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Communication

Analysis of circadian clock gene *BMAL1* in Pakistani congenital cataract families

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Abstract: In mice, mutations or targeted disruptions of the core circadian gene *Bmal1* have been implicated in early onset of ocular pathologies, including premature/congenital cataract development. The aim of the present study was to analyze probands of consanguineous Pakistani cataract families to identify the novel pathogenic variants in the *BMAL1* gene. We have studied 21 congenital cataract families. Ophthalmic examination was performed for the probands and available family members. Genomic DNA was isolated from peripheral blood. PCR and Sanger sequencing was performed for the entire coding region of the *BMAL1* gene. Targeted Sanger sequencing of *BMAL1* revealed a heterozygous variant c.41A>T; p.(Asp14Val) in one proband, but it did not co-segregate with the disease phenotype in the family. In addition, a nonsynonymous variant (rs2290037) was identified in five probands. Our study is the first one to analyze the role of *BMAL1* gene mutations in humans for their association with congenital cataract. Although we were unable to find the variants associated with congenital cataract families from Pakistan, more studies from other populations will be informative to further prove the role of *BMAL1* with the disease.

Keywords: Congenital cataract, consanguineous, circadian, mutation

1. Introduction

Cataract is defined as an opacity of the crystalline lens resulting in refractive index variation frequently caused by an abnormal intra-cellular aggregation of high-molecular-weight proteins. Cataract impairs visual acuity and circadian photoreception via the photosensitive retinal ganglion cells[1].

The World Health Organization (WHO) describes cataract as the major cause of blindness throughout the world, affecting 16 million people worldwide, particularly in developing countries[2]. The prevalence of congenital cataract (CC) is estimated to be 1–6 cases per 10,000 live births in developed countries, and 5–15 cases per 10,000 in the underdeveloped countries[3]. Inherited cataracts represent a major contribution to congenital cataracts[4-6]. Worldwide estimates

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show that approximately 200,000 children every year are affected by lifelong vision impairment due to cataract[7]. There are significant costs associated with the diagnosis and management of these children, as well as with their long-term rehabilitation, visual assistance, and lost productivity[4,5]. CC is particularly serious because it has the potential to result in permanent blindness at a very young age.

Circadian rhythms are biological processes widely distributed in mammalian tissues synchronized by a master-hypothalamic clock[8]. In mammals, the core clock genes, including *Bmal1*, *Clock*, *Cry*, and *Per*, are rhythmically expressed in the suprachiasmatic nucleus (SCN) - the master clock in the hypothalamus and also in almost all peripheral tissues, including the eyes[8]. An endogenous clock present in the mammalian retina[9,10], regardless of the absence of the master clock[11,12], is capable of maintaining autonomous rhythmic behavior. The clock genes control the expression of numerous target genes in a circadian manner, influencing many physiological and biochemical processes[13] such as *Bmal1* has been observed to be involved in the pathophysiology of cataract in mice[14-16]. In blind individuals, along with the loss of vision, the biological clock might no longer be fully synchronized, or entrained, by the light/dark cycle[17]. Extreme circadian desynchrony has been observed with daytime drowsiness due to elevated daytime melatonin levels and night-time insomnia due to circadian alerting[17,18]. Decreased potential for circadian photoentrainment is known to be associated with cataract development in humans[19].

Currently, over 48 genes have been recognized underlying the pathogenesis of congenital cataract. Mutations in the crystalline genes account for 50 % of the disease[20-22]. Almost 25% is caused by pathogenic variants in the connexin genes[23-25]. The remaining causative gene mutations are described in glucosaminyl (N-acetyl) transferase 2 (*GCNT2*)[26], beaded filament structural protein 2 (*BFSP2*)[27], aquaporin (*MIP*) [28,29], paired-like homeodomain 3 (*PITX3*) [30], avian musculoaponeurotic fibrosarcoma (*MAF*)[31], heat shock transcription factor 4 gene (*HSF4*)[32], lens intrinsic membrane protein (*LIM2*)[26,33], as well as many others, as delineated in the Cat-Map database (https://cat-map.wustl.edu/).

Previously, it has been reported that genes implicated in cataract development in humans, are also the key players in animal models like rodents and vice versa[34,35]. Recently, a core circadian clock protein BMAL1 has been implicated in the regulation of ocular aging[14-16]. More than 50 % of Bmal1 deficient mice, >50% developed before the 40th week of life[14]. Also, deletion of BMAL1 disrupts clock-dependent oscillatory gene expression and behavioral rhythmicity coincident with eye pathologies, reduced body weight, impaired hair growth, abnormal bone calcification, neurodegeneration, and a shortened life span[36-39].

Evidence for causative effects of circadian clock disruption on aging, cancer and other phenotypes has also been provided by many other studies[40-42].

In the current study we aimed to identify the novel variants in the *BMAL1* gene associated the CC phenotype in the consanguineous Pakistani families.

2. Materials and Methods

Subjects

The patients were recruited at the pediatric ophthalmology department of Al-Shifa Eye Trust Hospital, Rawalpindi, Pakistan. The study was approved by the Institutional Review Board of the Al-Shifa Eye Trust Hospital (Rawalpindi, Pakistan), and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained for study participation from the participants and/or their parents, as appropriate. Comprehensive, ocular, medical, and family histories were obtained from the parents/available family member. Detailed ophthalmic examination was

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performed for both affected and unaffected individuals of families. Blood samples were collected from affected and unaffected siblings, and from the parents. Genomic DNA was extracted using QIAGEN DNA Blood Midi Kit (QIAGEN, Germantown, Maryland, USA).

PCR and Sanger Sequencing

Genomic DNA of the probands (n=21) of the consanguineous cataract families were analysed using PCR. PCR products were analyzed on 2% agarose gels followed by Sanger sequencing using ABI BigDye chemistry (Applied Biosystems Inc., Foster City, CA, USA), and were processed through an automated ABI 3730 Sequencer (Applied Biosystems, Inc.) using standard protocols. Primers for the *BMAL1* gene (NM_001351814.1) were designed using Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/) to cover exon/intron boundaries up to 100 base pairs into introns. Primer sequences and polymerase chain reaction (PCR) conditions are available on request.

Data processing

The obtained sequences were aligned with the reference sequence (NM_001351814.1) using CodonCode Aligner (version 6.1) (CodonCode Co., Centerville, MA, USA). Intra-familial segregation analysis was also performed upon the identification of variant in the exon 5 of the *BMAL1* gene in the respective family.

Pathogenicity of the identified variants was evaluated by publicly available tools including PhyloP, Grantham and polymorphism phenotyping v-2 (PolyPhen-2) (version 2.1.0 r367) (http://genetics.bwh.harvard.edu/pph2/) MutationTaster (http://www.mutationtaster.org/), and sorting intolerant from tolerant (SIFT, http://sift.bii.a-star.edu.sg/) to predict the functional impact of the sequence variants on the encoded protein. To determine the amino acid conservation among different species, protein sequences were obtained from using UniProt (https://www.uniprot.org/uniprot/O00327) database. Kalign (2.0) was used for the multiple nucleotide and amino acid sequence alignment.

3. Results and Discussion

Mutation Identification

Sanger sequencing of n=21 probands of CC families revealed a heterozygous missense variant c.41A>T; p.(Asp14Val) in the *BMAL1* exon 5 in one proband (Figure 1B). This particular variant c.41A>T; p.(Asp14Val) was predicted to be deleterious by SIFT, damaging by PolyPhen-2 and disease causing by Mutation Taster. The wildtype nucleotide and amino acid residues were highly conserved with a phyloP score 4.73, and grantham score 152 respectively. The nucleotide and amino acid residues were found to be highly conserved among different orthologous species (Figure 1C and 1D). The p.Asp14Val variant was present with allele frequency of 0.000065 (2/30576 individuals) exclusively in the South-Asian population (http://gnomad.broadinstitute.org). The segregation analysis was performed but the variant did not segregate with the disease phenotype (Figure 1A).

In addition, we detected a nonsynonymous variant (rs2290037) in intron 7 in n = 5 probands with a higher allele frequency 0.06626 (15570/234984 individuals).

The current study was performed to evaluate the role of *BMAL1* variants in the pathogenesis of CC. The identified heterozygous variant c.41A>T; p.(Asp14Val) did not segregate with the disease phenotype. Our study is the first one to evaluate the role of a circadian disease gene, in particular *BMAL1*, for its association with CC in humans. Previously, homozygous b*mal1*$^{-/-}$ mice were observed with the cataract phenotype. Although we were unable to find any association with

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BMAL1, yet, it is known that the BMAL1 gene plays an important role in normal lens physiology[16].

Figure 1

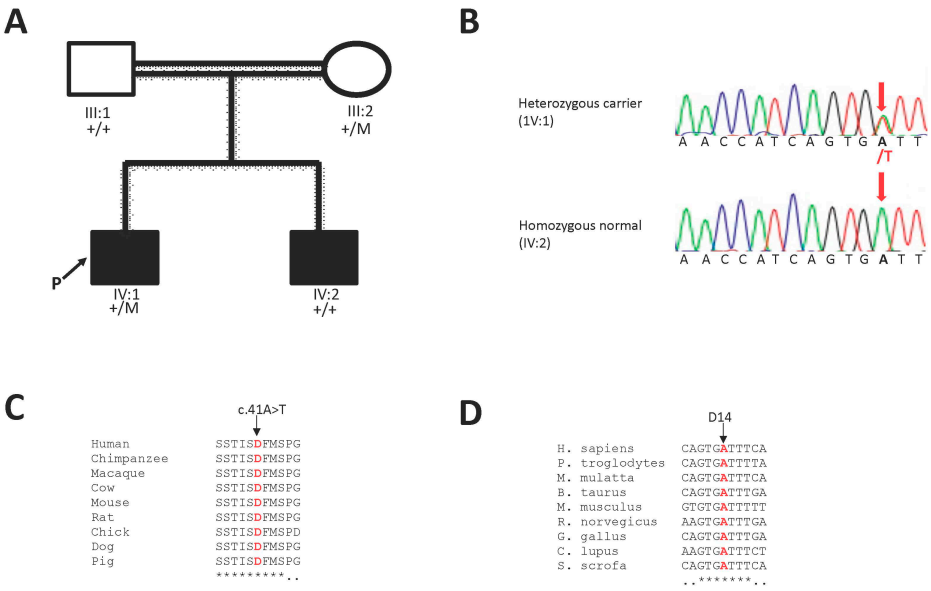

Figure 1. (A) Pedigree and segregation of a novel missense mutation c.41A>T; p.(Asp14Val) in the *BMAL1* gene in a recessive congenital cataract family.(B). DNA chromatogram of the *BMAL1* fragment for the affected individual (IV:1) carrying heterozygous genetic mutation. (C). Multiple sequence alignment of c.41A to show the nucleotide conservation among different species. (D). Multiple sequence alignment of the region of the BMAL1 protein surrounding the novel Asp14Val mutation in various species. The aspartic acid residue (indicated with an arrow) is highly conserved among all species analyzed.

Circadian clock genes are known to influence disease susceptibility due to their pleiotropic activities on gene expression or by involvement in multiple pathways or via their direct involvement with circadian clock function[42].

Premature aging phenotypes described in the control of mutation rate, regulation of reactive oxygen species (ROS) homeostasis, apoptosis, stress responses, and the insulin/IGF pathway, these are also controlled directly by the core clock components[43-51]. Our hypothesis raises an intriguing question about the inter-relationship between the circadian system and premature ocular aging in humans. However, our results could not reveal any strong links between *BMAL1* gene and the development of CC in humans.

Since our cohort of patients was relatively small, we cannot fully exclude the involvement of BMAL1 in human aging and/or cataract. There is also a remote possibility that the observed phenomenon is specific for mice only. Therefore, we recommend to perform mutation analysis in circadian clock genes in cataract in addition.

Author Contributions: U.B and A.A.B.B. conceived and designed the experiments; U.B. performed the experiments; S.M, S.N.S., M.I.K. recruited patients and collected samples; S.M, M.P.F.S. and A.A.B.B. contributed reagents/materials/analysis tools; and U.B. wrote the manuscript. All authors have read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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