**Abstract**

**Purpose** To investigate eight previously unreported Pakistani families with genetically undefined OCA for mutations in *TYR*.

**Methods** Sanger sequencing of *TYR* has been performed in eight families with OCA phenotype. Mutation analysis was performed to establish the pathogenic role of novel mutation. Bioinformatics analysis was performed to predict the structural and functional impact on protein due to the mutation.

**Results** In this study, we identified six likely pathogenic variants of *TYR* (c.272G>A, c.308G>A, c.346C>T, c.715C>T, c.832C>T and c.1255G>A), including one novel variant (c.308G>A; p.Cys103Tyr), segregating as appropriate in each family. Cys103 lies in the highly conserved region of the tyrosinase enzyme, and p.Cys103Tyr is predicted to disturb enzymatic function via alteration of the configurational orientation of *TYR* leading to a more rigid polypeptide structure. We have also reviewed the mutation spectrum of TYR in Pakistani ethnicity. Published data on OCA families proposed that ~40% have been associated with genetic variations in the *TYR* gene. The mutations reported in this study have now been described with varying frequencies in Pakistani families, including very rare/unique mutations.

**Conclusion** A literature review of *TYR* gene mutations in Pakistani populations, combined with our genetic data, identified a number of gene mutations likely to represent regional ancestral founder mutations of relevance to Pakistani populations, in addition to sporadic and recurrent ‘hotspot’ mutations present repeatedly in other regions worldwide.

**Keywords**: Oculocutaneous albinism, *TYR*, tyrosinase, novel mutation, in silico analysis, Pakistan

**Introduction**

Non-syndromic oculocutaneous albinism (nsOCA) is an autosomal recessive disorder characterized by the partial or complete loss of pigmentation in the skin, hair, and ocular tissues that is due to a decrease or absence of melanin production [1]. Other clinically important features associated with OCA are foveal hypoplasia, misrouting of the optic nerves at the chiasm, photophobia, nystagmus, and vision impairment [2]. The prevalence of albinism worldwide has been estimated at 1 in 17,000, indicating that approximately 1 in 70 people are carriers of the OCA allele globally [3].

Non-syndromic OCA is further divided into 7 subtypes (OCA1-7) on the basis of genetic testing. Oculocutaneous albinism type 1 (OCA1) is the most common OCA variant affecting almost 50% of affected individuals worldwide [4, 5]. Clinically, OCA has a broad clinical presentation, with OCA1A being the most severe subtype with absolute lack of melanin biosynthesis throughout life resulting in completely white hair and skin. Other OCA subtypes (OCA1B, OCA2, OCA3, OCA4 and OCA7) display a degree of melanin pigmentation with advancing age, giving rise to a wide range of skin, hair and eyes colour although pigmentation remains typically less than unaffected individuals. Due to the indistinguishable clinical phenotypes and the involvement of different genes, molecular diagnosis has become an important tool to accurately identify the type and severity of OCA to aid genetic diagnosis, counselling and for considering therapeutic development [6, 7].

To date, the pathogenic variants in six genes, TYR, OCA2, TYRP1, SLC45A2, SLC24A5, and C10orf11, have been identified in individuals with nsOCA [8]. In a Pakistani family, possible locus for a form of nsOCA on chromosome 4q24 [9] has been previously identified, for which the gene remains undefined. In published population studies, however, the detection rate of alleles causing albinism varies from 60% to 90% [10, 11]. OCA type 1 is associated by mutation in the tyrosinase *TYR* (MIM# 606933) gene, which encompasses five coding exons and is located on chromosome 11q14.3. Tyrosinase enzyme has a pivotal role in the biosynthesis of melanin in melanocytes as it catalyses the first two reactions of the melanin synthesis pathway: formation of L-3,4-dihydroxyphenylalanine (L-DOPA) by hydroxylation of tyrosine and DOPAquinone production by the oxidation of L-DOPA. These two steps are crucial for melanin synthesis [12], and defects in tyrosinase catalytic activity due to *TYR* mutation may result into complete absence or decreased pigmentation of skin, eyes and hair, depending on residual enzyme activity [13]. OCA1A (MIM# 203100) is the most severe clinical phenotype characterised by an almost complete absence of skin, hair and iris pigmentation, and associated with pathogenic or null alleles in *TYR*. Less severe *TYR* mutations may manifest as OCA1B (MIM# 606952), which results in a milder, extremely variable phenotype due to decreased but not completely abrogated tyrosinase activity and low-moderate levels of melanin pigmentation in affected individuals [4, 14].

**Material and methods**

**Patients and family members**

This study entails the genetic investigation of eight Pakistani families recruited with informed consent with ethical approval (Ethical board of University of Health Sciences, Lahore). All families originate from the Punjab Province, with distinct ethnic backgrounds (1-Khokhar (Sahiwal), 2-Chadhar Jutt (Chiniot), 3-Arain (Gujranwala), 4-Malik Awan (Lahore), 5- Mughal (Lahore), 6-Gujjar (Lahore), 7-Saraki Somro (Rahim Yar khan), 8-Turk Pathan (RYK). The families diagnosed with OCA and having 2 or more affected members were included in this study, while the families with ocular albinism and syndromic OCA were excluded. Following diagnosis of a pro-band in each pedigree, further clinical details for each family were obtained by visiting ophthalmologists from local collaborating hospitals. Clinical images of the affected individuals were taken with consent in order to document phenotypic features and confirm disease status. Videos were also taken for further study. Ophthalmic examinations were completed using the best locally available resources including: visual acuity testing using LogMAR Visual Acuity Chart (LVRC) Numbers Distance, colour vision testing using Ishihara charts and funduscopic examination by direct ophthalmoscopy. Findings were recorded on the specified data forms.

**Molecular genetic analysis**

Peripheral venous blood samples were taken in EDTA containing vacutainer tubes from each participating individual for genomic DNA extraction as previously described [4]. One affected individual from each family was sequenced for all five coding exons and associated intron-exon junctions in the *TYR* gene. Sequence reads were aligned to the human genome reference sequence [hg19] to observe base pair changes using BioEdit software, CLC sequence viewer (<https://www.qiagenbioinformatics.com/products/clc-sequence-viewer/>) and Chromas Lite (<http://technelysium.com.au/wp/chromas/>) software. DNA samples from the families of those probands who showed putative mutations in one affected individual were sequenced in order to confirm segregation with the disease phenotype. The *in silico* pathogenicity prediction tools applied were; SIFT (<0.05), PolyPhen2 Hum Var (possibly damaging and probably damaging) and GERP++ (>2) [ref. 14]. Crystal structure of human tyrosinase related protein (PDB ID 5M8Q) [15] was used as a template for the construction of three-dimensional structure of wild and mutant tyrosinase protein. The models were visualized using UCSF-chimera (<https://www.cgl.ucsf.edu/chimera/>). RAMPAGE was used for evaluation and validation of the modelled 3-D structures [16].

**Results**

**Clinical findings**

Eight families with congenital nsOCA were enrolled from different cities within the Punjab province of Pakistan (family 1 from the Sahiwal, family 2 from Chiniot, family 3 from Gujranwala, families 5, 6, 7 from Lahore, families 7 and 8 from Rahim Yar Khan). The apparent mode of inheritance in all families was consistent with an autosomal recessive disorder and all affected individuals exhibited the cardinal clinical features of OCA with white to golden blonde hair, pale to reddish white skin, decreased visual acuity of variable extent, nystagmus, strabismus and photophobia. Ophthalmological examination of all affected individuals revealed the classical ophthalmic features of albinism namely; foveal hypoplasia, nystagmus, strabismus and a hypo pigmented fundus. The clinical findings are summarised in Table 1.

**Genetic findings**

Sequencing of the coding regions of *TYR* gene revealed a novel missense mutation chr11:88911429G>A [hg19]; c.308G>A; p.Cys103Tyr in the first coding exon of *TYR* in pedigree of family 1 of OCA (Fig 1a-b) which co-segregated appropriately on sequencing (Fig 1c). *In silico* analyses were undertaken using various pathogenicity prediction tools such as PolyPhen-2 and SIFT, indicating that this variant is likely deleterious (Table 2). Five other *TYR* missense variants were identified; chr11:89018011G>A [hg19]; NM\_000372.4: c.1255G>A; p.Gly419Arg in families 2, 3 and 4, chr11:88911393G>A [hg19]; NM\_000372.4: c.272G>A; p.Cys91Tyr in family 7, and c.715C>T; p.Arg239Trp in family 8, and two nonsense mutations were also identified; c.346C>T; p.Arg116Ter in family 5, and g.89191214;NM\_000372.4: c.832C>T; p.Arg278Ter in family 6 (Fig. 2a-g). The *TYR* variant, c.308G>A, identified in this study is not listed in homozygous form in online gnomAD genomic database (<http://gnomad.broadinstitute.org>), and the variant was also absent in age and sex matched 150 chromosomes of Pakistani ancestry.

**Comparative homology and protein homology analysis**

Clustal W alignment of tyrosinase proteins from various species showed the conservation of residues cysteine at positions 103 among eight species. The conserved amino acids are shown with a dark grey background, and the non-conserved amino acids are shown with a white background (Fig. 1d). In case of wild type structure, the Cys103 established the disulphide bond with nearby Cys122 (Fig. 3a-b). Due to the substitution of Cystine with tyrosine at position 122 loss the disulphide bond (Fig. 3c). This structural disruption might influence the function of protein and thus the reason to cause OCA1. The RAMPAGE server generated the Ramachandran plot for wild type displayed 90.3% of residues are in the most favoured region, while 8.6% of amino acids reside in the generously allowed region (Fig. 3d). According to the Ramachandran plot generated for structure with missense mutation 91.3% of residues are found in the most favoured region, while 7.4% of amino acids reside in the generously allowed region (Fig. 3e).

**Discussion**

Our study highlights the importance of the genetic burden of *TYR* gene mutation to the prevalence of albinism families from Pakistan. Pakistanis have a rich anthropogenic background owing to successive waves of invasions and emigrations, although most groups did not intermingle with the original local population and practiced endogamy, giving rise to genetic isolates that persist today. Parental consanguinity has been documented to lead to an increased incidence of recessive genetic disorders [17]. In Pakistan, 62.7% of marriages are consanguineous, ~80% of which are between first cousins [18], and marriage within clans and high consanguinity in Pakistan are a common cause of increased incidences of recessive disorders, including OCA.

Tyrosinase, a copper containing oxidase, is the rate-limiting enzyme in melanin biosynthesis pathway. It catalyses the first two reactions of melanin synthesis pathway; formation of DOPA, and then DOPA quinone subsequently. About one third of OCA cases in Pakistan are due to mutation in *TYR* [7]. In the present study, we identified one novel and five previously reported mutations in *TYR* associated with OCA in families from different regions of Pakistan. The novel *TYR* mutation (c.308G>A; p. Cys103Tyr), was identified in a family from the Sahiwal district (Punjab Province) of Pakistan. Affected individuals in this family presented with typical features including white hair (dyed black at the time of sampling), reddish white to white skin with sunburn scars on face and arms, nystagmus, severe photophobia (cannot go outside in day time), de-pigmented transparent grey irides with decreased visual acuity. The variant is not listed in online genome databases indicating that this is likely to be very rare in this population. *In silico* pathogenic tools establish the deleterious effect of this novel mutation. Amino acid sequence alignment using the program ClustalW 2.1 showed high conservation of the Cys103 residue in related vertebrates. The mutation p.Cys103Tyr lies in the highly conserved region of this enzyme tyrosinase, predicted to disturb the enzymatic function. Due to this mutation, the configurational orientation of TYR was reformed and lead to the rigid structure in nature.

This study also identified previously reported mutations in seven OCA families, which include, c.272G>A, c.346C>T, c.715C>T, c.832C>T and c.1255G>A. To date, published data describes >200 OCA families from Pakistani populations which have undergone genetic analysis. Out of these families, 81 families (~40%) have been associated with genetic variations in the *TYR* gene (Supplementary Table 1). The mutations reported in this study have now been described with varying frequencies in Pakistani families, including very rare/unique mutations (Supplementary Table 1); c.346C>T and c.715C>T [19] in just two families [20], as well as more common mutations; c.832C>T in seventeen families [4, 7, 20-22], and c.1255G>A in sixteen families [4, 7, 19, 22]. While it is not possible to conclusively determine whether common *TYR* mutations represent mutation hotspots vs founder gene mutations without more detailed genetic analyses, evidence to support both mechanisms is present in the literature. Several of the *TYR* mutations described in our study are commonly associated with OCA in Pakistan, and likely represent both regional founder as well as recurrent (hotspot) mutations. For example, the c.832C>T; p.Arg278Ter c.1255G>A; p.Gly419Arg variants in *TYR* identified in families 2,3,4 and 6, account for 21%, and 19.75%, of all families with known *TYR* variants in Pakistan respectively [4, 7]. While the frequency of the c.832C>T variant is higher in the Pakistani population, the mutation has also been identified in many other populations worldwide indicating that it has likely occurred recurrently, although an increased frequency of the variant in some areas may indicate it has also accumulated as a regional founder mutation (Guayanan 12.5%; Jewish 2.6%; Japanese 22.2%; European 2.5%; Mexican 0.83%; Indian 0.83% and 4.34%; Eastern Indian 8.3%; Syrian 0.83%; Chinese 18.75%) [ref.22]. Consistent with this, the variant is listed in online genome databases, occurring (in gnomAD) more frequently in the South Asian population (allele frequency= 0.0013) than in other regions (for example, allele frequencies in African and European populations are 0.0001249 and 0.00002372 respectively).

The current study identified the c.1255G>A variant as the most common allele of *TYR* present in the Punjabi ethnic group. Three families from the present study, and five families from previous studies with c.1255G>A, originate from the same geographical area (Punjab) and the same ethnic group (Punjabi language group). This variant has also been documented in other Pakistani ethnicities including Sindhi, Kashmiri and Balochi backgrounds, and together accounts for 19.75%, of all families from Pakistan known to have *TYR* mutation (Supplementary Table 1). The high frequency of the variant in the Pakistani population is a similar to the frequency in the Indian population (Indian 20%; South-Indian 16.6%), although notably this mutation occurs much less commonly in the white Caucasian population (0.83%; of families identified) [23]. Together this may indicate that the c.1255G>A variant may represent a founder mutation which has accumulated in the Indo-Pakistan subcontinent region, and consistent with this the variant occurs most commonly in South Asian populations in online databases (GnomAD South Asian population is 0.0003899, as compared to European frequency of 0.00003967). Consistent with this, the variant has not as yet been reported in other populations outside of these regions.

We also determined that the c.230G>A variant has been reported in Japanese, Korean, Chinese, and European populations [12, 23, 24-27]. While this may indicate that the variant has occurred recurrently, it has only been reported in communities in the Pakistani Kashmir region [19], and so while occurring recurrently may again represent a regional founder mutation of importance in this area of Pakistan. Similarly, c.62C>T; p.Pro21Leu, c.103 T>C; p.Cys35Arg, c.1231T>C; p.Tyr411His [24], c.240G>C; p.Trp80Cys [3], c.308G>A; p.Cys103Tyr (associated with severe photophobia, present study) and c.593T>C; p.Ile198Thr [20] have only been reported in specific Pakistani communities, and not outside of Pakistan. This likely indicates that each variant may also represent regional founder variants (Table 3). Conversely, variants c.346C>T; p.Arg116Ter [19], c.649C>T; p.Arg217Trp [7, 23], c.896G>A; p.Arg299His [7, 22), and splice site variant c.1037-7T>A [7, 19-22] have all been reported in different ethnic groups from Pakistan, as well as from other countries. These (and several other) variants may therefore represent *TYR* mutations recurring worldwide.

Overall, the prevalence of TYR alleles in Pakistan (37%) is similar to frequencies in families from Europe (46%), although largely different to studies in different populations. For instance, TYR and OCA2 variants account for 70% and 10 % of OCA in a study of 127 patients from a Chinese population, with notable regional variation [23]. In India, a study of 82 OCA patients revealed approximately 60% prevalence of TYR mutation [28]. Similarly, in the US, Europe, Italy, Japan, and Korea, the alleles of TYR are the most common cause of OCA [25]. In contrast, variants in OCA2 account for ~80% of the OCA cases in an African population [29, 30].

In conclusion, it is clear that the clinical presentation of OCA is caused by a wide variety of mutations in multiple genes. Thus for economic and geographical reasons, it is not feasible to routinely perform Sanger sequencing of all the known OCA genes to detect underlying genetic defects. Together our data and studies highlight the importance and expand current knowledge of the molecular spectrum and specific frequencies of *TYR* gene mutation in OCA in Pakistani communities, indicating that a number of TYR gene mutations likely involve founder gene mutations. Due to the frequency of mutations in the *TYR* gene which comprises only five coding exons, targeted sequencing of *TYR* may be appropriate (where broader sequencing panels are prohibitively expensive) , to provide valuable information to aid the diagnosis and counselling of affected individuals and family members throughout Pakistan.

**Ethics approval and consent to participate**

This study was approved by the ethical approval committee institutional review board of the University of Health Sciences, Lahore, Pakistan.

**Consent for publication**

Written consent was obtained from all patients or their relatives for publication.

**Availability of data and materials**

Data supporting the conclusions of this article are included within the article.

**Author contributions**

MS, SA, MIU, SH provided samples and clinical details. GVH, MS, ID, JES, AN and MAS performed genetic studies, and analysed data alongside SL, and ID. SM, JES, AHC, ELB and MIU designed and conceived studies. SL aided compilation and analysis of clinical information, and edited the manuscript with AHC, ELB, JES and SM.

**Acknowledgments**

We are thankful to the administration of LRBT Hospital, Lahore for giving permission to access the data of patients with albinism. We are also grateful to the Wellcome Trust (209083/Z/17/Z) and the Warman Foundation and Higher Education Commission of Pakistan for funding this research project (21-1340/SRGP/R&D/HEC/2016) and also supporting IRSIP fellowship for MS.

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**Figure Legends**

**Fig. 1: a**. Family pedigree showing *TYR* c.308G>A variant and **b**. image of affected individuals. **c**. co-segregation analysis of this family establish the *TYR* variant chr11:88911429G>A [hg19]; c.308G>A; p.Cys103Tyr. Parental samples were heterozygous, and unaffected siblings were either WT or heterozygous carriers and **d**. amino acid alignment using ClustalW showing high conservation of the Cys103 residue across vertebrates.

**Fig. 2:** Pedigrees of 7 families with oculocutaneous albinism co-segregating for *TYR* (MIM# 606933) mutations. Presence or absence of the variant is indicated by a + or − sign respectively. **a-c.** Pedigree representation of three families (family 2-4) with c.1255G>A mutation, **d.** pedigree of family 5 with c.346C>T variant, **e.** family 6 with c.832C>T mutation, **f.** family 7 with c. 272G>A and **g.** family 8 with c. 715C>T mutation.

**Fig. 3: a**. Three dimensional structure of tyrosinase showing the position of residue Cys103 through stick model, **b**. Close up view of normal, and **c**. mutant type tyrosinase, **d.** the Ramachandran plot for the modelled wild type AAAS protein presenting 90.3% of residues are found in the most favoured regions, 8.6% of residues are in the most allowed regions, and 1.1% of residues are found the outlier regions, **e.** the Ramachandran plot for the modelled mutant variant c.308G>A, 91.3% of residues are found in the most favoured regions, 7.4% of residues are in the most allowed regions, and 1.3% of residues are found in the outlier regions.

**List of Tables**

**Table 1**. Clinical features observed in OCA families.

**Table 2**. Novel and reported *TYR* (NM\_000372.4) variants identified in OCA families of this study.

**Supplementary Table 1**. Frequencies, ethnicity and founder *TYR* mutations reported in Pakistan.