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Mutation Analysis of families with childhood congenital cataract of *CRYAA* Exon 2 gene at Khyber-Pakhtunkhwa (KPK), Pakistan

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Congenital cataract in children is a leading cause of reversible blindness around the Globe. The majority of cases in childhood congenital cataracts are hereditary. A congenital hereditary cataract is linked to several distinct gene mutations. Finding and identifying these genes, as well as mutations in them, can better help researchers to understand the genetic basis of disease. Aim of current research was to identify genetic mutations in *CRYAA* containing the exon 2 gene in a different region of Pakistan from four families affected with congenital cataracts. A minimum of two generations' family history and full medical history were taken and pedigree was drawn to identify the pattern of inheritance. Family members with affected traits were enlisted for the study. Blood samples from four selected families were collected for DNA analysis and DNA was extracted using the chloroform phenol technique. The DNA was taken and utilized as a templet for the amplification of a gene of about 350bp fragments amplifying exon 2 of the *CRYAA* gene. After PCR amplification, the samples were sequenced using sanger sequencing. The sanger sequence results was then compared to the NCBI reference sequence for exon 2 of the *CRYAA* gene. MUSCLE and Eugene tools were used to further analyze the sequence. There were no mutation identify in exon 2 of the *CRYAA* gene, according to the analysis. Childhood Congenital cataract has an autosomal recessive inheritance pattern. Exon 2 of the *CRYAA* gene is not responsible for congenital cataracts in the selected families, according to the findings. Other important genes for congenital cataracts could be investigated to determine that which gene is responsible for the disease in the research region. These findings show that no mutation in the *CRYAA* of Exon 2 protein cause it to function inaccurately, broadening the range of disease-linked mutations in rare cases of children with congenital cataract and allowing for better diagnosis and therapy.

Keywords: Childhood Congenital Cataract, Alpha crystallin, Autosomal dominant, Axial length, Nuclear cataract, Nystagmus

INTRODUCTION

Cataracts of the eye lens are the most common cause of blindness in the globe. The presence of total or partial lens opacification

within the first year of life is known as congenital cataracts (Bermejo et al. 2020). Childhood Congenital cataract is especially dangerous since it can obstruct vision development. Childhood Congenital cataracts affect 6.3 out of every 100,000 people, with 30% of cases being hereditary (Haargaard et al. 2015; Shiels et al. 2010).

Childhood Congenital cataracts are a clinically and genetically varied lens condition (Huang and He, 2010). Congenital cataracts are divided into numerous subtypes according to their morphology, including entire lens, nuclear, lamellar, cortical, polar, sutural, pulverulent, cerulean, and coralliform (Reddy et al. 2012). Although autosomal recessive and X-linked inherited variants have been documented, autosomal dominant inheritance (ADCC) is the most common method of transmission genetically. To date, more than 40 genes in the human genome have been linked to various kinds of congenital cataracts, including at least 26 genes linked to autosomal dominant or autosomal recessive congenital cataracts. Crystallin and connexin genes appear to be the most frequently linked to congenital cataracts, about half of the mutations identified in crystalline genes (*CRYAA, CRYAB, CRYBA1/A3, CRYBB1, CRYBB2, CRYBA4, CRYGC, CRYGD, and CRYGS*) and a quarter in connexin genes (*GJA3* and *GJA8*) (Hejtmancik, 2008).

Crystallin proteins, which make up to 90% of soluble proteins in the lens, are divided into three groups, α-, β- and γ- crystallins (Reddy et al. 2012). α -crystallin is a member of the small heat shock protein (sHSP) family that prevents partially unfolded proteins from aggregating (Hejtmancik, 2008). The α -crystallin protein family is made up of two proteins: αA-crystallin, which is encoded by the *CRYAA* gene, and αB-crystallin, which is represented by the *CRYAB* gene (World Medical Association Declaration of Helsinki, 2013). In the *CRYAA* gene, there have been a total of 26 mutations discovered thus far. Previous research has linked *CRYAA* gene mutations to congenital cataracts, either alone or in combination with other clinical diseases such microcornea and microphthalmia (Beyer et al. 2013). This indicated that αA-crystallin plays a critical function in the lens formation.

More than half of childhood congenital cataract cases are accompanied by other visual abnormalities as nystagmus, strabismus, microcornea or microphthalmia, persistent hyperplastic primary vitreous, congenital glaucoma, morning glory syndrome, and persistent pupillary membrane (Song et al. 2014). This study includes four Pakistani families with four-generation of childhood congenital cataracts with different morphology such as Amblyopia, nystagmus and microphthalmia and corneal opacity were found. By directly screening the candidate gene, we have found no mutation in αA-crystallin *(CRYAA)* of exon 2. The effects of no mutation on the αA-crystallin were further studied.

MATERIALS AND METHODS

Subjects Clinical Examination

We screened four families from a different regions of KPK, Pakistan with four-generation diagnosed with autosomal recessive congenital cataract (ARCC) and various ocular anomalies were observed in family 1 such as Amblyopia, nystagmus in family 2, microcornea in family 3 and corneal opacity in family 4 which were recruited at the Hayatabad Medical Complex, Peshawar. To confirm the affected status and identify whether there were any other ocular abnormalities, all the affected family members that participated in this study underwent complete ophthalmic examinations, including visual acuity, slit-lamp biomicroscopic fundus examination with dilated pupils, B-scan ultrasonography and axial length. The phenotype of the proband was recognized by ophthalmic operating microscope photography. A non-affected subject with their families of childhood congenital cataracts was also recruited. Written informed consent was obtained from all the participants enrolled in our study or from their guardians.

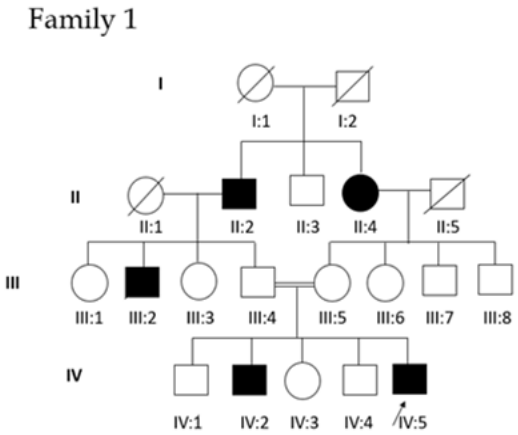
Extraction of Genomic DNA and Mutation Analysis

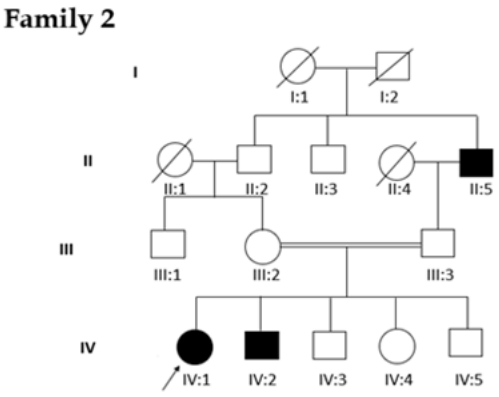
The genomic DNA of the participants was extracted from peripheral blood using Thermo fisher DNA Blood Mini Kit. *CRYAA Exon 2* and flanking regions of ARCC gene (accompanied ocular anomalies such as Amblyopia, nystagmus, microphthalmia and corneal opacity) of *CRYAA,* was amplified by genomic polymerase chain reaction (PCR). The functional candidate gene was determined from the Online Mendelian Inheritance in Man (OMIM) database). The primer for *CRYAA_ex2* NM_000496 was F 5'CTTGGTGTGTGGGAGAAGAGG 3' (Forward) and 5' CTTCAACCCTGGGAGAGGGA 3' (Reverse). Each reaction mixture (25μl) contained 2μl of genomic DNA, 3μl of $Mgcl_2$, 3μl of 10X buffer, 2.5μl of dNTPs, 2μl of Forward and

Reverse primer, 0.5μl of Taq Polymerase and 12 μl of water taken in each tube and mixed. The following PCR condition was used for DNA amplification: 95°C for 5 min; followed by 35 cycles at 94°C for 40 sec, 57°C for 30 sec (annealing temperature difference according to primer), 72°C for 45 sec, and a final extension at 72°C for 7 min. The PCR products of the parents, probands from each family and one unaffected member were sequenced. The sequencing results were analyzed using Chromas 2.33 and were compared with the reference sequence in the NCBI database (http://www.ncbi.nlm.nih.gov/) (Ahmad and Khan, 2021; Zafar and Iftikhar, 2021; Shah and Khan, 2021). Bioinformatics use in-Silico approaches to identify the location or specific gene (Bashir and Ahmad, 2021; Jan, and Ahmad, 2021; Jan and Khan, 2021; Bashir and Ullah, 2021), therefore we screened the mutations in the *CRYAA* from the family members and unaffected members to confirm the mutation.

RESULTS

The slit-lamp examination revealed polymorphic cataracts in Family 1 (Fig. 2A). The proband in this family 1 showed opacities involving the nucleus and peripheral cortex, and the image of individual IV:2 represented a bilateral nuclear cataract in the central lens and opacities involving the peripheral cortex. The images of individual IV:5 revealed a nuclear cataract. The phenotypes of the two individuals denoted Amblyopia. The slit lamp image of the proband in Family 2 revealed congenital zonular cataracts with sutural opacities (Fig. 2B). All affected individuals (IV:1 and IV:2) in this family exhibited congenital zonular cataracts with sutural opacities and the ocular abnormality of the patient was associated with microphthalmia . The proband in family 3 presented opacities involving the bilateral posterior polar cataract and the slit lamp image of individual IV:1 and IV:2 revealed a bilateral posterior polar cataract. The phenotypes of the two individuals were the same and associated with corneal opacity. The slit lamp image of the proband in Family 3 revealed a posterior polar cataract (Fig. 2C). The image of the proband in Family 4 shows nuclear cataracts (Fig. 2D). All affected individuals (IV:1 and IV:2) in this family exhibited nystagmus, and the B-scan ultrasonography examination of the proband in this family revealed nuclear cataracts in the left and right eyes.





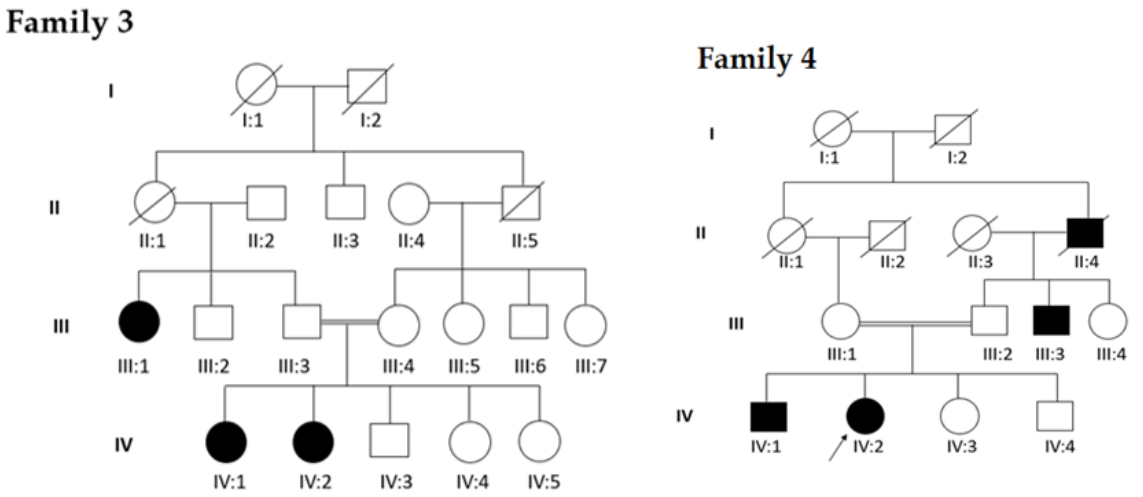


Figure 1: Four Pakistani families with Autosomal recessive cataracts. Family 1, 2,3 and 4 showing four generations of pedigrees. The arrow indicates the proband in each family and affected. Circles denote females and squares denote males. Black squares and circles indicate family members exhibiting congenital cataracts and white squares and circles are unaffected individuals.

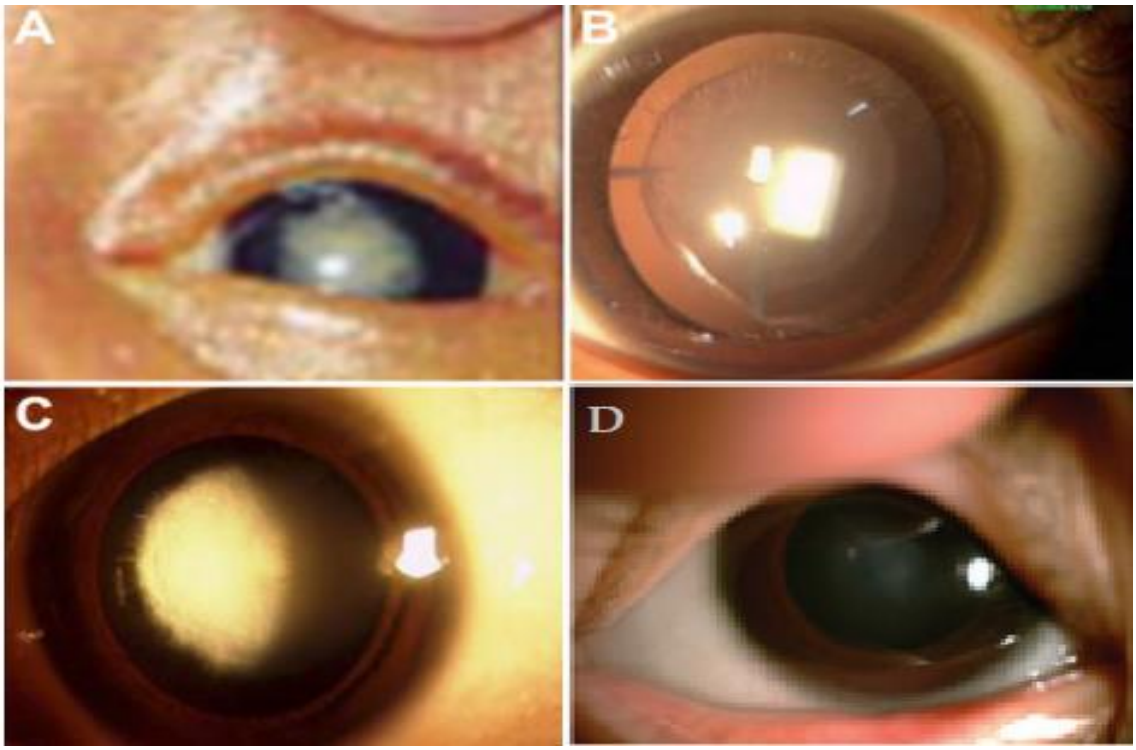


Figure 2: Different types of cataracts. A: Nuclear cataract: The eye picture shows the opacity of both lenses in one of the patients. B: Zonular with sutural cataract in one of the patients. C: Polar cataract phenotype shown by one of the patients. D: Nuclear cataract phenotype found in one patient

Reference sequence of Exon 2 *CRYAA* gene

GTTCGATCCGACCGGGACAAGTTCGTCATCTTCCTCGATGTGAAGCACTTCTCCCCGGAGGACCTCA

CCGTGAAGGTGCAGGACGACTTTGTGGAGATCCACGGAAAGCACAACGAGCGCCAG

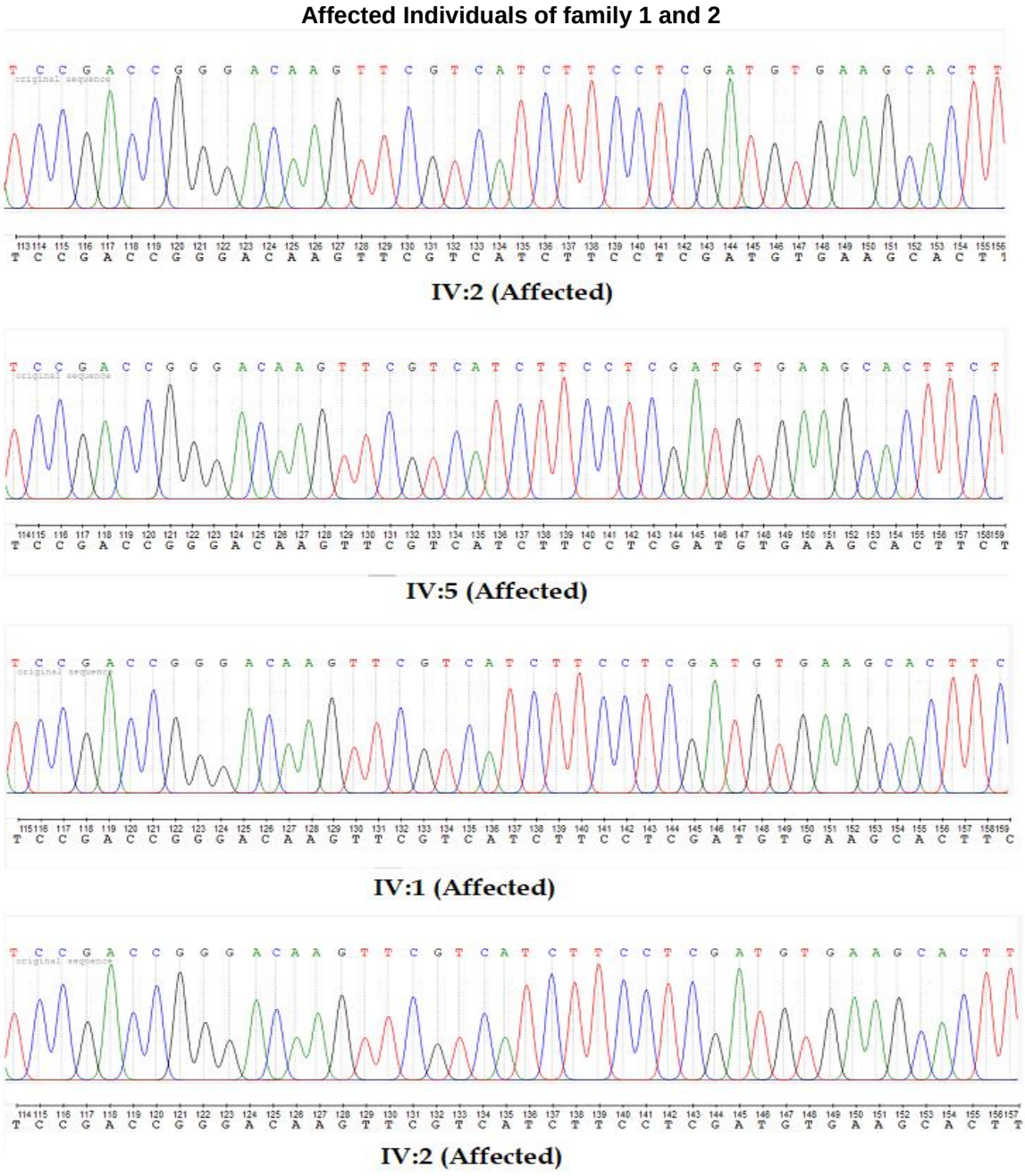


Figure 3: Partial genome sequence of *CRYAA* Exon 2. Sequences of an affected family member of families 1 and 2. The sequence indicates no mutation.

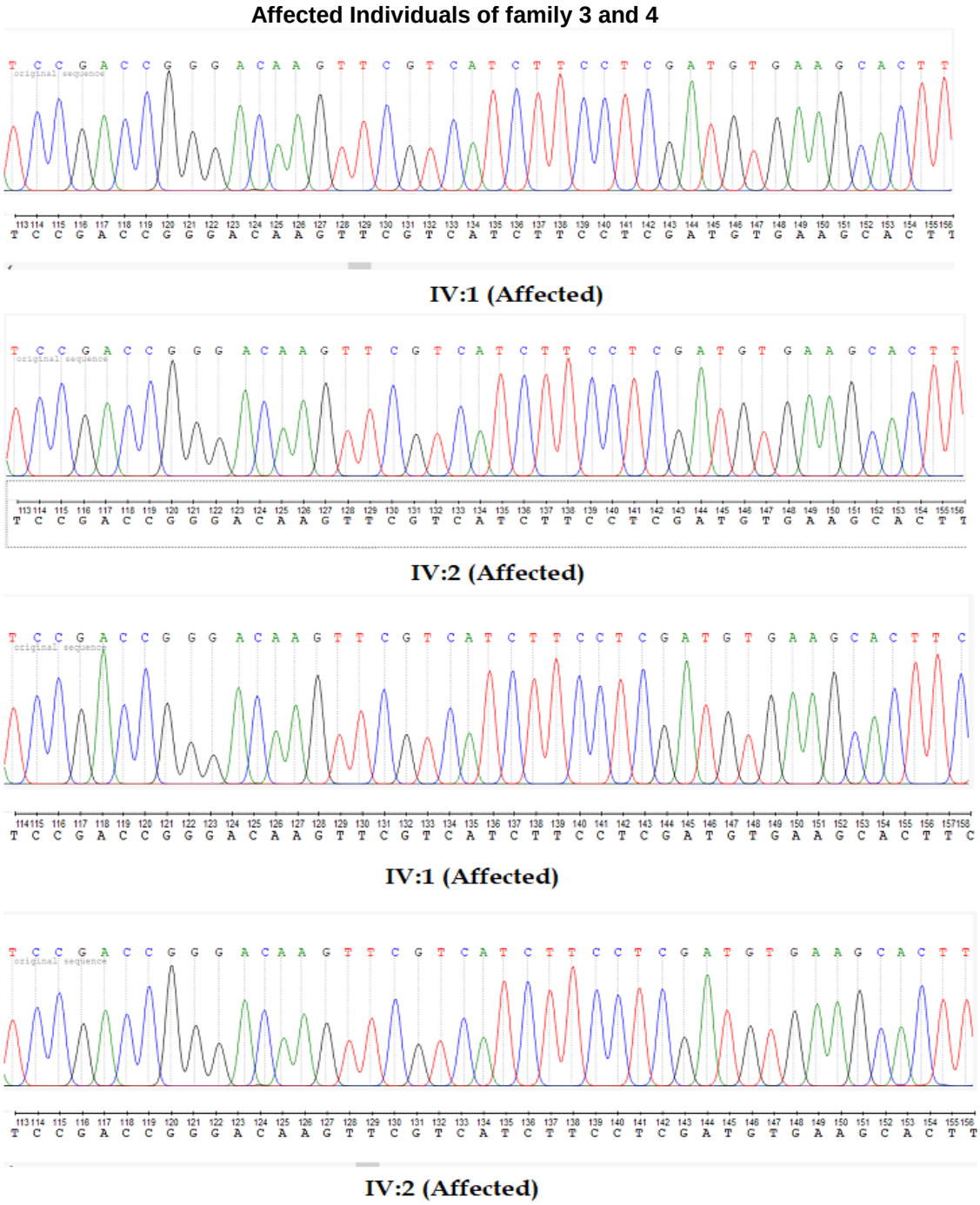


Figure 4: Partial genome sequence of *CRYAA* Exon 2. Sequences of an affected family member of family 3 and 4. The sequence indicates no mutation.

DISCUSSION

The lens is an avascular organ that depends on continuing transparency to allow the normal light transmission to focus images on the retina. Epithelial cells, which form a single layer along the front surface, and fiber cells, which make up the bulk of the organ, make up the lens. Crystallins are tiny soluble proteins found in high concentrations in lens fiber cells, which develop from epithelial cells during the life cycle of the organism. The majority of the metabolic, synthetic,

and active transport machinery in the lens is located to the surface cells, and mature fiber cells have modest metabolic activities. To establish and maintain lens transparency, lens crystallin and a complex cell-cell communication mechanism are required. Damage to the lens cells and/or proteins can produce opacities, which can cause vision loss and finally blindness (Beyer et al. 2013).

Crystallin is a key structural protein found in the lens of vertebrates, accounting for 90-95 percent of water-soluble crystalline. The lens gets its transparency and diopter from proteins that are organized in a regular pattern. Human lens crystallins are classified into three families: alpha, beta, and gamma, with beta and gamma crystallins being superfamily. Alpha crystallins are made up of two gene products: alpha-A and alpha-B, which stand for acidic and basic crystallins, respectively. Alpha crystallins can be produced by heat shock, which belongs to the small heat shock protein family. They function as molecular chaperones, but unlike real chaperones, they do not renature proteins and release them. Instead, they hold the proteins in huge soluble aggregates made up of 30-40 subunits, with the alpha-A and alpha-B subunits arranged in a 3:1 ratio (Graw, 2009).

The *CRYAA* gene has a unique position on chromosome 21q22.3 (Hawkins et al. 2015; Ahmad and Qadus et al. 2020), which comprises 3 exons and encode the alpha crystalline, a protein composed of 251 amino acids consisting of 9% of water-soluble crystalline. A total of 13 mutations of the *CRYAA* gene have been defined, which the most common are missense or nonsense mutations. Some α-A crystallin mutations have been reported previously, consisting of R12C, R21W, R21L, R49C, G98R, R54C, R116C and R116H (13-20). Concerning secondary and tertiary changes of structure, all the mutants of the *CRYAA* gene identified show variable degrees of secondary and tertiary structural changes, which can be central to protein unfolding/misfolding and later to the formation of protein aggregates (Raju and Abraham, 2011).

In the present study, four families were studied from different regions of KPK, Pakistan and screened with *CRYAA* Exon 2, but they found no mutation. It may be possible that these families were not responsible to identify the mutation in *CRYAA* Exon 2 gene for this targeted region and cannot carry the pathogenic variation along with their family background. Thus, the genetic investigation of these families and their recruitment combined with other associated additional families can provide a further and a better description of the pathophysiology of the disease as well as provide additional information for better use of generating therapeutic procedures for the identification of other responsible genes for childhood congenital cataract disease.

CONCLUSION

The sequence analysis of *CRYAA* exon 2 in patients and control subjects showed no mutation in these families. The results of this study may establish a phenotype-genotype correlation and help to detect a more precise and accurate prognosis or diagnosis, carrier screening for the transfer of congenital cataracts within families vertically as well as horizontally and also in making the effective approaches for genetic counseling in this regard.

CONFLICT OF INTEREST

The authors declared that the present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

MH and KM designed and performed the experiments and also wrote the manuscript, MK, AS & HUK. SK, MSK performed data analysis and proofreading of the article. MQ designed experiments and reviewed the manuscript. All authors read and approved the final version.

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