.

Research funding: For this research, a grant was obtained from Pakistan Science Foundation (PSF), vide reference *No. PSF/Res/P-UHS/Med (492)*.

Conflict of interest: Nil

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