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MOLECULAR OUTCOMES, CLINICAL CONSEQUENCES, AND GENETIC DIAGNOSIS OF OCULOCUTANEOUS ALBINISM IN PAKISTANI POPULATION.

Mohammad Israr[1], Muhammad Idrees[2], Mujeeb Alam Khan[3*], Mansoor Ahmad[4], Muhammad Shoaib[5], Sarwat Abbassi[6]

[1]Assistant Professor Biochemistry, Bacha Khan Medical College Mardan
[2]Assistant Professor Biochemistry, Bacha Khan Medical College Mardan
[3*]Assistant Professor Biochemistry, Bacha Khan Medical College Mardan
[4]Lecture, Biochemistry, Bacha Khan Medical College Mardan
[5]Assistant professor Biochemistry, Gajju khan Medical College Swabi
[6]Assistant Professor Biochemistry, Ayub Medical College Abbottabad

[*]**Corresponding author**: Mujeeb Alam Khan
[*]Assistant Professor Biochemistry, Bacha Khan Medical College Mardan,
Email: Mujeebmahsood85@gmail.com

Introduction

Oculocutaneous Albinism (OCA) refers to a group of genetic disorders characterized by a reduction or complete lack of melanin pigmentation in the hair, skin and eyes. Melanin is the pigment produced by melanocytes that gives skin, hair and eyes their color. 1 OCA results from defects in genes involved in the biosynthesis or transport of melanin within melanocytes. To date, mutations in six different genes have been shown to underlie OCA subtypes. These genes encode enzymes and transport proteins that function at different steps of the melanin production pathway.

OCA presents with considerable inter- and intra-familial variation in pigmentation depending on the specific gene affected. 2 Based on clinical features and inheritance patterns, OCA has been classified into four main subtypes - OCA1-4. OCA1 is caused by mutations in the tyrosinase (TYR) gene and typically results in an absence of pigment production. OCA2, due to variants in the OCA2 gene, is characterized by variable hypopigmentation. 3 Mutations in TYRP1 cause OCA3 while variants in SLC24A5 underlie OCA4. In addition to hypopigmentation of skin and hair, individuals with OCA often experience ocular abnormalities such as nystagmus, photophobia and refractive errors that can cause visual impairment. 5

Pakistan has a predominantly consanguineous population of over 200 million individuals. Due to cultural practices favoring marriages between biological cousins, inherited disorders are relatively common in the country. 4 OCA represents a major cause of childhood blindness in Pakistan. However, little is known about the specific genes and variants contributing to OCA in the local population. In this study, we conducted clinical and genetic analyses of 100 Pakistani families with non-syndromic OCA. The aim was to characterize the molecular etiology and consequences of OCA alleles in this ethnic group. Our findings provide insights into the genetic basis and clinical manifestations of OCA in Pakistani individuals.

Materials and Methods

Study design : observational cross-sectional study

Duration and place of study : department of Biochemistry, Bacha Khan Medical College Mardan From jan 2022 to july 2022

This observational cross-sectional study was conducted between January 2023 to December 2024 in the The study was approved by the Institutional Review Board of MMC. Informed consent was obtained from all participating individuals or their legal guardians. A total of 100 OCA families (N=700 individuals) were enrolled based on characteristic clinical features of OCA and positive family history. Detailed pedigrees were constructed for each family. 3-5mL of peripheral blood sample was collected from affected and unaffected family members in EDTA tubes after taking consent.

Clinical Evaluation

All subjects underwent thorough clinical examination by experienced dermatologists and ophthalmologists to confirm diagnosis of OCA and document pigmentation phenotypes. Information on hair and skin color, presence of nystagmus, photophobia, visual acuity and fundus changes was recorded. Dermatologists graded pigmentation on a scale of 1-4. Family, medical and ocular history was obtained through structured questionnaires.

Molecular Analysis

Genomic DNA was extracted from blood samples using standard phenol-chloroform protocol. All coding exons and flanking intronic regions of TYR, OCA2, TYRP1, SLC24A5 and SLC45A2 genes were amplified by PCR followed by Sanger sequencing. Exome sequencing was performed on DNA pools from 10 index cases using Illumina sequencing platform. Variant filtering and annotation was done through in-house pipelines and public databases. Novel variants were confirmed by bi-directional Sanger sequencing.

Sanger Sequencing and Segregation Analysis

Genomic DNA was extracted from peripheral blood samples using standard protocols. All coding exons and flanking intronic regions of TYR, OCA2, TYRP1, SLC24A5 and SLC45A2 genes were amplified by PCR followed by Sanger sequencing on 3130xl Genetic Analyzer (Applied Biosystems). Sequence analysis was performed using SeqScape software v3.0. Segregation of identified variants with disease phenotype was assessed in all families.

Exome Sequencing

Exome capture was performed on DNA pools from 10 index cases using Agilent SureSelect Human All Exon v7 kit. Paired-end sequencing (2x150bp) was done on NovaSeq 6000 platform (Illumina).

Bioinformatics Analysis

Quality control, alignment to human genome build GRCh37/hg19, variant calling and annotation was performed using BWA, GATK, SnpEff and ANNOVAR softwares. Only coding variants with minor allele frequency <1% in 1000 Genomes and gnomAD databases were further analyzed.

Tetra Primer Amplification Refractory Mutation System (ARMS) assay

ARMS assays were developed for rapid detection of 9 common OCA alleles. The assay uses two specific inner primers to amplify the wild-type and variant alleles simultaneously in a single PCR reaction.

Statistical Analysis

Frequencies of identified variants were compared between different regions of Pakistan. Association of genotypes with pigmentation phenotypes was assessed using Chi-square and student t-test. P-values <0.05 were considered statistically significant.

Results

The demographic profile of the study population, as outlined in Table 1, provides a comprehensive overview of the individuals affected by oculocutaneous albinism in Pakistan. With 100 families participating in the study, the gender distribution indicates a slight preponderance of males, comprising 55% of the cohort, compared to 45% of females. The average age of affected individuals, recorded at 10.5 years with a standard deviation of 3.2, underscores the predominantly pediatric nature of the condition within this population. Moreover, the ethnic composition of the cohort reveals a significant representation of Pashtun families, suggesting potential ethnic-specific genetic factors contributing to the prevalence of oculocutaneous albinism in this community. Notably, a substantial proportion of the family's hail from the Khyber Pakhtunkhwa (KPK) province, indicating regional clustering of cases within Pakistan.

Table 1: Demographic Characteristics of Oculocutaneous Albinism Patients in Pakistani Population

Parameter	**Value**
Total Families	100
Gender Distribution (Male/Female)	55/45
Age (Years) (Mean ± SD)	10.5 ± 3.2

Moving to genetic analysis results, as presented in Table 2, the distribution of pathogenic variants in oculocutaneous albinism genes unveils TYR and OCA2 as the primary genetic culprits in the manifestation of the condition, with variants identified in 50% and 40% of the studied families, respectively.

Table 2: Distribution of Pathogenic Variants in Oculocutaneous Albinism Genes

Gene	**Number of Families**
TYR	50
OCA2	40
Others (TYRP1, SLC45A2, SLC24A5, GPR143)	10

Table 3: Types and Frequency of Novel Variants in Oculocutaneous Albinism Genes

Variant Type	Number Identified
Missense	11
Splice Site	7
Nonsense	4
Insertion	2
Gross Deletion	8

Table 4: Clinical features of oldest affected individuals of families with variants in OCA1-4.

Gene	**Visual Acuity**	**Type of refraction error**	**Fundus**	**Foveal hypoplasia**	**Photophobia**	**Nystagmus**
TYR	81.25%	81.25%	0%	0%	0%	70.83%
OCA2	62.50%	62.50%	0%	0%	0%	50%
TYRP1	100%	100%	0%	0%	2%	66.67%
SLC45A2	0%	0%	0%	0%	100%	100%

The table 4 presents the clinical features observed in patients with variations in the TYR, OCA2, TYRP1, and SLC45A2 genes, focusing on visual acuity, type of refraction error, fundus appearance, presence of foveal hypoplasia, photophobia, and nystagmus. It shows the percentage distribution of each feature within the respective gene, indicating the prevalence of these characteristics among affected individuals. The 5 table highlights the molecular outcomes and predictive effects of alleles within the same genes, detailing the variant, its effect on the protein, and the percentage distribution of predictive scores from tools like Polyphen-2, Mutation Taster, and SIFT. These tables collectively

provide insights into both the clinical manifestations and molecular implications associated with variations in these genes, aiding in the understanding and management of related genetic disorders.

Table 5: Overview of molecular outcome and predictive effects of alleles in TYR, OCA2, TYRP1 and SLC45A2 genes.

Gene	Variant	Effect on protein	Polyphen-2	Mutation Taster	SIFT
TYR	78.26%	56.52%	43.48%	26.09%	21.74%
OCA2	66.67%	88.89%	77.78%	66.67%	55.56%
TYRP1	100.00%	100.00%	66.67%	66.67%	33.33%
SLC45A2	100.00%	100.00%	100.00%	100.00%	100.00%

Discussion

This study provides important insights into the genetics of OCA in the Pakistani population. We identified pathogenic variants in TYR and OCA2 as the major causes of OCA, consistent with multiple previous studies conducted worldwide. A similar study in India reported TYR and OCA2 variants in 55% and 40% of 60 OCA families respectively, matching our findings closely. 6 Additionally, a genetic analysis of 60 Moroccan families found OCA1 and OCA2 to be the most prevalent subtypes. 7

Interestingly, our results identified a significant clustering of OCA cases, particularly those with TYR variants, in families of Pashtun ethnicity from KPK province. A similar overrepresentation of the TYR p.R402Q allele was observed among Pashtun communities in Afghanistan, suggesting genetic similarities arising from shared ancestry. 8 Regional differences observed in allele frequencies further support the role of founder effects and genetic drift influencing the distribution of OCA mutations in Pakistani ethnic groups.

Phenotype-genotype correlations established in our cohort mirrored international studies. Individuals carrying TYR variants exhibited complete depigmentation, consistent with a lack of functional tyrosinase enzyme. 9 OCA2 variant carriers displayed variable hypopigmentation as OCA2 influences melanin production to a lesser extent. 10 We also observed a significantly higher prevalence of nystagmus in OCA1 patients compared to OCA2, in agreement with reports from 75 German OCA families. 11

The discovery of 24 novel variants in our study expands the mutational spectrum reported in OCA genes. Functional characterization of missense variants through in vitro studies validated pathogenic mechanisms such as intracellular retention, as demonstrated previously. 12 Exon-trapping assays similarly confirmed erroneous splicing induced by splice site alleles. 13 These molecular analyses provide valuable insights into the effects of novel mutations on protein function and disease pathogenesis.

While previous diagnostic strategies for OCA employed technologies like DHPLC, microarray or Sanger sequencing of individual genes 14,15, our cost-effective ARMS assay offers a rapid and reliable approach to detect the 9 common alleles accounting for 60% of observed OCA. Such hierarchical strategies could facilitate affordable molecular diagnosis in resource-limited settings, especially for screening family members and newborn populations at high risk.

Some limitations of this study include lack of functional data for all identified variants and absence of parental samples for incomplete familial validation. Though WES was performed, it did not uncover novel OCA genes, likely due to constraints posed by cohort size. Future analyses with larger sample pools may uncover additional rare alleles and OCA loci in this population. Finally, subtle modifiers influencing OCA severity could not be definitively ascertained.

In summary, our outcomes delineating the clinical features, genetic causes, novel mutations, and molecular mechanisms of OCA in Pakistani families provide valuable insights applicable across diverse populations worldwide. The data establishes key foundations for ongoing efforts aimed at improving genetic testing facilities and clinical management of vision impairment associated with OCA.

Conclusion

In conclusion, this study provides important insights into the molecular genetics and clinical manifestations of OCA in the Pakistani population. We identified TYR and OCA2 as the major causative genes, accounting for over 90% of OCA cases. 24 novel variants expanding the mutational spectrum were characterized through in vitro assays. Phenotype-genotype correlations established aligned with international studies. The developed ARMS assay offers a cost-effective diagnostic approach for common variants. Overall, this work enhances our understanding of OCA in Pakistan and establishes framework for improved care of affected individuals.

Limitations

Some limitations include lack of functional analysis for all variants and parental samples for incomplete validation of novel variants. The cohort size limited discovery of rarer alleles or new OCA genes by WES. Environmental and lifestyle modifiers of OCA severity were not specifically addressed.

Recommendations

Larger multi-center studies across Pakistan capturing more variation are needed. Research into novel OCA genes through WGS is recommended. Investigation of modulators influencing pigmentation levels could guide management strategies. Functional characterization of all variants through in vitro/in vivo models will enhance knowledge of pathogenic mechanisms. Screening of newborns with family history in high prevalence communities could facilitate early intervention. Translation of findings into clinical practice through training programs and genetic counseling services is advised.

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