

Mutation Analysis of a Pakistani Oculocutaneous Albinism Family Identifies a Novel Splice Site Defect in *OCA2* Gene

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ABSTRACT

Oculocutaneous albinism (OCA) is a multi-systemic and rare genetic disorder of pigmentation. It occurs due to defects in the melanin synthesis pathway. OCA is characterized by hypopigmentation of hair, dermis, and ocular tissue. The clinical consequences of OCA includes whitish skin, whitish to golden hair and photophobia. Genetic studies have so far reported seven non-syndromic OCA genes, among which Pakistani OCA families mostly segregate *TYR* and *OCA2* gene mutations. In the present study we aimed to investigate the genetic factor of OCA in a consanguineous family from Pakistan. Genetic analysis was performed through microarray genotyping and homozygosity-by-descent (HBD) mapping, whole exome sequencing (for mutation identification) and Sanger sequencing (for variant segregation). Homozygosity analysis revealed a 1.2 Mb HBD region on chromosome 15 between markers rs4778147 to rs8036234 (chr15:27752745-28962131bp), which harbors the previously reported *OCA2* gene. The subsequent whole exome sequencing identified a novel splice site defect at splice donor site, wherein G was replaced by T at genomic position 28171268 (NM_000275.2:c.2079+5G>T) within intron-19 of the *OCA2* gene. This change presumably may either includes few intronic nucleotides or skip exon-20 in the mature mRNA. Our study further supports the evidence of high incidence of *OCA2* gene mutations in Pakistani families.

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Authors' Contribution

HG, RH and AM performed experiments, data analysis and bioinformatic predictions. EUK, WS and NA recruited the family and did clinical analysis. MAK, AHS and JBV remained involved in data analysis, manuscript drafting and project supervision.

Key words

Oculocutaneous albinism, *OCA2*, Homozygosity-by-descent mapping, Whole exome sequencing, Splice site defect

INTRODUCTION

Autosomal recessive non-syndromic oculocutaneous albinism (OCA) is an uncommon condition of defective melanin synthesis. The clinical features of OCA patients comprise of white milky skin, whitish to golden hair and deterioration of retinal cells. Worldwide, OCA affects 1 in 17,000 individuals, which means that ~1 in 70 people are carriers of the defective OCA allele (Gul *et al.*, 2019; Shahzad *et al.*, 2017). Molecular genetic studies have so far reported seven genes (*TYR*, *OCA2*, *TYRP1*, *SLC45A2*, *SLC24A5*, *C10ORF11*, and *MC1R*) and an uncharacterized locus (*OCA5*) responsible for causing

non-syndromic OCA subtypes. The product of these OCA genes are involved in the melanin bio-synthesis pathway (Khan *et al.*, 2015). Among the known OCA genes, *TYR* and *OCA2* are the most commonly reported defective genes globally, and in Pakistani families as well. Genetic studies have shown that the splice mutation c.1045–15 T>G in *OCA2* is the most frequent pathogenic allele (11.3% prevalence) among OCA patients (Gul *et al.*, 2019; Jaworek *et al.*, 2012). Clinical diagnosis of different OCA subtypes is unable to distinguishable between the genes because of its overlapping and variable manifestations. Hence, in this scenario, molecular diagnosis is considered as the gold standard for determining the OCA subtype.

Herein this investigative study, a consanguineous OCA family from Pakistan was analyzed, resulting in the identification of a previously unreported splice donor site defect (NM_000275.2:c.2079+5G>T) within intron-19

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of the *OCA2* gene. This change presumably may either add non-coding nucleotides into the mature mRNA, or may lead to skipping of exon 20, either way resulting in a frame-shift and premature protein truncation.

MATERIALS AND METHODS

The study was approved by the institutional ethical review board of Gomal University, Dera Ismail Khan, Pakistan, and the samples were enrolled after executing the written research ethics agreement and informed written consent by participants. Thereafter, blood samples were obtained for DNA extraction and onward genomic studies. DNA qualitative and quantitative assessment was carried out through NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, Berlin, Germany). Clinically, the disease was diagnosed through apparent OCA features like whitish skin, un-pigmented hairs, and presence of eye abnormalities (nystagmus, photophobia, and reduced vision).

Homozygosity scanning

HBD mapping (finding Homozygous by Descent regions) was performed through SNP microarray using Illumina Infinium CoreExome-24 Kit following the Infinium HTS Assay (15045738A). Selected individuals were used for whole genome SNP genotyping (III-1, III-2 and III-3). The data were analyzed for finding HBD regions using Homozygosity Mapper (Seelow *et al.*, 2009) and FSuite (Gazal *et al.*, 2014). Each homozygous region was checked at the genotype level to confirm its haplo-identity. This strategy helped in excluding the false positive results, while the remaining regions were selected for further analysis.

Whole exome sequencing (WES) and data analysis

WES was performed for the single affected individual of family (III-2). Sequencing library was synthesized using ThruPLEX DNA-seq 96D kit (No. R400407, Rubicon Genomics, Ann Arbor, MI) and SureSelect XT2 Human All-Exon Version 5 target-enrichment system (Agilent Technologies, Santa Clara, CA). Paired-end sequencing (125 bp sequence read) was performed on an Illumina HiSeq 2500 platform (HiSeq SBS Kit v4 [250 cycle]). The raw data analysis was performed through the SNAP Aligner [1.0 beta 18 for Linux (for BAM files), GATK [v. 4.0 (for vcf files) (McKenna *et al.*, 2010), and ANNOVAR [v. 1.04, (for variant annotation) (Wang *et al.*, 2010).

Sanger sequencing and mutation analysis

After finding the pathogenic variant, Sanger sequencing was performed to check its segregation with

the disease phenotype within the whole family. For Sanger sequencing, the sequencing PCR product was analyzed on ABI 3730XL instrument (Applied Biosystem, USA). For mutation analysis, the Sanger data was analyzed using FinchTV Program, Version 1.4.0 (<http://www.geospiza.com>). The software was used to read the sequence chromatogram and sequence alignment with human reference genome (hg19). In addition to this, mutations analysis was also performed through BLAT tool of UCSC Genome Browser (<http://www.genome.ucsc.edu>).

Bioinformatic analysis

To predict the functional impact of different variants on protein and its association with albinism disorder, various bioinformatic tools were employed i.e. SIFT (deleterious = ≥ 0.05 ; tolerated = ≤ 0.05) (Ng Pauline and Henikoff, 2003), PolyPhen-2 (Adzhubei *et al.*, 2013), I-Mutant 2.0 (decrease stability = (DDG < 0; increase stability = DDG > 0), I-Mutant 3.0 (decrease = ≤ 0.5 kcal/mol; Increase = ≥ 0.5 kcal/mol). Splicing defect prediction was performed with the help of Human Splicing Finder [<http://www.umd.be/HSF/>] (Desmet *et al.*, 2009), and the 'splice site prediction by neural network' through the Berkeley Drosophila Genome Project (BDGP; https://www.fruitfly.org/seq_tools/splice.html; (Reese *et al.*, 1997).

RESULTS

General description of family

The current family was ascertained from among the Pashto ethnic population of Swat District. The family pedigree consists of three generations, with four affected individuals in all generations. The pedigree analysis showed possible autosomal dominant inheritance pattern, although autosomal recessive inheritance was equally plausible given the consanguineous nature of the family. The apparent clinical features of patients include golden hair color, white skin color with reddish pigmentation (Fig. 1). The additional ocular symptoms include brown iris color, photophobia, nystagmus, strabismus and weak eye sight.

Molecular analysis of family under investigation

The current family was previously published in JPMA (Gul *et al.*, 2017), wherein the exact genetic mutation was not reported due to low coverage of exome, including splice junctions. Moreover, at that time, microarray based genome-wide SNP genotyping was not performed. Therefore, the family underwent re-sequencing for whole exome including splice junctions with enhanced coverage as well as whole genome homozygosity scan.

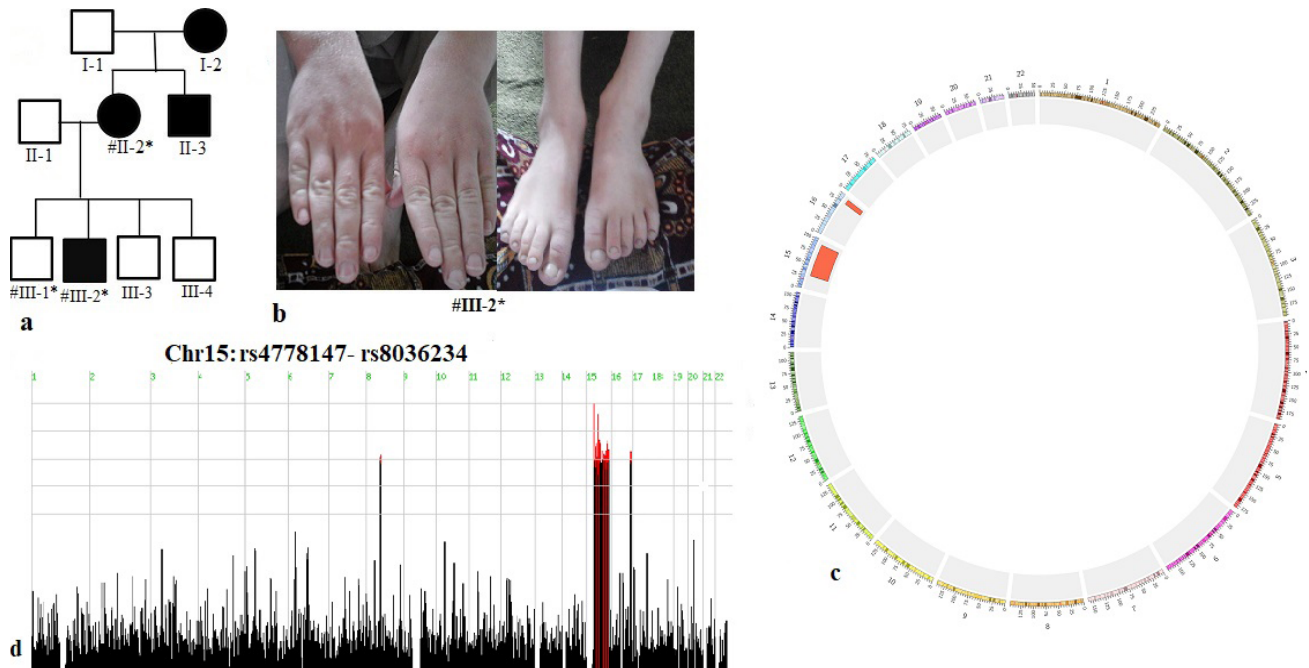


Fig. 1. a) Family's pedigree showing autosomal dominance, having total of four affected individuals in all generations. b) Picture of affected albino individual reveals the hypopigmentation of skin. c) FSuite HBD map of family indicating HBD region at chromosome 15. d) HomozygosityMapper's image showing analyzed result of SNP genotyping of family depicts HBD region at chromosome 15.

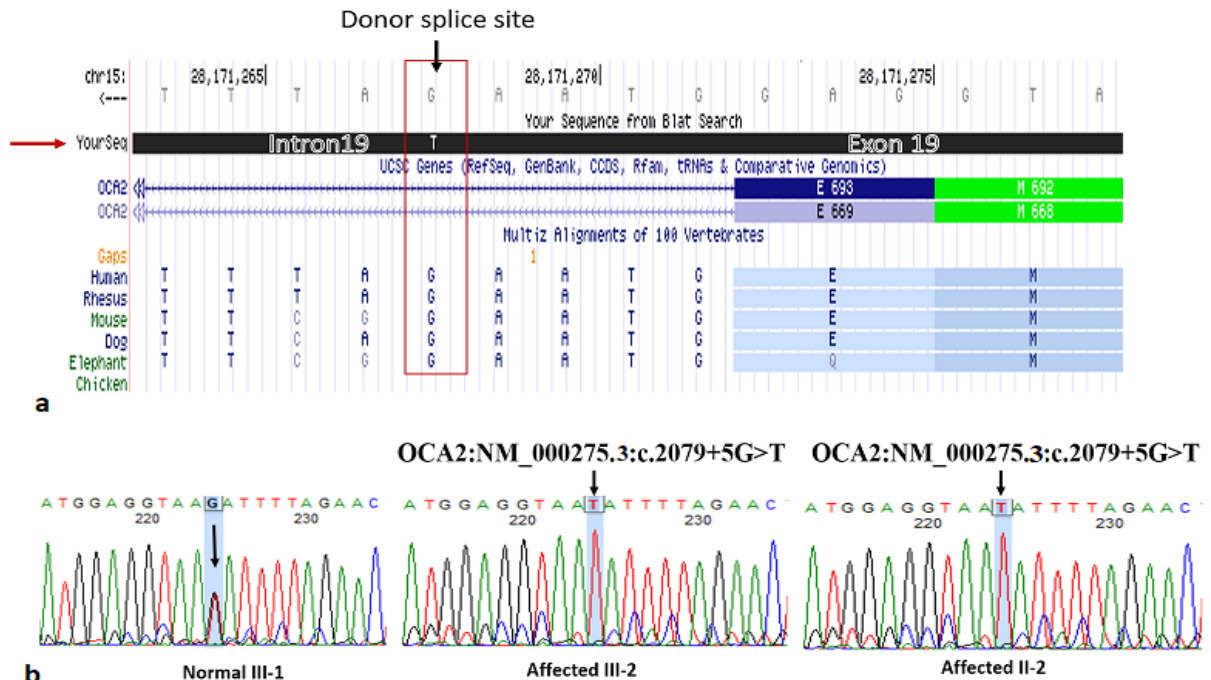


Fig. 2. Family's affected individual's sequence in UCSC genome browser depicts substitution of G with T and G is conserved in each species at position 2079+5. B) Sequence chromatograms of affected and normal individuals of family reveals the homozygous substitution of G nucleotide with T at position 2079+5 while the normal sibling was heterozygous at this position.

Table I. Reported Splice site mutations in *OCA2* gene.

Sr. No	Nucleotide change/ Splice site mutations	Location	Effect on protein/ skip- ping of exon	Ethnic group	Reference
1	c.1951+1G>A			Philippines/ Northern European	(Oetting <i>et al.</i> , 2005)
2	c.1503+5G>A		Exon 14	Italian proband	(Rimoldi <i>et al.</i> , 2014)
3	c.1364+1G>A	IVS13+1		Japanese	(Okamura <i>et al.</i> , 2016)
4	c.2079+1G>A	IVS19+1	Exon 19	Japanese	(Okamura <i>et al.</i> , 2016)
5	c.1501-2A>T			African	(Kerr <i>et al.</i> , 2000)
6	c.808-3C>G	IVS7		Chinese	(Wang <i>et al.</i> , 2017)
7	c.2080-2A>G			Chinese	(Wang <i>et al.</i> , 2017)
8	c.808-2A>G	IVS7		Chinese	(Hu <i>et al.</i> , 2014)
9	c.2080-1G>A				
10	c.1182+1G>A		Exon11-12	Chinese	(Yang <i>et al.</i> , 2019)
11	c.1784+1G>A			Korean	(Park <i>et al.</i> , 2012)
12	c.1784+2T>C			Caucasian	(Passmore <i>et al.</i> , 1999)
13	c.1045-15T>G	IVS9	Exon 10	Pakistani	(Jaworek <i>et al.</i> , 2012)
14	c.1045-2A>G	IVS9			(Sengupta <i>et al.</i> , 2010)
15	c.2433-5T>G				
16	c.2338+2T>G				
17	c.2339-2A>C			Chinese	(Biyuan <i>et al.</i> , 2017)
18	c.1842+1G>T			Caucasian	(Lee <i>et al.</i> , 1994a)
19	c.1842-1G>T		Acceptor splice site	Kuna	(Carrasco <i>et al.</i> , 2009)
20	c.1637-2A>G				
21	c.1636+1G>A				(Suzuki <i>et al.</i> , 2003)
22	c.1044+1G>A			Chinese	(Wattanasirichaigoon <i>et al.</i> , 2008)
23	c.807+1G>T				
24	c.574-19A>G			Tanzanian	(Spritz <i>et al.</i> , 1995)
25	c.1239+5G>A			African-American	(King <i>et al.</i> , 2003a)
				West Indian	
				Puerto Rican	
26	c.1050-3C>G	IVS7-3C>G		Puerto Rican	(Chiang <i>et al.</i> , 2009)

Genome-wide homozygosity analysis revealed a 1.2 Mb long HBD region on chromosome 15 between markers rs4778147 to rs8036234 (chr15:27752745-28962131bp; hg19) (Fig. 1). Exome sequence analysis of individual III-2 identified substitution of C by A at position 28171268 (15q13.1) in the 19th intron of the *OCA2* gene that is predicted to affect the donor splice site. Sequence chromatograms of affected and normal siblings of family revealed the homozygous substitution of G nucleotide by T at cDNA position 2079+5, while unaffected sibling was shown to have carrier status of G/T (Fig. 2). The currently identified variant is not reported in genomAD,1000

Genome, the Human Gene Mutation Database and ClinVar. The splicing prediction tool at the BDGP predicts a reduction of the splice donor score from 0.97 to 0.53 (out of 1.0). HSF predicts a reduction from 95.9 to 83.59 (out of 100).

DISCUSSION

OCA is a recessively inherited pigmentation disorder of skin, hair and retinal pigment epithelium, which is caused due to defects in melanin biosynthesis (Márquez-García and Montoliu 2013). Genetically speaking, defect

in seven genes have been implicated in OCA, among which *TYR* and *OCA2* have the highest incidence rate. The current study involved the identification of a novel splice site defect NM_000275.2:c.2079+5G>T in *OCA2* gene. Epidemiologically, *OCA2* is the most widespread form of albinism worldwide; which in South African Black is found to be 1:3600, (Kromberg and Jenkins 1982; Stevens *et al.*, 1997) 1:10 000 in Black and 1:36000 in Caucasians (Witkop *et al.*, 1989), and 1:10 000 in the Irish (Froggatt, 1960). The *OCA2* locus is mapped on chromosome 15q11.2-q1211 (Lee *et al.*, 1995). The *OCA2* gene encodes an 838 amino-acid long protein that is also known as P protein, which is required for melanin pigment synthesis by mediating Cl⁻ specific anion conductance (Bellono *et al.*, 2014; Duffy *et al.*, 2007). Though initially reported as an integral melanosomal protein, *OCA2* is potentially associated with transport of some molecules into the melanosome and regulates melanosomal pH (Brilliant, 2001) with an additional possible role of processing and trafficking of melanosomal proteins (Chen *et al.*, 2002; Toyofuku *et al.*, 2002).

The *OCA2* protein belongs to the Na⁺/H⁺ antiporter family. It acts as a precursor to melanin synthesis and controls the process of tyrosinase and tyrosinase-related protein, also maintains the pH of the melanosomes (Bellono *et al.*, 2014; Ni-Komatsu *et al.*, 2006). So far, 184 *OCA2* mutations have been reported in HGMD (Human Gene Mutation Database, professional 2019.4), including 104 nonsense/missense mutations, 26 splicing mutations, 1 regulatory mutation, 32 indels 20 gross indels, and 1 complex rearrangements.

In this study, the genetic defects underlying *OCA2* in two albino patients (III-2, II-2) identified a novel splicing mutation (NM_000275.2:c.2079+5G>T). Before this study, twenty-six (26) splice mutations have been reported globally (Table I). While, literature survey has shown that it is the second splicing mutation being reported in the Pakistani population. Previously, Jaworek *et al.*, 2012 reported one splice site mutation c.1045-15T>G in a consanguineous Pakistani family. The clinical synopsis of current patients is in agreement with previously published *OCA2* phenotypes. Hence, the phenotyping in present family will help in genotype-phenotype correlation analysis. Splicing mutations usually result in errors during the splicing process and may lead to improper intron removal and thus causes alteration of the open reading frame.

CONCLUSIONS

Exome sequence analysis in a Pakistani OCA family found a novel splice site defect. The current finding

supported the evidence of high incidence of *OCA2* gene mutation in Pakistan. Although, variant pathogenicity is strongly supported by *in silico* analysis, however, functional studies are required to investigate the effect of mutation on mRNA and protein levels.

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Miss Hadia Gul is supported by Higher Education Commission of Pakistan under its IRSIP scholarship scheme to visit University of Toronto, Canada.

Statement of ethics

The study was performed after taking approval from institutional ethical review board of Gomal University, Dera Ismail Khan, Pakistan, to ensure the privacy and ethical rights. Moreover, the data has not been published or submitted elsewhere, except the part of Miss Hadia Gul's Ph. D thesis.

Statement of conflict of interest

The authors have declared no conflict of interest.

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