Original Article

Molecular Analysis of OCA1 and OCA2 Genes in Sindhi Inbred Families

PJO – Official Journal of Ophthalmological Society of Pakistan

Jalpa Bai[1], Hina Shaikh[2], Ghulam Mujtaba Sohu[3]
Ashok Kumar Narsani[4], Ali Muhammad Waryah[5]
[1-5]*Liaquat University of Medical and Health Sciences, Jamshoro*

ABSTRACT

Purpose: The purpose of this study was to identify the common mutations of (Oculocutaneous albinism)OCA1 and OCA2 genes in Sindhi Inbred Families.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: Liaquat University of Medical and Health Sciences (LUMHS), Jamshoro, Pakistan, from October, 2020 to September, 2022.

Methods: Forty-four patients of eight families with clinically diagnosed OCA and Ocular albinism (OA) with or without family history were recruited for this study and all affected individuals other than OCA were excluded from the study. A single missense substitution was identified in the OCA1 and OCA2 using Poly Phen 2, Mutation Taster and I-Mutant software.

Results: Out of 8 randomly chosen OCA afflicted families, there were two carriers and two affected individuals identified in family III. In exon 4 of the OCA1 gene, a common mutation (homozygous) c.1255 G>A (p. Gly419Arg) was identified. In three-generation pedigree for the albinism family VII was identified, including two affected, one carrier, and two normal people. Participants in this family who carried the 1045-15 T>G mutation in the OCA2 gene were affected.

Conclusion: Albinism affected individuals in Pakistan have varying phenotypic and genetic presentations. This is due to the fact that the population of Pakistan and those of Sindhi ancestry are heavily inbred, consanguineous, segregated, and afflicted by hereditary diseases.

Key Words: Oculocutaneous albinism, Ocular albinism, Genes, Melanin, Mutation, Hereditary, Pakistan.

How to Cite this Article: Bai J, Shaikh H, Sohu GM, Narsani AK, Waryah AM. Molecular Analysis of OCA1 and OCA2 Genes in Sindhi Inbred Families. 2024;40(3):233-239. **Doi: 10.36351/pjo.v40i3.1813**

Correspondence: Jalpa Bai
Liaquat University of Medical and Health Sciences, Jamshoro
Email: jalpa.bai@outlook.com

Received: February 16, 2024
Accepted: May 25, 2024

INTRODUCTION

Oculocutaneous albinism (OCA) is a hereditary disease caused by lack of enzymes necessary for the production of melanin.[1] Melanin has been identified to protect skin from Ultraviolet radiation. People with darker skin produce more melanin than those with lighter skin. Eye, hair, and skin hypopigmentation are caused by its poor production. Oculocutaneous Albinism (OCA), which affects the eyes, skin, and hair presents in two ways. Syndromic Oculocutaneous Albinism (OCA) and non-syndromic Oculocutaneous Albinism. Syndromic OCA is the more severe form of OCA and has systemic associations, while the non-syndromic (ns) OCA is a class of autosomal recessive diseases marked by a decrease in melanin levels in skin, hair and eyes. Severity varies depending on the type of Lysosome-related organelles (LROs), which includes a number of signs and symptoms such as visual acuity, nystagmus, foveal hypoplasia, and bleeding problems. Due to the existence of a single defective gene or heterozygous mutations in the OCA

genes of two separate alleles, OCA carriers can pass the disease on to their offspring, establishing the disease heterogeneity in many global populations.[2] Loss of pigment causes a pale complexion, red eyes that reflect light or are light brown or blue-green and fair or white hair. Because of the total absence of pigment in hair, eyes and skin as well as the slight depigmentation of the eyes, albinism exhibits a wide range of phenotypic variation. Numerous infections and neurological problems are among the OCA signs and symptoms. Pudlak Hermansky (PHS), Chediak Higashi (CHS), Syndrome Griscelli, Prader Willi (PWS), Breen Cross McKusick, Elejalde, and Syndrome Waardenburg type II (WS2) are just a few examples of the syndromic albinism.[3]

Approximately, one in 17,000 to 20,000 people worldwide are affected by the OCA.[4] The eight subtypes of the nsOCA include OCA1, OCA2, OCA3, OCA4, OCA5, OCA6, OCA 7 and OCA8. It was identified that the OCA1 phenotype has a high mutation detection frequency. OCA1 affects 1 in 40,000 people worldwide and is the most common in America (70% of cases), China (sporadic albinism), the Indian subcontinent (familial albinism) and the Caucasian population. OCA2 allele frequency is 1 in 1000 in sub-Saharan Africa, which is higher than the global average.[5] One in 39,000 people worldwide have OCA2 type of OCA, which is the most prevalent variety. OCA1 and OCA2 with common genetic alterations are also present in Indo-Pak families. German, Japanese, and Indo-Pakistani populations have been documented to have the OCA3 (1 in 8500 individuals).[6] One in 100,000 people have OCA4, which affects the Japanese population more frequently than the populations of Asia and Europe by 24%.[7] While China, Eastern India and Atlantic Island were frequently recorded with OCA6 and OCA7, the actual prevalence was unknown. OCA5 was observed in the consanguineous Pakistani communities. Around the world, HPS was detected in 1 in 500,000 cases of syndromic OCA, as in 1 in 12,000 cases, PWS in 1 in 15,000 cases, and CHS in 500 cases or less.[8,9]

Keeping in view the international data, the goal of this study was to reveal the mutational spectra and common alleles in Sindhi inbred families that carry the OCA1 and OCA2 genes.

METHODS

This descriptive study was conducted at the Department of Molecular Biology and Genetics, Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan. The study was approved by the Institutional Ethics Committee of Liaquat University of Medical and Health Sciences. All study procedures were conducted in accordance with the ethical rules of the Helsinki Declaration and Good Clinical Practice.[10] Before enrolling in the study, written informed consent was obtained from all study participants and consent was obtained from parents or guardians of participants under age 18 years.

A total of8 families were recruited having two or more than two affected individuals from Sindh Pakistan. All the recruited participants were clinically diagnosed OCA and OA. Individuals with conditions other than OCA were excluded from study. After enrolling the Adobe Illustrator (1.0) software was used For Pedigrees drawing of the selected families Figure 1).

Venous blood of 10ml was collected from each participant, parents, and close relatives such as siblings under an aseptic environment with a sterile syringe. Falcon tubes containing 400µl of anticoagulant 0.5M ethylene diamine tetra acetic acid (EDTA) was used for storing blood at -80°C till the commencement of DNA extraction and further analysis.

The EDTA vacutainers filled with whole blood of the study participants (n=44) of 8 families were utilized for the isolation of DNA using standard nonorganic Grimberg protocol.[11] After DNA extraction, quantification of DNA was done assessing the Optical Density (OD) of DNA at 260 nm wavelength in UV-visible spectrophotometer. For quantity and quality assessment of genomic DNA, gel electrophoresis was executed on 0.8% agarose gel (Figure 2).

For a specific allele PCR, a 1.5% agarose gel was created. The findings were viewed, and bands were identified based on size. Its ladder was run in an isolated well with a PCR product acting as a control band to confirm the required size of the product.[12]

Using software called primer1, the ARMS (amplification system of refractory mutation) assay was created. DNA genomic containing the desired mutation was chosen from the UCSC genome browser and placed in the window.[13]

Following three phases (denaturation, annealing temperature, and extension), particular DNA segments were amplified using PCR technique. Various PCR

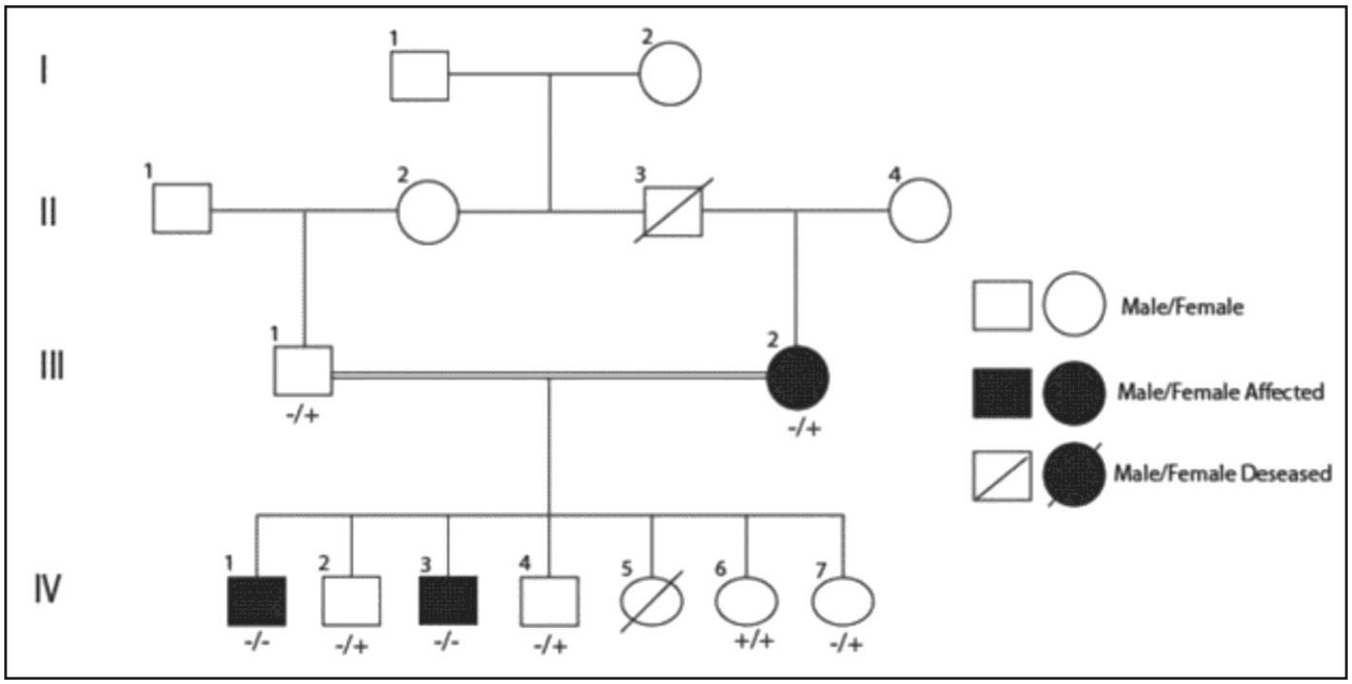

Figure 1: Sample Pedigree Drawing.

programs were used to optimize the genes of various primers. Initialization took place for two minutes at 95°C. Within 30 seconds at 95°C, the double DNA strand was split into a single strand. DNA templates were joined with primers at 62/60°C for 40 seconds every 30 cycles. Primer nucleotides were joined with their complementary counterparts. With the aid of various enzymes, including DNA polymerase, the freshly synthesized strands were lengthened, and multiple DNA segment copies were created at 72°C for 50 seconds. The last extension was performed for 5 minutes at 72°C, and the last hold was performed at 25°C (infinity).[14]

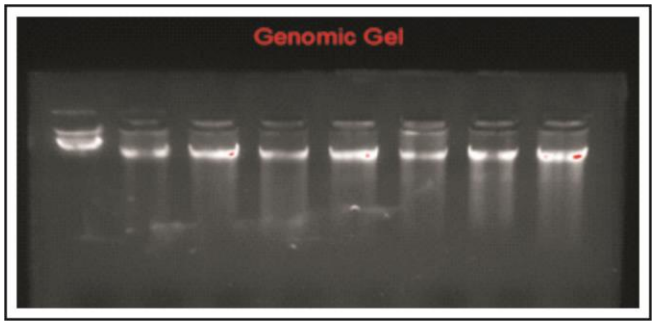

Figure 2: Genomic DNA analysis on 0.8% Agarose Gel.

RESULTS

The enrolled families were screened for identification of mutations in the selected gene. The ages of the affected individuals ranged from 1 to 60 years. Homozygous mutations in the TYR and OCA2 genes were recognized in the OCA.

Table 1: *Optimization Recipe of ARMS assay PCR.*

S. No.	Reagents	Volume
1.	Buffers	2µl
2.	DNTPs	2µl
3.	Taq Polymerase	0.6µl
4.	Printer forward inner	0.5µl
5.	ward outer	0.5µl
6.	Printer reverse inner	1µl
7.	Printer reverse outer	1µl
8.	DNA	2µl
9.	Water	10.4µl
	Total Volume	**20µl**

The albinism family III enrolled from Rawat Magsi Thatta City in Sindh Pakistan. This three-generation pedigree consists of two affected in two loops as shown in figure 3 A and was drawn after questioning numerous family members. Affected individuals are shown in (Figure 3 B&C). Common mutation c.1255 G>A (p. Gly419Arg) was presented in Figure 4. Two affected individuals II:1, II:4 and

four normal individuals were available at the time of screening.

The albinism family VII was enrolled from Matiari city situated in Sindh province of Pakistan. The enrolled family belonged to Sindhi ethnicity. This three-generation pedigree consists of two affected in one loop and was drawn after questioning numerous family members (Figure 5 A). The two affected III:1 and III:2 individuals and three normal individuals were available at the time of screening. Affected individuals are shown in (Figure 5 B& C), while common mutation c.1045-15 T>G was presented in (Figure 6).

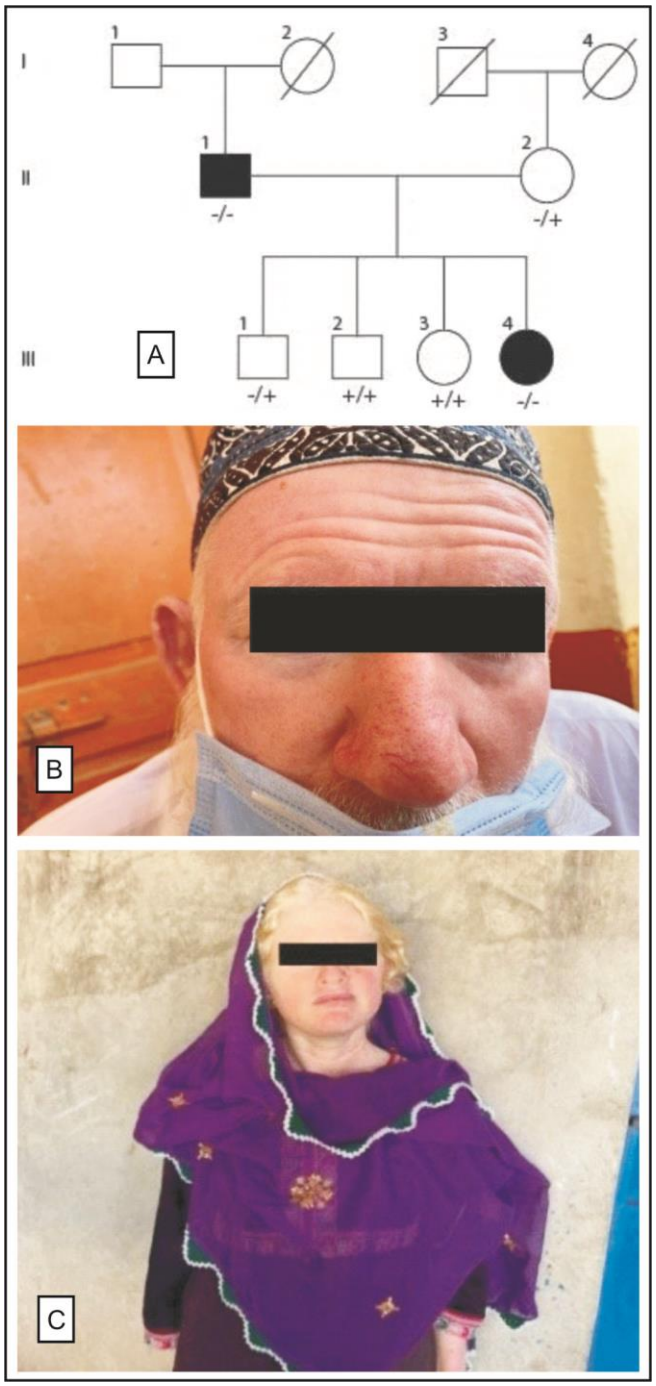

Figure 3 (A): Pedigree showing the affected individuals are Homozygous while their parents are heterozygous. **(B & C):** Affected individuals Oculocuteneous Albinism.

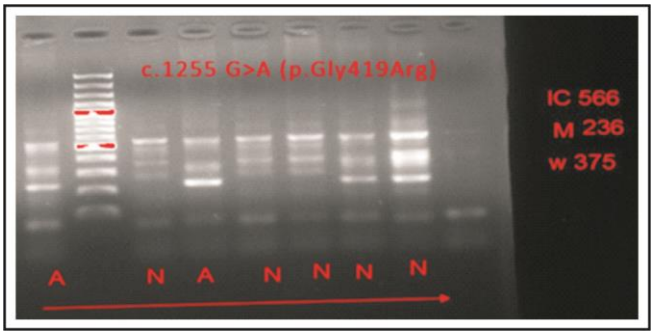

Figure 4: Gel Picture indicating the mutation c.1255 G>A (p. Gly419Arg).

DISCUSSION

In this study, we tested a total of eight OCA families in the Sindh province of Pakistan and we found two mutations in the OCA1 and OCA2 genes in two distinct families, namely family number three and family number seven. The ratio of the OCA1 and OCA2 genes is 1:1. In the albinism family III, there were identified to be two carriers and two affected individuals. In exon 4 of the TYR gene, the common mutation (homozygous) c.1255 G>A (p. Gly419Arg) was identified. Two affected people were found to possess the allele genotype AA, while two carriers were identified to carry the allele genotype GA, according to the mutation c.1255 (G>A (p. Gly419Arg). An albinism family VII with a three-generation lineage was identified. It was made up of two affected, one carrier, and two healthy people. According to mutation 1045 T>G, two affected people have the allele genotype GG, while the carrier has the allele genotype TG. There were no mutations found in any of the remaining 06 families.

OCA2 and TYR are the most often found OCA-causing genes in families of different ethnic backgrounds worldwide.[15] Tyrosinase, an enzyme produced by melanocytes and encoded by the TYR gene, produces the melanin pigment that gives eyes, skin, and hair colour. In the gene TYR, mutations with more than 600 variants have so far been identified.[16]

According to reports, the TYR is a pathogenic mutation in around 44% of the Pakistani population, compared to 46% in the European population, 70% in the Chinese population, and 60% in the Indian population.[17-19] The OCA phenotype is based on a higher prevalence of pathogenic TYR mutations in other ethnic populations such the Japanese, Italians, Americans, and Koreans.

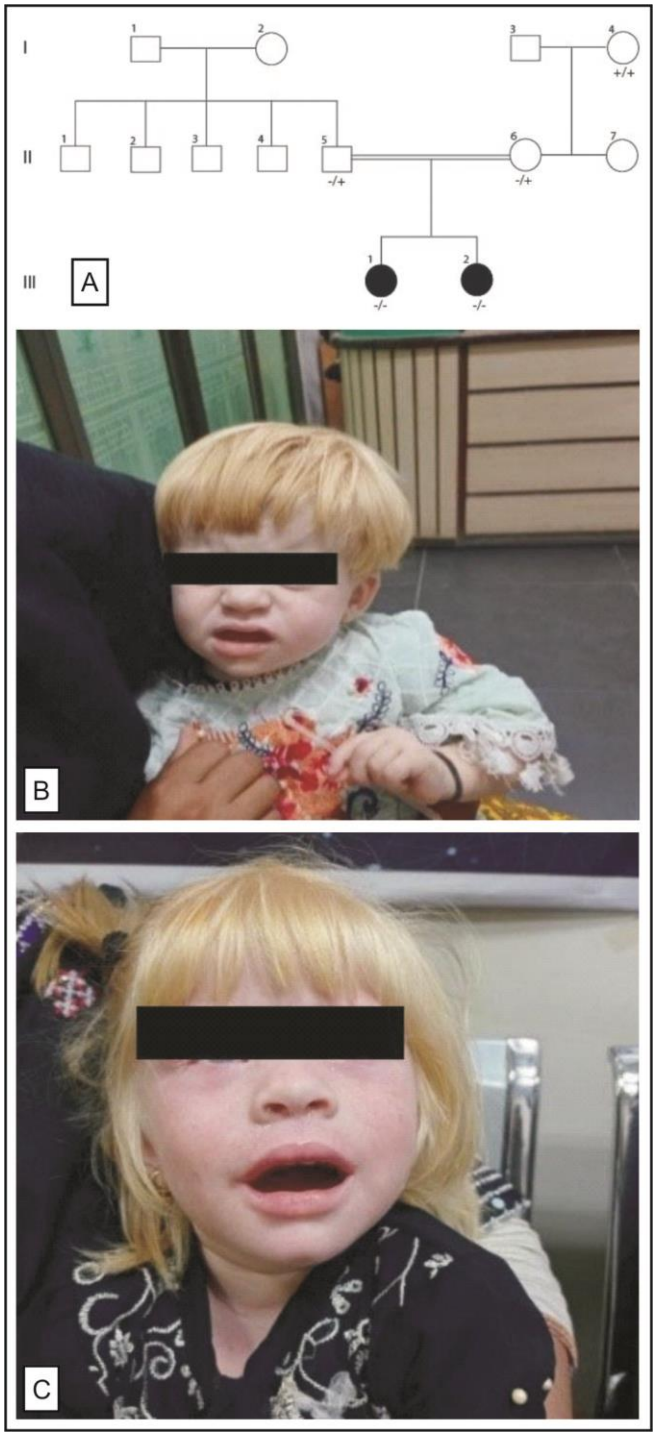

Figure 5(A): Pedigree of consanguineous family showing the affected individuals are Homozygous while their parents are heterozygous. **(B & C):** Affected individuals Oculocuteneous Albinism.

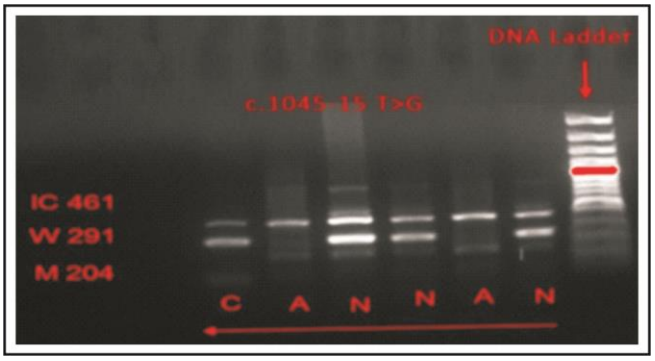

Figure 6: Gel Picture indicating the mutation in c1045-15 T>G.

In the TYR gene family III of our analysis, we found the known mutation c.1255 G>A (p.Gly419Arg) in exon 4. These general traits of the family were light brown hair, white complexion, and light grey iris, as well as photophobia, nystagmus, foveal hypoplasia, and albinism. It was identified that OCA families with more than 200 members currently exist in multiethnic groupings, which has been verified by other studies.[17]

Genetic diseases are rising in Pakistan due to high consanguinity. With a 23% frequency in OCA families from multiethnic communities including Balochi, Sindhi, Saraiki, Kashmiri, and predominately Punjabi ethnic community of Pakistan, it was identified that c.1255G>A (p.Gly419Arg) is the most prevalent pathogenic TYR variation.[20]

The pedigrees of the remaining families from the Sindhi ethnicity of the various groups did not indicate an OCA gene mutation in the current study. However, the traits of hair, skin, and eye albinism that were present in all the families may indicate that it will arise in the coming generation. These newly identified OCA gene mutation cases could aid in the development of novel molecular diagnostic tests for the Pakistani population as well as personalized healthcare and genetic counselling.

CONCLUSION

Genetic makeup of the Pakistani population is diverse, and the affected individuals have varying phenotypic and genetic presentations. This is due to the fact that

the population of Pakistan and those of Sindhi ancestry are heavily inbred, consanguineous, segregated, and afflicted by hereditary diseases. The discovery of new cases may provide important new sources for the genes producing new diseases and aid in the creation and adoption of efficient therapeutical strategies.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Amber Mehmood and Muhammad Idrees Rao at Molecular Biology & Genetics Department LUMHS, Jamshoro.

Funding: None to declare.

Patient's Consent: Researchers followed the guidelines set forth in the Declaration of Helsinki.

Conflict of Interest: Authors declared no conflict of interest.

Ethical Approval: The study was approved by the Institutional review board/Ethical review board **(LUMHS/REC/-158/2021).**

REFERENCES

1. **Yang Q, Yi S, Li M, Xie B, Luo J, Wang J, et al.** Genetic analyses of oculocutaneous albinism types 1 and 2 with four novel mutations. BMC Med Genet. 2019;**20(1):**106. Doi: 10.1186/s12881-019-0842-7.
2. **Butler MG, Hossain WA, Cowen N, Bhatnagar A.** Chromosomal Microarray Study in Prader-Willi Syndrome. Int J Mol Sci. 2023;**24(2):**1220. Doi: 10.3390/ijms24021220.
3. **Shah QA, Ahmad S, Hasnain MJ, Pervez MT.** Oculocutaneous Albinism-A review. Pure Applied Biol. 2022;**11(4):**911–918.
4. **Wright CY, Norval M, Hertle RW.** Oculocutaneous albinism in sub-Saharan Africa: adverse sun-associated health effects and photoprotection. Photochem Photobiol. 2015;**91(1):**27-32. Doi: 10.1111/php.12359.
5. **Gopalakrishnan S, Negiloni K, Suganthan RV, Velu S, Raman R.** Low-vision intervention for oculocutaneous albinism in a Tertiary Eye Care Hospital in India. Saudi J Ophthalmol. 2023;**37(1):**38-42.Doi: 10.4103/sjopt.sjopt_266_21.
6. **Jablonski NG.** Living color: The biological and social meaning of skin color. University of California Press; 2012. ISBN978-0-520-25153-3
7. **Karim S, Saharti S, Alganmi N, Mirza Z, Alfares A, Turkistany S, et al.** Two Novel Homozygous *HPS6* Mutations (Double Mutant) Identified by Whole-Exome Sequencing in a Saudi Consanguineous Family Suspected for Oculocutaneous Albinism. Life (Basel). 2021;**12(1):**14. Doi: 10.3390/life12010014.
8. **Mendiola AJP, LaSalle JM.** Epigenetics in Prader-Willi Syndrome. Front Genet. 2021;**12:**624581. Doi: 10.3389/fgene.2021.624581.
9. **Shakil M, Harlalka GV, Ali S, Lin S, D'Atri I, Hussain S, et al.** Tyrosinase (TYR) gene sequencing and literature review reveals recurrent mutations and multiple population founder gene mutations as causative of oculocutaneous albinism (OCA) in Pakistani families. Eye (Lond). 2019;**33(8):**1339-1346. Doi: 10.1038/s41433-019-0436-9.
10. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;**310(20):**2191-2194. Doi: 10.1001/jama.2013.281053.
11. **Grimberg J, Nawoschik S, Belluscio L, McKee R, Turck A, Eisenberg A.** A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. Nucleic Acids Res. 1989;**17(20):**8390. Doi: 10.1093/nar/17.20.8390.
12. **Arcot Sadagopan K, Teng CH, Hui G, Lin DL.** Ocular findings and a comparative study of hair, skin and iris color in Chinese patients with albinism. Ophthalmic Genet. 2023;**44(1):**54-69. Doi: 10.1080/13816810.2022.2135109.
13. **Zweig AS, Karolchik D, Kuhn RM, Haussler D, Kent WJ.** UCSC genome browser tutorial. Genomics. 2008;**92(2):**75-84. Doi: 10.1016/j.ygeno.2008.02.003.
14. **Lee YH, Wang MY, Yu XX, Unger RH.** Glucagon is the key factor in the development of diabetes. Diabetologia. 2016;**59(7):**1372-1375. Doi: 10.1007/s00125-016-3965-9.
15. **Okamura K, Hayashi M, Abe Y, Kono M, Nakajima K, Aoyama Y, et al.** NGS-based targeted resequencing identified rare subtypes of albinism: Providing accurate molecular diagnosis for Japanese patients with albinism. Pigment Cell Melanoma Res. 2019;**32(6):**848-853.Doi: 10.1111/pcmr.12800.
16. **Xu Y, Zhang XH, Pang YZ.** Association of Tyrosinase (TYR) and Tyrosinase-related Protein 1 (TYRP1) with Melanic Plumage Color in Korean Quails (Coturnix coturnix). Asian-Australas J Anim Sci. 2013;**26(11):**1518-1522. Doi: 10.5713/ajas.2013.13162.
17. **Ullah MI.** Clinical and Mutation Spectrum of Autosomal Recessive Non-Syndromic Oculocutaneous Albinism (nsOCA) in Pakistan: A Review. Genes (Basel). 2022;**13(6):**1072. Doi: 10.3390/genes13061072.

18. **Jaworek TJ, Kausar T, Bell SM, Tariq N, Maqsood MI, Sohail A, et al.** Molecular genetic studies and delineation of the oculocutaneous albinism phenotype in the Pakistani population. Orphanet J Rare Dis. 2012;**7:**44. Doi: 10.1186/1750-1172-7-44.
19. **Dumitrescu AV, Tran J, Pfeifer W, Bhattarai SV, Kemerley A, Dunn TV, et al.** Clinical albinism score, presence of nystagmus and optic nerves defects are correlated with visual outcome in patients with oculocutaneous albinism. Ophthalmic Genet. 2021;**42(5):**539-552. Doi:10.1080/13816810.2021.1933544.
20. **Jah Samdani A, Khan Khoso B.** A unique albino village of Bhatti Tribe in Rural Sindh, Pakistan, with Oculocutaneous Albinism manifestations: An epidemiological study. Iranian J Dermatol. 2009;**12(2):**42–46.

Authors Designation and Contribution

Jalpa Bai; Research Assistant: *Design, Literature search, Data analysis, Manuscript preparation.*

Hina Shaikh; Research Assistant: *Data analysis, Statistical analysis.*

Ghulam Mujtaba Sohu; Ophthalmologist: *Literature search.*

Ashok Kumar Narsani; Professor: *Manuscript editing, Manuscript review.*

Ali Muhammad Waryah; Professor: Concepts, Design, Manuscript review.