·Letter to the Editor·

Homozygosity mapping of a consanguineous Pakistani family affected with oculocutaneous albinism to *Tyrosinase* gene

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Dear Sir,

I am Haiba Kaul, from the Department of Biochemistry, University of Health Sciences, Lahore, Pakistan. I write to present a study of oculocutaneous albinism (OCA) in consanguineous Pakistani families.

OCA is a genetic defect of melanin biosynthesis that mainly affects eyes, skin and hair. It is a congenital condition and the affected individuals have reduced or completely absent melanin pigment in their eyes, skin and hair. Clinical manifestations of the disease included visual problems that are atypical expansion of retina and unusual prototypes of nerve relations established in eye and brain that might lead to visualization issues [1]. Other features include heritable nystagmus, decreased pigmentation of iris (iris luminousness), diminished pigmentation of the retinal epithelium, foveal hypoplasia, and compressed visual acuity[2].

In OCA, diverse genetic heterogeneity has been documented and seven loci have been associated with the disease (OCA1-7). Among these loci, four genes: tyrosinase (*TYR*), pink eyed dilution for P-protein (P), tyrosinase-related protein (*TYRP1*), and solute carrier 45 subunit A2 (*SLC45A2*) are well documented to cause different types of OCA type (OCA1-4) respectively. Mutations in two novel genes *SLC24A5*[3] and *C10orf11*[4] are recently discovered resulting into OCA-6 and OCA-7 respectively. OCA-5 locus has been discovered but its gene is yet to be identified[5]. Of the various types of OCA, OCA1 (MIM 203100) results due to mutations in *TYR* (MIM 606933) which resides on chromosome 11q14.3 [6]. Mutations that resulted in complete lack of *TYR* activity are known as OCA1A, whereas mutations retaining some enzyme activity outcome in another type of albinism called OCA1B.

Due to the strong consanguinity culture in Pakistan, diseases segregating in recessive mode are quite common and thus the incidence of albinism is much greater in our population as compared to the non-consanguineous populations. Till today, very few studies are being conducted to explore OCA genes harboring in Pakistani families. In a family study, mutations were reported in *TYR* alleles and in *TYRP1* genes in Pakistani patients [7]. Recently, a study reported novel locus, OCA5, in a consanguineous Pakistani family[6].

This study was undertaken with the aim to decipher the genetic basis of OCA in consanguineous Pakistani families using linkage analysis approach. Prior to the start of this study, ethical approval was taken from institutional review board (IRB) of the University of Health Sciences, Lahore, Pakistan. We enrolled ten families affected with OCA which belonged to the Punjabi ethnic group with at least two affected in each kindred. Affected members of the enrolled families were physically and clinically examined at the Layton Rehmatulla Benevolent Hospital, Lahore, Pakistan. Blood samples in EDTA containing vacutainers were collected from the affected and unaffected members of the enrolled families. Genomic DNA was extracted from all the samples collected using a modified phenol chloroform method as described by Kaul *et al*[8]. Genotyping was carried out by using microsatellite markers for four known OCA loci (OCA1-OCA4). These include (*TYR*, *OCA2*, *TYRP1* and *SLC45A2*). Highly polymorphic short tandem repeat (STR) markers were selected from Marshfield maps (http://www.marshfieldclinic.org/research/pages/index.aspx) and the National Center for Biotechnology Information (http://www.

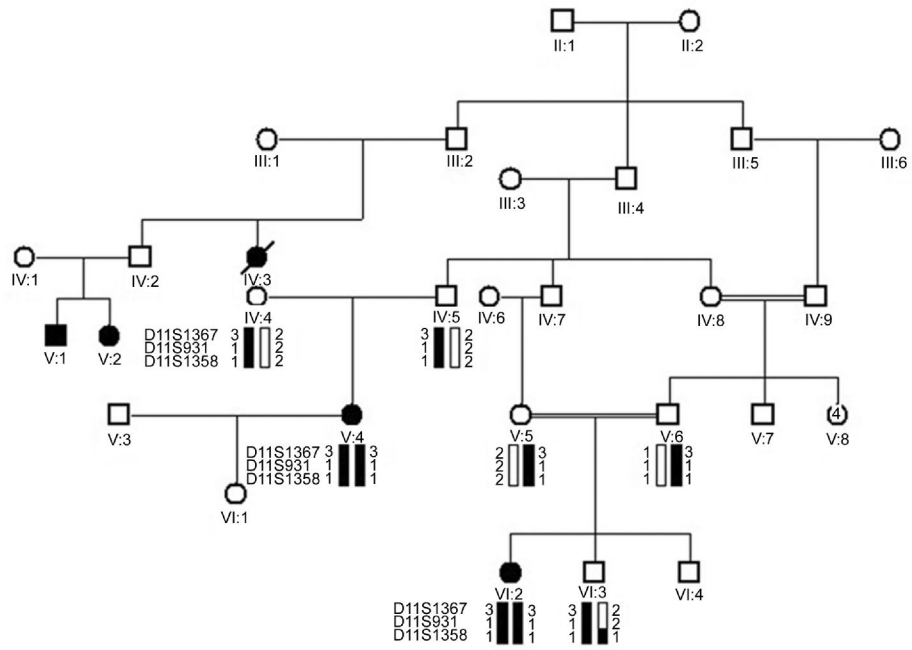

Figure 1 Haplotype of AL03 for markers (D11S1367, D11S931 and D11S1358) spanning *TYR* gene Squares: Males; Circles: Females; Filled squares and circles: Affected individuals; Blank squares and circles: Unaffected individuals; Single lines: Non-cousin marriage; Double lines: Consanguinity.

ncbi.nlm.nih.gov/). These primers were commercially synthesized with forward primers labeled with fluorescent FAM dye. Polymerase chain reaction (PCR) and linkage protocols were used according to previous standards [9]. Analysis of specific genotypes were assigned using Peak Scanner™ Software v1.0 software (Applied Biosystems). Peak scanner sizes different nucleic acid fragments that identify peaks and fragment sizes for application specific capillary electrophoresis assays. This data was used to construct haplotypes of the families using Cyrillic® software. Statistical scoring using, logarithm of odds (LOD) score was calculated to evaluate the linkage of respective OCA locus. Two-point linkage analysis was performed using the FASTLINK version of MLINK from the LINKAGE Program Package[10]. An autosomal recessive mode of inheritance with complete penetrance and a disease allele frequency of 0.001 were used for the analysis.

Out of ten families selected for this study, one family AL03 was mapped to *TYR* gene on chromosome 11q14.3. AL03 belongs to a remote village of Punjab province of Pakistan. The family belongs to the Mughal caste and seldom marries out of the family and thus is highly consanguineous. The pedigree was drawn up to six generations with five affected (one deceased) individuals segregating disease in an autosomal recessive manner (Figure 1). The affected individuals (V-4, VI-2) were examined by medical physicians at the local hospital. These individuals showed phenotypes of white hair, nystagmus and decreased visual acuity but are able to perform daily work and study with the use of visual aid devices. Physical examination and clinical investigations established OCA phenotype.

Haplotype analysis revealed that in family AL03 both the affected individuals V-4 and VI-2 were homozygous for the alleles of three markers D11S1367, D11S931 and D11S1358 (Figure 1). The gene *TYR* resides between markers D11S1367, D11S931 and D11S1358. Two-point linkage analysis was performed using the FASTLINK version of MLINK. The highest LOD score of 1.80 (θ=0.00) was obtained with D11S1367 for AL03. The results of linkage evidently showed that there is association of *TYR* gene in this family.

TYR gene encodes *TYR*, copper containing enzyme, which catalyzes conversion of tyrosine to melanin and thus is important for melanin biogenesis [7]. Mutations of *TYR* gene have been documented in various populations worldwide[11]. However, molecular analysis of OCA in Pakistani families has not been carried out on large scale. There are few reports regarding mapping of OCA genes in Pakistani families [12]. Apart from the linkage of one OCA family, nine other families were failed to link to any of four genes: *TYR*, pink eyed *P*, *TYRP1*, and *SLC45A2*. This depicts that a high genetic heterogeneity is present in our population for OCA. We can therefore conclude that the remaining families might harbor genetic defect underlying in other 3 gene/loci. Conversely, there is a high probability that a new gene that still remains to be identified in OCA pathology might be responsible for the disease.

In conclusion, this study reports a family designated as AL03 with two members affected with OCA linked to *TYR* gene. None of other nine families screened were found linked to the genes screened for OCA.

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Conflicts of Interest: Shakil M, None; **Ullah MI,** None; **Hussain S,** None; **Firasat S,** None; **Mahmood S,** None; **Kaul H,** None.

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