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Original Article

Evaluation of crystallins as a candidate gene for cataract

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ABSTRACT

Congenital Cataract is the leading cause of visual impairment worldwide, affecting ocular lens. Of the cataract mutations reported to date, about half the mutations occur in crystallins. The present study was carried out to investigate whether the variations in the human crystallins are related to congenital cataract in the Kashmiri population. Blood samples were collected and genomic DNA was extracted. Genotyping of SNPs in coding regions of the CRYAA, CRYAB, CRYBA1/A3, and CRYGC were performed by PCR and direct sequencing. No pathological mutation was detected upon sequencing of the coding regions in subjects with congenital cataract. This study found no evidence that *crystallin gene is* responsible for congenital cataract in these patients.

Keywords: Congenital cataract, Mutation, Sequencing.

INTRODUCTION

Cataract is an opacification of the lens resulting from alterations in lens cellular architecture or in lens proteins, or both. Congenital cataract is a clinically and genetically heterogeneous lens disease responsible for a significant proportion of visual impairment and blindness in childhood [1,2]. The commonness of congenital cataract is evaluated to shift from 0.6 to 6 per 10,000 live births with an occurrence of 2.2–2.49 per 10,000 live births [3]. It is assessed that globally, 20 million children under the age of 16 years suffer from cataract, and among these, 200,000 (15%) are severely visually impaired or blind [4,5]. Pediatric cataracts are responsible for 7.4% to 15.3% of childhood blindness in developing countries like India [6-8].

Crystallin genes represent about 90% of the water soluble proteins in lens and the encoded proteins represent around 30% of lens mass, these proteins assume important roles in maintaining the lens transparency [9] and are good candidate genes for screening in congenital cataract patients. These proteins contribute to the transparent, refractive properties of the lens and are encoded by several different families of genes [*10*, *11*]. The α-, β- and γ-crystallins are most common in the lenses of major vertebrates, and exhibit a spatial and temporal expression pattern characteristic for each class during lens development [*12*].

Congenital cataract is the most important treatable cause of pediatric blindness in developing countries like India. On the basis of recent studies, mutations in about half of affected families occur in crystallins, a quarter in connexins, and the remainder is evenly split between membrane proteins, intermediate filament proteins, and transcription factors. However, the relative contribution of these classes of genes to congenital cataracts in India is still unclear. To clarify the role of crystallin gene mutations in relation to congenital cataracts in the Kashmiri population, we analyzed crystallins in 185 congenital cataract subjects. No mutation was identified in crystallins. The results indicate that crystallin genes are unlikely to be responsible for congenital cataract in these patients.

METHODS

Subjects: We included in the study a total of 300 individuals, 185 with congenital cataract and 115 with ethnically and age-matched normal individuals without any history of ocular or systemic disorders were enrolled as controls. Clinically diagnosed consecutive congenital

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cataract from Kashmir, were recruited from local hospital (Ophthalmology unit) as well from our ophthalmologist's clinic. These congenital cataract cases had no other ocular or systemic abnormalities. Detailed history was taken from parents regarding high fever, TORCHES ([*Toxoplasma gondii*; *T. gondii*], rubella virus [RV], cytomegalovirus [CMV], herpes simplex virus [HSV], syphilis [caused by *Treponema pallidum*]) infection, tuberculosis, exposure to radiation, and drug intake during gestation period. Metabolic tests like serum biochemistry for levels of blood glucose, calcium and phosphorous evaluations, RBC transferase and galactokinase levels and urine test for reducing sugars (galactosemia) and for amino acids (Lowe syndrome) were also done. Cases with known cause of congenital cataract were excluded from the study. Affected status was determined by a history of cataract extraction or ophthalmologic examination. They had no metabolic, genetic, or ocular disorder on examination by a ophthalmologist and an extensive history was taken regarding family, occupation of parents, any medical problem, and drug intake by parents. Informed consent in accordance with the Declaration of Helsinki was obtained from all participants or their parents and controls.

Mutation Detection: Genomic DNA was extracted from whole blood samples using standard protocols. Primer pairs for individual exons were designed using the primer3 program. The primer sequences used to amplify the coding exons of crystallins are shown in Table 1. PCR reactions were carried out in a total volume of 50 µl containing 50-100 ng of genomic DNA, 2-6 pmole of each primer, 1x PCR buffer (Sigma Aldrich) and 0.5 units of Taq DNA polymerase (Sigma Aldrich). Cycling conditions for PCR were as follows: one cycle of denaturation at 95 °C for 5 min, 30 cycles of denaturation at 95 °C for 45 s, annealing at 57°C for 45 s, and extension at 72°C for 45 s, and one final 6 min elongation cycle at 72 °C. PCR products were analyzed on 1.5% agarose gel and purified using purification kit or NaI. Samples that were amplified were sent for sequencing to confirm the presence of sequence variations. Sequence results obtained in fasta and pdf formats were analysed using ClustalX version 2 software (Thompson JD *et al*, 1997 & Larkin MA *et al*, 2007) and by Chromas Pro version 1.49 beta 2 software for the detailed inspection of individual chromatograms.

RESULTS

A total of 185 diagonsed with cataract and 115 control subjects participated in this study. The subjects described in this study, is from the Kashmir province of India, and a detailed medical and family history was obtained from all affected and unaffected members. According to the medical family records cataracts in all affected individuals developed in the early years of their lives. Clinical examination conducted with slitlamp microscopy mostly revealed congenital developmental lamellar cataract in affected individuals (Figure 1). No other ocular or systemic abnormalities were present in the family. Since, crystallin proteins have been associated with cataracts, we sequenced the exons of crystallins. However, mutation screening of coding regions of the CRYAA, CRYAB, CRYBA1, CRYBA3, CRYGC **did** not reveal any mutation.

DISCUSSION

The transparency and high refractive index of the lens are achieved by the precise architecture of the fiber cells and the homeostasis of the lens proteins in terms of their concentration, stability, and supramolecular organization [13]. Different crystallin genes have been recognized as the main candidates for certain hereditary forms of lens opacity in humans [14]. Mutations of crystallin genes represent 45% of the reported familial cataract mutations to date. We screened coding regions of crystallin genes by direct sequencing and could not detected any mutations.

In the present study, conducted for the first time in Kashmiri ethnic population, we did not detect any mutation in the in the Kashmiri families with congenital cataracts. The results suggest that *Crystallins* is unlikely to be the responsible gene for congenital cataracts in these patients.

CONCLUSION

This is a novel study carried out for the first time in Kashmir Valley, for screening mutation in crystallin genes. The sequence analysis of *crystallins didn't reveal* any variation, indicating that crystallin genes are excluded as a candidate gene responsible for congenital cataract in these patients.

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Conflict of Interest: The authors declare that they have no competing interests.

Author's Contributions:

Tabassum Rashid: Sample Collection & Processing, Performed all the lab work.

Aeijaz Ul Noor: Data Analysis & Preparation of Manuscript.

Khurshid I Andrabi: Designed the work, edited the manuscript, co-ordinated the group and overall invigilator of the study.

Figure 1:

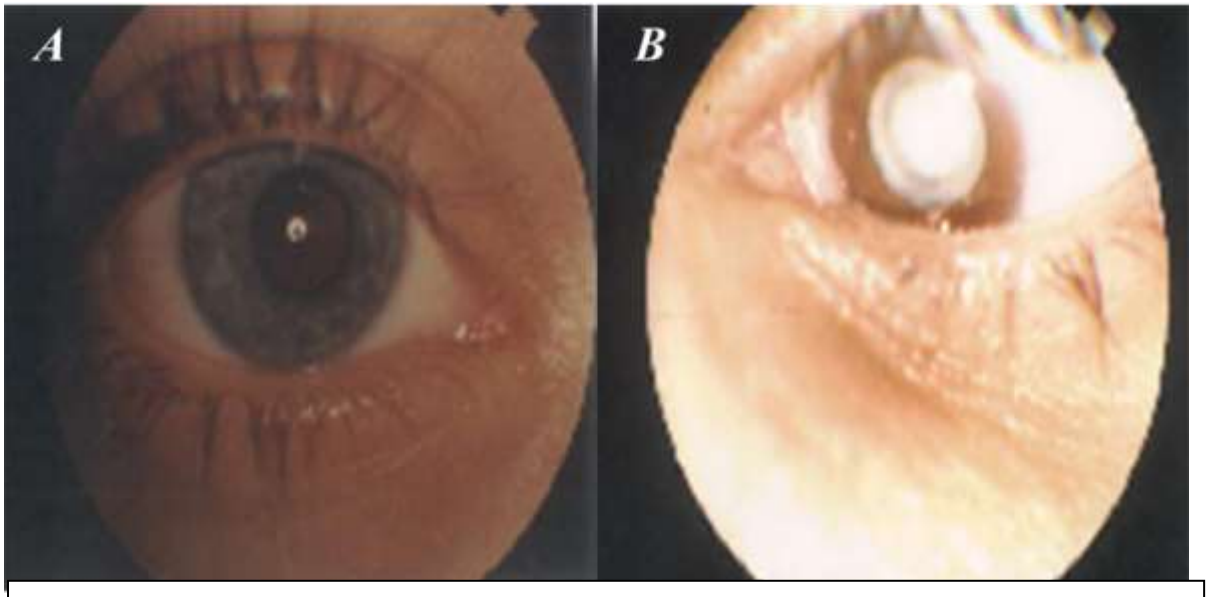

Figure 1. Slit lamp photographs of the individuals. A: Unaffected Individual and B: Individual

Table 1: Oligonucleotides used as primers for PCR amplification of CRYAA, CRYAB, CRYBA1/A3 and CRYGC and their annealing temperatures.

Gene	Forward Primer	Reverse Primer	Tm	Product Size
CRYAA Exon 1	CTCCAGGTCCCCGTGGTA	AGGAGAGGCCAGCACCAC	60	251
CRYAA Exon 2	CTGTCTCTGCCAACCCCAG	CTGTCCCACCTCTCAGTGCC	60	220
CRYAA Exon 3	GAGCCAGCCGAGGCAATG	GAGCCAGCCGAGGCAATG	58	308
CRYAB Exon 1	CTCACACTCACCTAGCC	GAAGTTAGGGGACGGAG	54	464
CRYAB Exon 2	TTTGCCCTCTTTCTCCC	TCATCCCATCTAAGGCG	52	399
CRYAB Exon 3	TAAGGGGAAATCAGGATGCCT	AAATCCTTGGAGCCCTCTAA	55	500
CRYBA1/A3 Exon 3	GGTAACAGAAAGCACAG	ACCAAGCCACTAAGAAC	50	422
CRYBA1/A3 Exon 4	GCTCTACTGGGATTGGC	TGGGCTCTTGAGTATCC	54	513
CRYGC Exon 1 & Exon 2	CAACGCAGCAGCCATCC	CCTCCCTCCCTGTAACC	56	512
CRYGC Exon 3	TGCCACAACCTACCAG	GGAAATTGGTAGTGTAAG	52	392

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