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Molecular and structural analysis of genetic variations in congenital cataract

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Objective: To determine the relative contributions of mutations in congenital cataract cases in an Indian population by systematic screening of genes associated with cataract.

Methods: We enrolled 100 congenital cataract cases presenting at the Dr. R. P. Centre for Ophthalmic Sciences, a tertiary research and referral hospital (AIIMS, New Delhi, India). Crystallin, alpha A (*CRYAA*), *CRYAB, CRYGs, CRYBA1, CRYBA4, CRYBB1*, *CRYBB2*, *CRYBB3,* beaded filament structural protein 1 (*BFSP1*), gap function protein, alpha 3 (*GJA3*), *GJA8*, and heat shock transcription factor 4 gene genes were amplified. Protein structure differences analysis was performed using Discovery Studio (DS) 2.0.

Results: The mean age of the patients was 17.45±16.51 months, and the age of onset was 1.618±0.7181 months. Sequencing analysis of 14 genes identified 18 nucleotide variations. Fourteen variations were found in the crystallin genes, one in Cx-46 (*GJA3*), and three in *BFSP1*.

Conclusions: Congenital cataract shows marked clinical and genetic heterogeneity. Five nucleotide variations (*CRYBA4*:p.Y67N, *CRYBB1:*p.D85N, *CRYBB1*:p.E75K, *CRYBB1*:p.E155K, and *GJA3*:p.M1V) were predicted to be pathogenic. Variants in other genes might also be involved in maintaining lens development, growth, and transparency. The study confirms that the crystallin beta cluster on chromosome 22, Cx-46, and *BFSP1* plays a major role in maintaining lens transparency. This study also expands the mutation spectrum of the genes associated with congenital cataract.

Cataracts are the most common cause of visual impairment and account for 10% of all childhood blindness worldwide [1]. The prevalence of cataract, depending on regional socioeconomic development, is 1−6 cases per 10,000 live births in industrialized countries [2,3] and 5−15 per 10,000 in the poorest areas of the world [4,5]. Various etiological factors have been identified, including infection, metabolic disorders, and genetic defects. Hereditary cataracts are clinically highly heterogeneous and show considerable interfamilial and intrafamilial variability [6]. Hereditary congenital cataract may be inherited as autosomal dominant, autosomal recessive, or X-linked traits and thus shows marked genetic heterogeneity.

Congenital cataract is a clinically and genetically heterogeneous disorder [7]. Different mutations in the same gene can cause similar cataract patterns while the highly variable morphologies (total, polar, zonular, and capsular) of cataracts within families suggest that the same mutation in a single gene can lead to different phenotypes [6,8]. To date, more than 40 genetic loci have been linked to congenital cataracts [9]. Among these candidate genes, crystallin and connexin genes represent a major proportion of the mutations identified in congenital cataract and have been associated with cataracts of various morphologies [10], including genes encoding crystallins (crystallin, alpha A [*CRYA*], *CRYB*, and *CRYG*) [11], lens-specific connexins (*Cx43*, *Cx46*, and *Cx50*) [12,13], cytoskeletal structural proteins (beaded filament structural protein 1 [*BFSP1*]) [14], and heat shock transcription factor 4 gene (*HSF4*) [15].

The α-, β-, and γ-crystallins represent more than 90% of the lens-soluble proteins in humans, encompassing almost 35% of the mass and accounting for the optical transparency and high refractive index [16,17]. In the human lens, α-crystallin makes up about 40%, β-crystallin 35%, and γ-crystallin 25% of the total crystallin protein. Lamellar and nuclear cataracts are the most common types of hereditary congenital cataract [18,19]. The HSF4 transcription factor is the predominant HSF expressed postnatally in ocular lens [20]. Specific interactions between HSF4 and HSE in the promoters of β-crystallin (causes autosomal dominant congenital cataract when mutated) [21], Hsp70, and Hsp82 have been demonstrated [20]. The aim of our study was to determine the relative contributions of mutations in congenital cataract cases in an Indian population with systematic screening of 14 genes associated with cataract.

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METHODS

Patient ascertainment and clinical examination: After receiving ethical approval from the institutional review board (IRB#00006862; All India Institute of Medical Sciences, Delhi, India), 100 clinically diagnosed congenital cataract cases from northern India who presented at the Dr. R. P. Centre for Ophthalmic Sciences (AIIMS, New Delhi, India) were enrolled in this study. In this study 69% of the patients were found to male as compared to 31% female. Mean age of the patients is 17.45±16.51 and the mean age of onset of disease was 1.618±0.7181. Affected status was determined by a history of cataract extraction or ophthalmologic examination. A total of 100 normal individuals without any history of ocular disorders were enrolled as controls. Patients with a history of intrauterine infection such as rubella, TORCHES (TOxoplasmosis, Rubella, Cytomegalovirus, HErpes simplex, Syphilis), and traumatic cataract were excluded from the study. Informed consent in accordance with the Declaration of Helsinki was obtained from all participants or their parents.

Polymerase chain reaction and deoxyribonucleic acid sequencing: Brief description for how the blood was drawn and how samples were preserved prior to use is as follow: For DNA isolation 2-3 ml of peripheral blood was collected in EDTA vials from each case. The samples were stored in -80 °C prior to use. Genomic DNA was extracted from the blood samples of the patients with congenital cataract and controls, using an organic method described by Sambrook et al. [22]. The exon-intron regions of all the genes (*CRYAA, CRYAB, CRYGs, CRYBA1, CRYBA4, CRYBB1*, *CRYBB2*, *CRYBB3, BFSP1, GJA3, GJA8*, and *HSF4*) were amplified. PCR amplifications for all primer sets (Table 1) were performed in a 40 µl volume containing 1.0 µl of 20 mM stock solution for each primer (Eurofins Genomics India, Bangalore, India), 100 ng genomic DNA, 1 unit Taq polymerase (Banglore Genei, Bengaluru, India), 0.1 mM each deoxynucleotide triphosphate (dNTP), and 4 µl 10X PCR buffer (with 15 mM $MgCl_2$). Amplified PCR products were purified using a gel/PCR DNA fragments extraction kit (Geneaid Biotech, Sijhih City, Taiwan). Purified PCR products were sent for sequencing to Molecular Cloning Laboratories (South San Francisco, CA). All sequence variants were compared to the Human Genome Reference Sequence provided by the National Center for Biotechnology Information (NCBI), using ClustalW2 (multiple sequence alignment program for DNA; European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, UK).

Bioinformatics analysis: MutationTaster, a free, web-based application was used for rapid evaluation of the disease-causing potential of DNA sequence alterations [23]. The Sorting Intolerant From Tolerant (SIFT) analysis tool was also used to predict the functional impact of the missense changes identified in this study [24]. Positions with normalized probabilities <0.05 are predicted to be deleterious, and those ≥0.05 are predicted to be tolerated. Another free, web-based application, PolyPhen-2, structurally analyzes an amino acid polymorphism and predicts whether that amino acid change is likely to be deleterious to protein function [25,26]. A PolyPhen-2 *s*core of >2.0 indicates the change is probably damaging to protein function. Scores of 1.5–2.0 are possibly damaging, and scores of <1.5 are likely benign. Three web-based applications were used to predict the pathogenicity of non-synonymous variations. The variations were considered pathogenic only when the outcome of two out of three applications suggested the variations were disease causing.

Protein modeling: The normal and mutant proteins were analyzed for their structure. Prediction of structure differences between the wild-type and mutant proteins was performed using Discovery Studio (DS) 2.0 (Accelrys, San Diego, CA) [27]. The first step in the homology modeling method is to find a suitable homologous structure (template). Thus, structural differences between the wild-type and mutant were predicted in mutants when a suitable template or homolog structure was present. The model structure of the three mutant proteins was developed and refined with minimization programs in the presence of the CHARMm force field in a manner similar to the structure of the A4 protein.

Statistical analysis: Pearson χ^2/Fisher's exact test was applied to compare the two groups (cases versus controls). P values less than 0.05 were considered significant. All tests were performed with SPSS software for Windows (version 11.5; SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

A total of 100 patients with sporadic congenital cataract were enrolled in this study. The age range of the patients was 1 month to 3 years. The mean age of onset was 1.618±0.7181 months, as the age at which the disease was first noticed by the child's parents or by a clinician. Out of 100 patients, 80 had bilateral congenital cataract, and 20 had unilateral congenital cataract. All cases enrolled were sporadic, and the male to female ratio was 2.2:1 (69 men and 31 women). Different forms of cataracts with variable degrees of opacification were observed. Nuclear cataract (72%) was the most prevalent phenotype found. The other phenotypes observed were zonular with nuclear (19%), total cataract (05%), Zonular/lamellar (03%) and anterior polar cataract (01%).

Table 1. Oligonucleotides of mutated genes used as primers for PCR amplification.

Gene Name	Primer Name	Primer Sequence	Anne Temp (°C)	Product Size (bp)
CRYBA4	Ex-2-F	5′-TAGCCCAGTCACTCCTGGAC-3′	57 °C	213
	Ex-2-R	5′-GCCTTGATTGCACCTCTGTG-3′		
	Ex-3-F	5′-TTTGCAATCCCTGCTTTACC-3′	57 °C	423
	Ex-3-R	5′-ATGGCACCCTCCTACTGTTGG-3′		
	Ex-4-F	5′-AAAAATGTCTCCAGCCATCG-3′	57 °C	314
	Ex-4-R	5′-AGCTTGAAGTGGCGACATGAG-3′		
	Ex-5-F	5′-AAATGGCAAGGTTTCTGGTAC-3′	57 °C	297
	Ex-5-R	5′-GCCTCAGTGTTCTCCTCTGG-3′		
	Ex-6-F	5′-AGGGAATGGCATGATCAAAG-3′	57 °C	335
	Ex-6-R	5′-TGCTGGGTTCACACAGGTTAC-3′		
CRYBB1	Ex-2-F	5′-ACAGGATGTGGGGCTATGAG-3′	59 °C	380
	Ex-2-R	5′-GTGCGGAGGAGTAAGAGGTG-3′		
	Ex-3-F	5′-CATTTCACAAACTGTGGCTCA-3′	62 °C	379
	Ex-3-R	5′-GGACATAATGTATGTGCCAGGA-3′		
	Ex-4-F	5′-GTAGGGAGTGGGGGCTTCTA-3′	62 °C	286
	Ex-4-R	5′-CTCCTTCTTGCCCTTGTCAG-3′		
	Ex-5-F	5′-GCTCATCTCTCTCGCTCCAC-3′	61 °C	298
	Ex-5-R	5′-TCTGATTCTGCCTGTGCTTG-3′		
	Ex-6-F	5′-TCAATGAAGGACAGGCTGGT-3′	62 °C	381
	Ex-6-R	5′-TCCAGGAGAAATTTTGGCTTT-3′		
CRYBB2	Ex-2-F	5′-CAGAGGGGAGTGGTCTCAAG-3′	59 °C	244
	Ex-2-R	5′-ATGCCAAGCCCATTTTACAG-3′		
	Ex-3-F	5′-TCAGCATCCTTTGGGTTCTC-3′	59 °C	299
	Ex-3-R	5′-CAAGGGTAGATTCCCCCACT-3′		
	Ex-4-F	5′-AACCCTAGGGGTCAACATCA-3′	62 °C	297
	Ex-4-R	5′-CTCCAAGGTGGCAGAGAGAG-3′		
	Ex-5-F	5′-GAGTGATGTGTGGGACATGC-3′	62 °C	377
	Ex-5-R	5′-CAGAGGTCAGCAGAGCACAC-3′		
	Ex-6-F	5′-GGCTTCACCCTTCCTAGTGG-3′	59 °C	399
	Ex-6-R	5′-CAAAGACCCACAGCAGACAA-3′		

Gene Name	Primer Name	Primer Sequence	Anne Temp (°C)	Product Size (bp)
GAJ3	Ex-1A-E	5′-TGCGGACCCGGCACTCAGC-3′	62 °C	383
	Ex-1A-R	5′-TCCATGCGCACGATGTGCAGTCA-3′		
	Ex-1B-F	5′-CTGTTCATCTTCCGCATTTTGG-3′	62 °C	603
	Ex-1B-R	5′-TCTTCTTCCAGCCCAGGTGGTA-3′		
	Ex-1C-F	5′-AAGCTCAAGCAGGGCGTGAC-3′	62 °C	624
	Ex-1C-R	5′-CTAGATGGCCAAGTCCTCCGG-3′		
BFSP1	Ex-1-F	5′-GGGCCTCCGGTGTTTATTTA-3′	58 °C	589
	Ex-1-R	5′-ATCGACAGGGGACCGAGAGAC-3′		
	Ex-2-F	5′-AAAGGAGAGGGCATCGTACC-3′	58 °C	238
	Ex-2-R	5′-AACCTGCACTTCCACCATTC-3′		
	Ex-3-F	5′-CAGGTGGTCTGTGTGCACAT-3′	58 °C	249
	Ex-3-R	5′-TCGGCTTACCTGATCAAACC-3′		
	Ex-4-F	5′-RGCCATTCCTGTTCTCATCT-3′	58 °C	250
	Ex-4-R	5′-GCCCTTCCCTGGGAGTCT-3′		
	Ex-5-F	5′-ACCTTCTCTGCCCTTTTCCT-3′	58 °C	227
	Ex-5-R	5′-CACCTCCATGAAACATGTGG-3′		
	Ex-6-F	5′-CCTTTTCCTGGGTGAGGTCTG-3′	58 °C	366
	Ex-6-R	5′-GGCACACAATAGGCACTCAA-3′		
	Ex-7-F	5′-CTTGCCCCTGACCTCTGTT-3′	58 °C	199
	Ex-7-R	5′-AAGAGAGCCGCTTGGTTTTT-3′		
	Ex-8-F	5′-TTCCAACCAGCGTATTTTCTTT-3′	58 °C	699
	Ex-8-R	5′-TCAGGGCCTTCCAGCTCT-3′		
	In5-Ex-7-F	5′-CATCTTCCAGGGTGTCCAG-3′	58 °C	316
	In5-Ex-7-R	5′-AAGAGAGCCGCTTGGTTTTT-3′		

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Mutations in more than 40 genetic loci have been linked to congenital cataracts. Of these mutations, approximately half involve crystallins, one-quarter involve connexins, and the remaining involve other genes [9]. Direct sequencing analysis of 14 genes (*CRYAA, CRYAB, CRYGs, CRYBA1, CRYBA4, CRYBB1*, *CRYBB2*, *CRYBB3, BFSP1, GJA3, GJA8*, and *HSF4*) identified 18 nucleotide variations (Table 2), 14 of which were in crystallin genes (*CRYBA4, CRYBB1*, and *CRYBB2*), one in Cx-46 (*GJA3*), and three in *BFSP1*. Five nucleotide variations (Figure 1; *CRYBA4*:p.Y67N, *CRYBB1*:p.E75K, *CRYBB1*:p.D85N, *CRYBB1*:p.E155K, and *GJA3*:p.M1V) were predicted to be pathogenic in in silico analysis. No variation was detected in the *CRYAs, CRYGs, CRYBA1, CRYBB3, GJA8*, and *HSF4* genes.

Functional changes in crystallin molecular properties could cause the breakdown of the lens microstructure and result in changes in the refractive index and increased light scattering [28]. Out of the 14 variations observed in the crystallin genes (Table 2), eight were observed in the *CRYBA4* gene, four in the *CRYBB1* gene, and two in the *CRYBB2* gene. Billingsley et al. [29] identified *CRYBA4* as a cataract gene in a large Indian family with an autosomal dominant cataract phenotype. A total of 102 nucleotide variations (Table 3) have been reported in the *CRYBA4* gene, but only three have been associated with cataract [30] (Table 4). Two non-synonymous, novel variations (*CRYBA4:*p.T84A, *CRYBA4:*p.Y67N) were found in exon 4 of the *CRYBA4* gene. Computational assessment showed *CRYBA4:*p.Y67N was pathogenic whereas *CRYBA4:*p.T84A was polymorphic. *CRYBA4:*p.Y67N was found in two cases affected with bilateral nuclear cataract whereas *CRYBA4:*p.T84A was found in four patients. Two had zonular cataract, and two had nuclear cataract. None of the variations (*CRYBA4:*p.T84A, *CRYBA4:*p.Y67N) were found in the controls. The mutation *CRYBA4:*p.Y67N located in the neighboring β strand of the N-terminal domain whereas the *CRYBA4:*p.T84A mutation is situated in the β crystallin A4 protein in the loop region.

For modeling studies, the crystal structure of the human *CRYBA4* protein (PDB id: 3LWK) was used as the template. The complete model structure including the missing region of the native human crystallin βA4 as well as its mutant (*CRYBA4:*p.T84A, *CRYBA4:*p.Y67N) were developed using the homology modeling approach to study the effect of the mutations on the crystallin's structure and function. The *CRYBA4* protein model structure is dominated by β strands. The two domains interact through intramolecular contacts

Table 2. Nucleotide variations found in congenital cataract patients.

S.No.	Nucleotide Change	Locus	Codon Change	Amino acid Change	Type of Mutation	POLYPHEN/SIFT / Mutation Taster
1.	g.27021536*	CRYBA4	ACG>GCG	T84A	NS	0.823/0.02/PM
2.	g.27021532*	CRYBA4	GGC>GGA	G82G	SYN	NA
3.	g.27021497*	CRYBA4	CGA>AGA	R71R	SYN	NA
4.	**g.27021485* #**	**CRYBA4**	**TAC>AAC**	**Y67N**	**NS**	**3.084/0.00/DC**
5.	rs5761637T>A^	CRYBA4	TTT>TTC	F57F	SYN	NA
6.	rs4276A>G^	CRYBA4	intronic	NA	NA	NA
7.	rs73880140C>T^	CRYBA4	intronic	NA	NA	NA
8.	rs2071862G>A^	CRYBA4	intronic	NA	NA	NA
9.	**g.G27008082A* #**	**CRYBB1**	**GAC>AAC**	**D85N**	**NS**	**1.689/ 0.02/DC**
10.	**g.G27008112A*#**	**CRYBB1**	**GAA>AAA**	**E75K**	**NS**	**2.002/0.00/DC**
11.	rs57400078C>A^	CRYBB1	intronic	NA	NA	NA
12.	**g. A26997943G*#**	**CRYBB1**	**GAA>AAA**	**E155K**	**NS**	**2.088/0.00/DC**
13.	g. G25617606A*	CRYBB2	GAT>AAT	D4N	NS	0.552/0.49/**DC**
14.	g.G25617414A*	CRYBB2	CAG>CAA	Q6Q	SYN	NA
15.	**c. A178G #**	**GJA3**	**ATG>GTG**	**M1V**	**NS**	**2.864/0.00/TP**
16.	g.G17475531A*	BFSP1	GAC>AAC	D395N	NS	1.398/0.01/PM
17.	g.G17475444A*	BFSP1	GAA>AAA	E424K	NS	0.521/0.92/**DC**
18.	rs147241220 A>G^	BFSP1	CTA>CTG	L44L	SYN	NA

(Abbrevations: *Novel variations, ^Reported-Ensembl, SYN-synonymous, NS-Non synonymous, A-Not applicable, PM-polymorphism, TP-Truncated protein, DC-disease causing), #-Pathogenic variations

Table 3. Genetics variations found in the CRYBA4, CRYBB1, CRYBB2 and GJA8 genes (Ensembl).

Type of Variants	Gene Name				
	CRYBA4	CRYBB1	CRYBB2	GJA3	BFSP1
Stop gained	1	1	–	–	–
Splice site	2	2	–	–	6
Essential splice site	3	1	–	–	–
Synonymous coding	9	10	2	29	36
Non-synonymous coding	16	15	7	–	43
Within non-coding gene	52	–	–	–	53
Frameshift coding	–	1	–	2	4
Intronic	54	13	14	3	219
5 prime UTR	–	1	1	–	9
Upstream	–	–	–	–	7
downstream	–	–	–	–	5
All	102	42	24	34	339

mediated by loop regions. The missing loop regions were generated from residues Asn83 to Pro87, and residues 180–183 lie in the N-terminal domain and the C-terminal domain, respectively (Figure 2). The model structures of the mutants (*CRYBA4:*p.T84A, *CRYBA4:*p.Y67N) were developed by the Build Mutant protocol, and the mutant was refined similarly to the wild-type protein structure.

The mutation *CRYBA4:*p.Y67N does not affect the conformation of the region housing the mutation (Figure 3). Tyrosine (Tyr) is larger and more hydrophobic than Asparagine (Asn). Thus, the bulky Tyr67 side chain restricts the

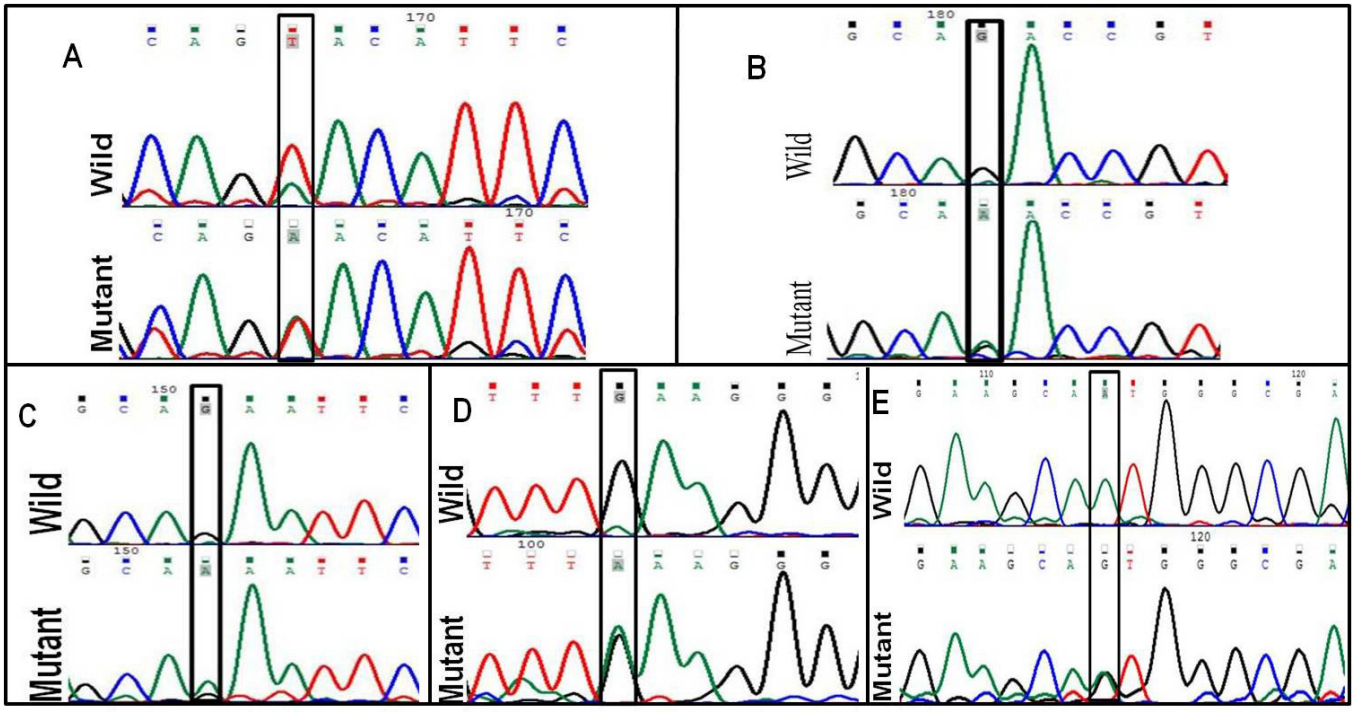

Figure 1. Deoxyribonucleic acid sequence electropherogram of pathogenic variations. (**A**) crsytallin beta a4, CRYBA4:p.Y67N (T>A), (**B**) crystalline beta b1, CRYBB1:p.D85N (G>A), (**C**) CRYBB1:p.E75K (G>A), (**D**) CRYBB1:p.E155K (A>G), and (**E**) gap function protein, alpha 3, GJA3:p.M1V (A>G).

Table 4. Summary of the mutations identified in CRYBA4, CRYBB1, CRYBB2 and GJA3 genes with different congenital cataract phenotypes belonging to different populations (Cat-Map).

Gene	Exon/ Intron	DNA Change	Coding Change	Pattern of Inheritance	Origin	Cataract Phenotype
CRYBB2	Ex2	c.5C>T	p.A2V	AD	China	Congenital posterior subcapsular
	Ex2	c.54G>A	p.K18KfsX17		India	Congenital zonular
	Ex2	c.62T>A	p.I21N		China	Nuclear
	Ex2	c.92C>G	p.S31W	AD	China	Coronary
	Ex4	c.177G>C	p.W59C	AD	India	Total
	Ex5	c.383A>T	p.D128V	AD	Germany	Nuclear, “ring-shaped” cortical
	Ex5	c.(433C>T; 440A>G; 449C>T)	p.(R145W; Q147R; T150M)	?	Denmark	?
	Ex5	c.436G>A	p.V146M	AD?	China	Nuclear (Microcornea)
	Ex6	c.452G>C	p.W151C	AD	India	Central nuclear
	Ex6	c.463C>T, c.471C>T	p.Q155X	AD	USA	Cerulean
	Ex6	c.463C>T, c.471C>T	p.Q155X	AD	Switzerland	Central zonular pulverulent
	Ex6	c.463C>T, c.471C>T	p.Q155X	AD	India	Sutural cerulean
	Ex6	c.463C>T, c.471C>T	p.Q155X	AD	China	Progressive polymorphic
	Ex6	c.463C>T, c.471C>T	p.Q155X	AD	Chile	Variable
	Ex6	c.463C>T, c.471C>T	p.Q155X	AD	China	Progressive polymorphic coronary
	Ex6	c.463C>T, c.471C>T	p.Q155X	?	India	Cortical, pulverulent
	Ex6	c.463C>T, c.471C>T	p.Q155X	AD	China	Cerulean
	Ex6	c.477C>A	p.Y159X	?	Denmark	?
	Ex6	c.607G>A	p.V187M	AD	Lesotho	Nuclear (Strabismus)
	Ex1	c.2T>A	p.M1K	AR	Somalia	Nuclear, pulverulent
	Ex2	c.171delG	p.G57GfsX107 (p.N58TfsX106)	AR	Israel	Nuclear
CRYBB1	Ex6	c.658G>T	p.G220X	AD	USA	Central sutural pulverulent
	Ex6	c.667C>T	p.Q223X	AD	China	Nuclear progressive
	Ex6	c.682T>C	p.S228P	AD	China	Nuclear (Nystagmus)
	Ex6	c.698G>A	p.R233H	AD	China	Nuclear (Nystagmus)
	Ex6	c.757T>C	p.X253RextX27	AD	UK	Nuclear cortical riders (Microcornea)
CRYBA4	Ex4	c.190G>T	p.G64W		China	Congenital nuclear (Microcornea)
	Ex4	c.206T>C	p.L69P	AD	India	? (Microphthalmia)
	Ex4	c.281T>C	p.F94S	AD	India	Lamellar

Gene	Exon/ Intron	DNA Change	Coding Change	Pattern of Inheritance	Origin	Cataract Phenotype
GJA3	Ex2	c.-39C>G		Cx	China	Age-related nuclear
	Ex2	c.5G>A	p.G2D	AD	China	Nuclear pulverulent, Posterior polar
	Ex2	c.7G>T	p.D3Y	AD	Honduras	Zonular pulverulent
	Ex2	c.32T>C	p.L11S	AD	Denmark	“Ant-egg”
	Ex2	c.56C>T	p.T19M	AD	India	Posterior polar
	Ex2	c.82G>A	p.V28M	AD	India	Total, anterior capsular, cortical
	Ex2	c.96C>A	p.F32L	AD	China	Nuclear pulverulent
	Ex2	c.98G>T	p.R33L	AD	India	Granular embryonal
	Ex2	c.130G>A	p.V44M	AD	China	Central nuclear (punctate cortical)
	Ex2	c.130G>A	p.V44M	AD	USA	?
	Ex2	c.134G>C	p.W45S	AD	China	Nuclear
	Ex2	c.139G>A	p.D47N	AD	China	Nuclear
	Ex2	c.176C>T	p.P59L	AD	USA	Nuclear punctate
	Ex2	c.176C>T	p.P59L	AD	Denmark	?
	Ex2	c.176C>T	p.P59L	AD	China	?
	Ex2	c.188A>G	p.N63S	AD	UK	Variable pulverulent
	Ex2	c.226C>G	p.R76G	AD	India	Total
	Ex2	c.227G>A	p.R76H	AD	Australia	Nuclear lamellar pulverulent
	Ex2	c.227G>A	p.R76H	AD	Denmark	Lamellar, sutural
	Ex2	c.260C>T	p.T87M	AD	India	“Pearl-box”
	Ex2	c.415G>A	p.V139M	Cx	China	Age-related cortical
	Ex2	c.560C>T	p.P187L	AD	UK	Zonular pulverulent
	Ex2	c.559C>T	p.P187S	AD	China	Nuclear pulverulent
	Ex2	c.563A>C	p.N188T	AD	China	Nuclear pulverulent
	Ex2	c.1137insC	p.S380QfsX87	AD	UK	Punctate
	Ex2	c.1143_1165del23	p.381fs*48	AD	China	Punctate nuclear
BFSP1	Ex6	c.736–957del	p.T246del74fsX6	AR	India	Cortical progressive, juvenile onset

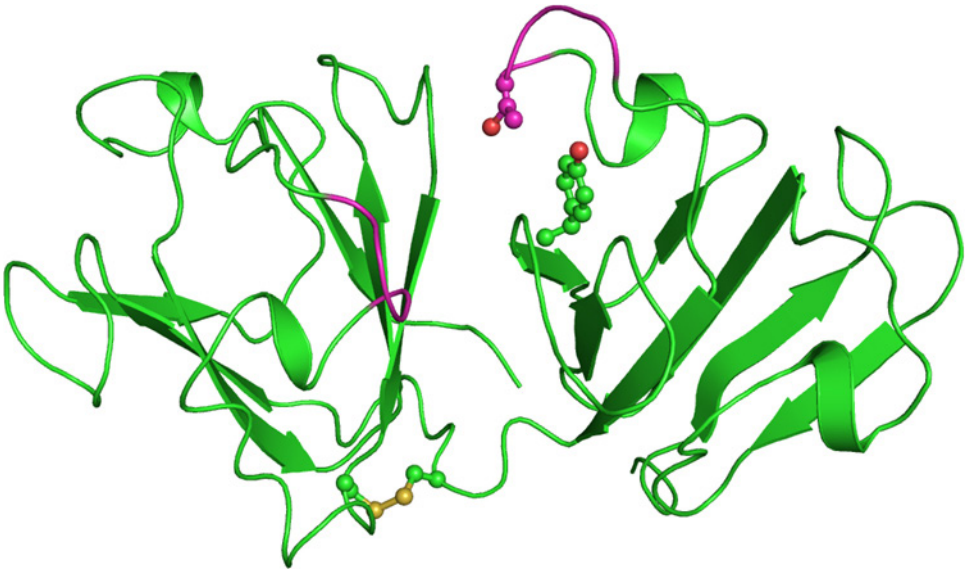

Figure 2. Cartoon representation of the model structure of the wild-type crystallin beta-A4 protein. The disulfide bridge and residues at the mutation site are shown as balls and sticks. The newly generated loops (residue 83–87 and 180–183) are in magenta.

movement of the loop, which imparts structural flexibility. As this protein is functional in its multimeric form, the increased flexibility in the mutant affects the stability of the oligomer as well as interactions with other partner proteins. The mutation (*CRYBA4:*p.T84A) is present in the modeled loop in the N-terminal domain of human crystallin β-A4. In this loop, two residues, Asn83 and Thr84, are involved in the hydrogen bond–mediated interactions with Gly159 and Gln161 of the C-terminal domain (Figure 4a). These interactions serve as a bridge between the two domains. The substitution of the hydrophobic Alanine (Ala) to Threonine (Thr) disrupts the intramolecular hydrogen bond, which reduces the inter-domain contact region as the Ala side chain is incapable of forming hydrogen bonds (Figure 4B). A slight broadening of the loop is also observed as the Asn83-mediated interactions are still conserved (Figure 4A,B). Thus, the mutation can lead to changes in the conformation of protein and its interaction with other partner proteins.

CRYBB1 is a major subunit of the β-crystallins and comprises 9% of the total soluble crystallin in the human lens [31]. A total of 42 nucleotide changes have been reported (Table 3). Of these, seven have been associated with congenital cataract [30] (Table 4). In this study, we detected four nucleotide variations (*CRYBB1:*p.D85N, *CRYBB1:*p.E75K, *CRYBB1:*p.E155K, and *CRYBB1:*rs57400078) in the *CRYBB1* gene. Three novel variations (*CRYBB1*:p.D85N, *CRYBB1:*p.E75K, and *CRYBB1:*p.E155K) were pathogenic according to the in silico analysis (Table 3). Most of the reported *CRYBB1* gene mutations occur in exon 6 (Table 4) [30], which encodes the Greek key IV and the COOH-terminal arm [32,33]. The *CRYBB1:*p.D85N and *CRYBB1:*p.E75K variations were found in the same patient with nuclear form of cataract whereas the *CRYBB1:*p.E155K change was detected in seven cases with different cataract morphology. The *CRYBB2* gene is one of the most important genes for lens transparency. We identified two novel variations (*CRYBB2:*p.D4N, *CRYBB2:*p.Q6Q) in the *CRYBB2* gene. The non-synonymous, novel change (*CRYBB2:*p.D4N) was found in one patient with lamellar cataract.

The crystal structure of the human *CRYBB1* protein (PDB ID: 1OKI) [34] was used as the template. The overall folds of *CRYBB1* and *CRYBA4* are similar (Figure 5) except in the loop region. The N-terminal domain of the *CRYBB1* protein harbors mutations (*CRYBB1:*p.E75K and *CRYBB1:*p.D85N) whereas *CRYBB1:*p.E155K occurs on the C-terminal domain. Both mutations are present on the surface of the protein (Figure 5) and are thus exposed to solvents and would be engaged in protein–protein interactions. The Glu75 (an acidic residue), a component of the β strand, is positioned to make two hydrogen bonds with the guanidine group of Arg60 present on the adjacent antiparallel β strand in the wild-type protein (Figure 6A). The mutation *CRYBB1:*p.E75K alters the environment and charge on the protein surface, disrupting the ionic interaction between Glu75 and Arg60 (Figure 6B). Thus, *CRYBB1:*p.E75K changes the electrostatic potential of the protein surface, which could affect interactions with other interacting partner proteins.

The other change (*CRYBB1:*p.D85N) occurs in the single turn helix conformation of the *CRYBB1* protein. Asp85 is involved in hydrogen bonding with the amide nitrogen of the Asn82 side chain present on the adjacent loop (Figure 7A).

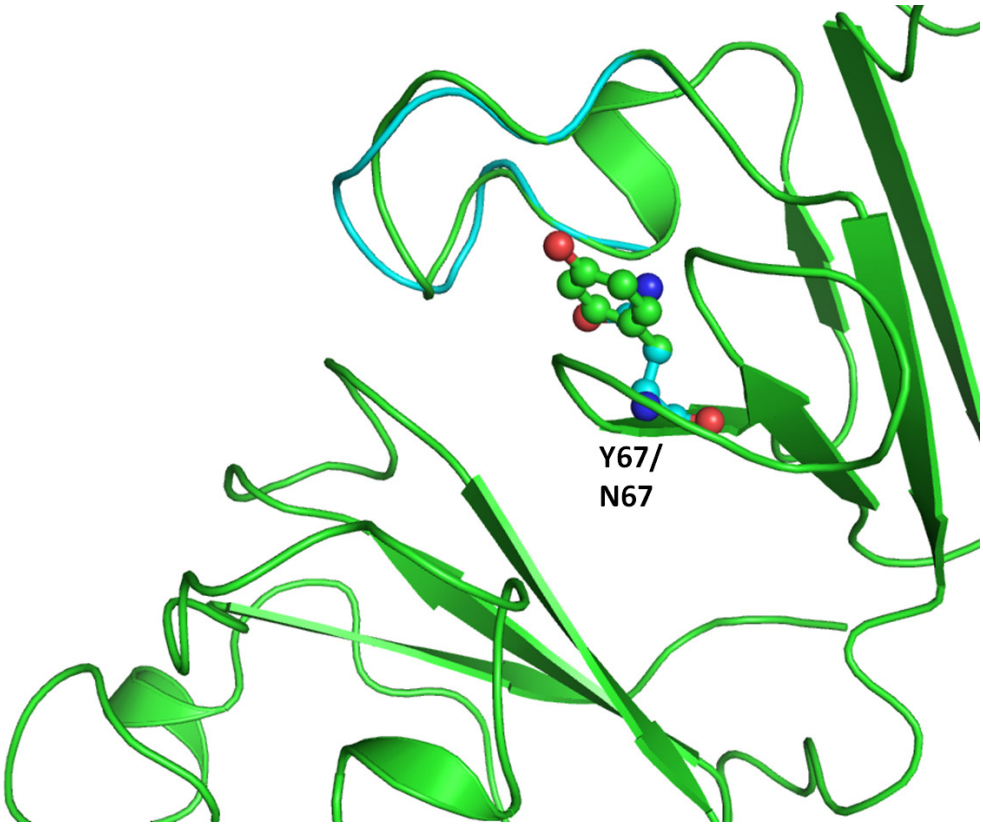

Figure 3. Superimposition of the model structure of the crystallin beta-A4 protein mutant (Tyr67Asn; in cyan) on the wild-type (in green).

Aspartate (Asp) and Asn differ only in the side chain group, with a carboxyl group in the former and an amide in the latter. Thus, in the *CRYBB1:*p.D85N mutant, the hydrogen bond–mediated interaction is retained (Figure 7B). In the other mutation (*CRYBB1:*p.E155K) in the *CRYBB1* protein, Glu155, located on a surface loop, forms two hydrogen bonds, one with the side chain amide nitrogen and the other with the main chain nitrogen of Asn162 present on the same loop (Figure 8A). Since the change occurs on the protein surface, the elongated side chain does not perturb the protein conformation. However, to accommodate the change and impart stability to the loop, the amide group of Asn162 flips by approximately 180°. This results in the formation of a hydrogen bond with the side chain nitrogen atom of the mutated residue Lys155 (Figure 8B). The change in negatively charged Glu155 with positively charged Lys155 affects the electrostatic potential of the surface, which could be vital for binding with other interacting partners. Thus, the modeling studies indicate that the mutation in the *CRYBA4* and *CRYBB1* proteins alters the internal conformation of the protein and reduces the stability of the proteins. Thus, the observed mutations could affect the function of the protein, including its ability to bind to its interacting partners.

The eye lens is an avascular structure, and intercellular transport of small biomolecules (<1 kDa) is mediated through connexins (Cx) that encode gap junction channel proteins [35,36]. *GJA3* encodes a protein containing 435 amino acids and is present in specialized lens fibers, which constitute the majority of the lens [37]. Similar to other connexin proteins, connexin 46 (Cx46) consists of four transmembrane domains (TM1–TM4), two extracellular loops (E1 and E2), a cytoplasmic loop (CL) between TM2 and TM3, and cytoplasmic N-terminal (NT) and C-terminal (CT) domains [38]. We detected a non-synonymous variation *GJA3:*p.M1V, resulting in the formation of a truncated protein, which leads to opacification in the lens. We found this variation in a patient with anterior polar cataract but not in any of the controls.

BFSP1 and *BFSP2* are highly expressed intermediate filaments and when mutated can cause cataract [39]. A total of 339 variations (Table 3) have been reported in *BFSP1*, but only one has been associated with an autosomal recessive mutation arising from a consanguineous marriage [40] (Table 4) [30]. We detected three nucleotide variations, two of which were novel and non-synonymous (*BFSP1:*p.D395N, *BFSP1:*p.E424K); the other one (*BFSP1:*p.L44L) is reported. None of the mutations were pathogenic. Modeling studies of

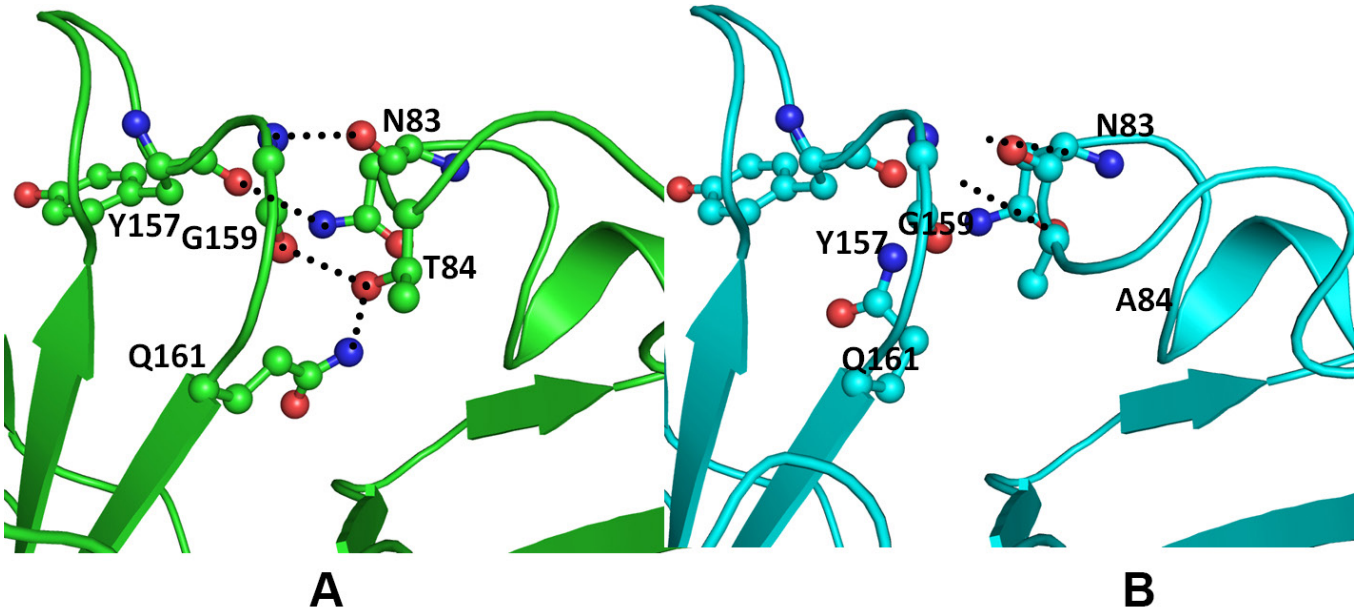

Figure 4. Model structure representation of wild (green) and Thr84Ala mutant (cyan) crystallin beta-A4 (*CRYBA4*) protein. **A**: The Thr84 hydroxyl group forms a hydrogen bond with the main chain carbonyl oxygen of Gly159 and the amide nitrogen of the Gln161 side chain. Asn83 is involved in the hydrogen-bonded interaction with the Gly159 main chain nitrogen atom and the Tyr157 main chain oxygen. **B**: The Ala84 mutant cannot be involved in this interaction. The important interacting residues are rendered as balls and sticks, and the hydrogen bonds are depicted as black dotted lines.

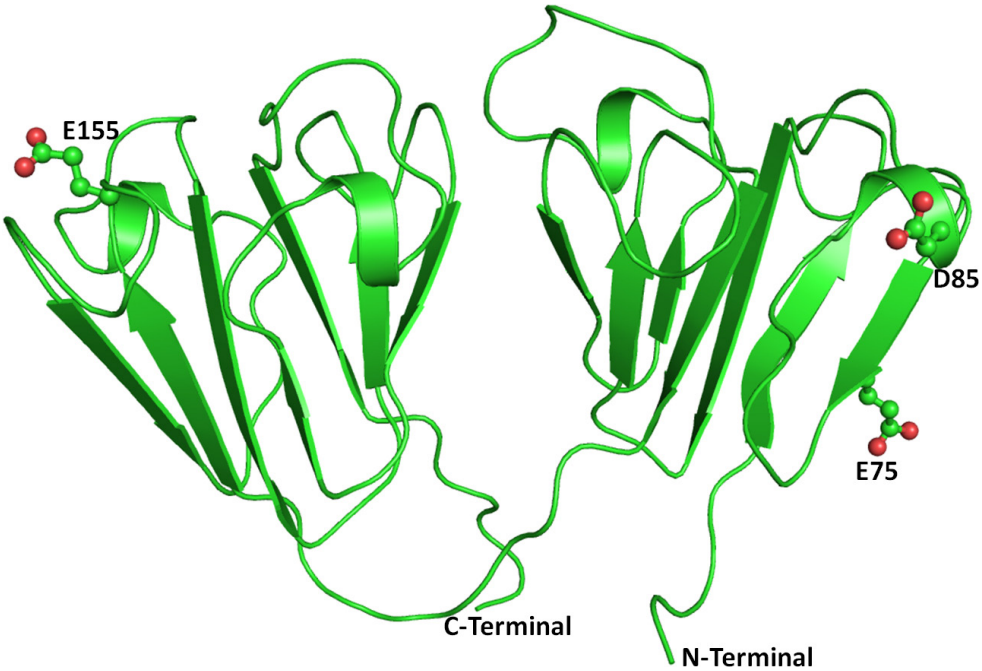

Figure 5. Cartoon representation of the crystal structure of the wild-type beta crystallin B1 protein. The residues at the mutation site are shown as balls and sticks.

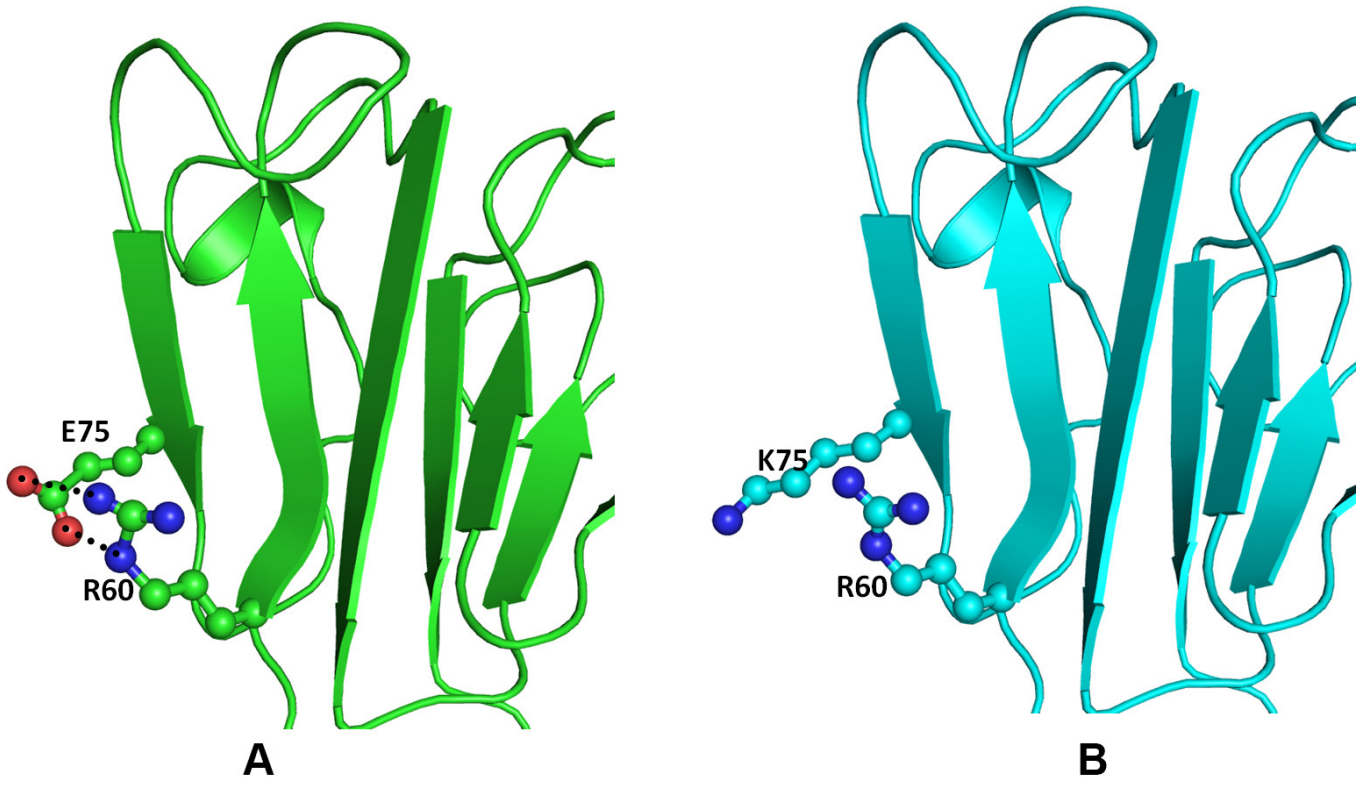

Figure 6. Model structure representation of the wild and mutant (Glu75Lys) proteins. **A**: Beta crystallin B1 (*CRYBB1*) protein showing the important residues (balls and sticks) and the hydrogen bonds (black dotted lines). **B**: The contacts are lost in the mutant.

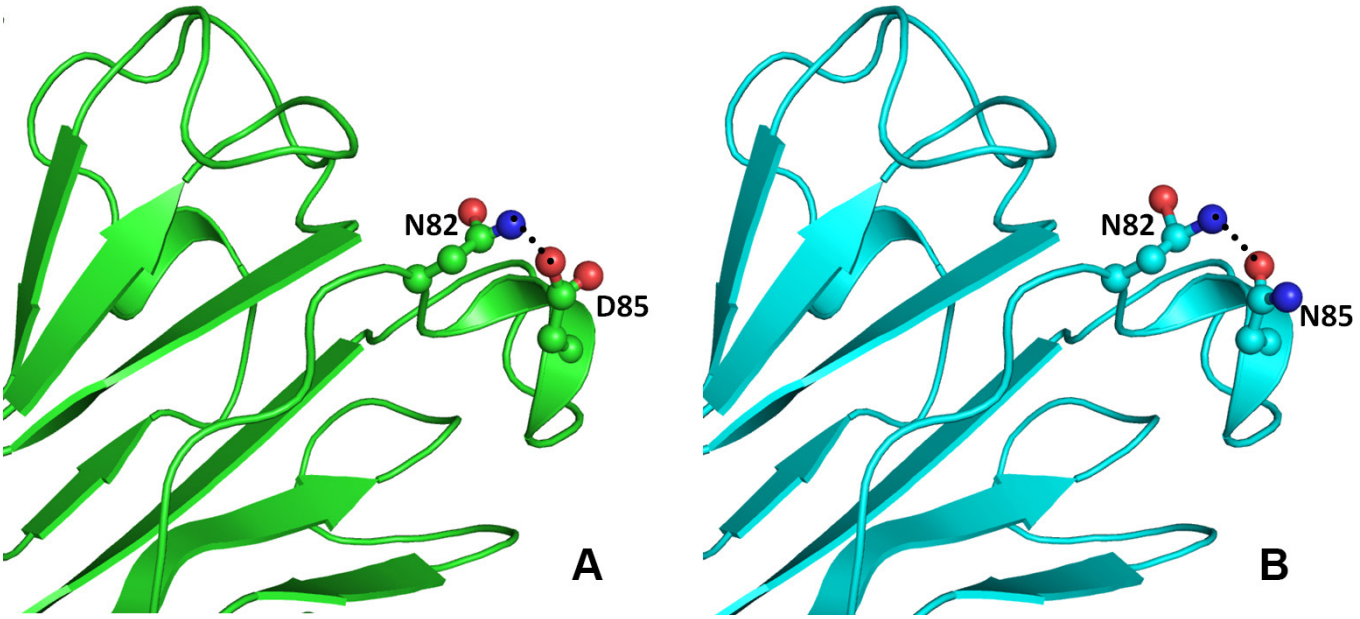

Figure 7. Model structure representation of the wild and mutant (Asp85Asn) proteins. In both structures (**A** and **B**), the interaction of residues as balls and sticks and hydrogen bonds as black dotted lines in beta crystallin B1 protein is same.

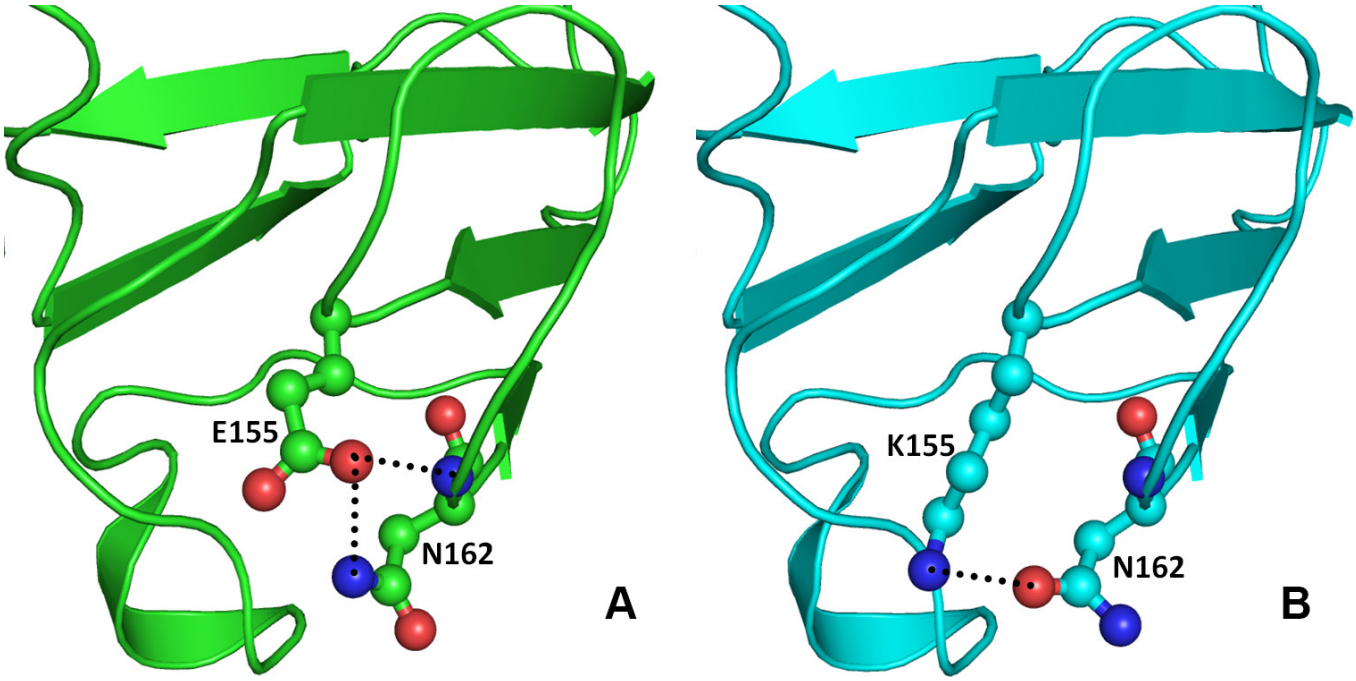

Figure 8. Model structure representation of the wild and mutant (Glu155Lys) proteins. **A**: Glu155 forms two hydrogen bonds, one with side chain amide nitrogen and another with main chain nitrogen of Asn162 which stabilizes and maintains the loop conformation essential for interactions with other proteins. **B**: In mutant (Glu155Lys) proteins, Asn162 flips by approximately 180° and forms hydrogen bond with the side chain nitrogen atom of the mutated residue Lys155.

the non-synonymous changes were not possible due to the lack of a suitable homolog model. The chi-square test and Fisher's exact test were used to compare differences in the frequency of the sequence variants, between the controls and individuals with congenital cataract. Chi-square analysis and Fisher's exact tests did not show any significant difference between the groups for any of the sequence variants.

This study identified variations in 100 patients with congenital cataract in a northern Indian population. Pathogenic changes in the crystallin family accounts for 10% of the population whereas a study in southern Indian patients with congenital cataract [41] reported only 16.6% variations in the crystallin family. Connexins account for 1% of the population compared to the 5.5% reported by the earlier study [41]. Five variations (*CRYBA4*:p.Y67N, *CRYBB1*:p.D85N, *CRYBB1*:E75K, *CRYBB1*:E155K, *GJA3*:p.M1V) detected in this study are predicted to cause cataract. This study further confirms that the crystallin beta cluster on chromosome 22, *GJA3*, and *BFSP1* plays a major role in maintaining lens transparency. The disease showed marked clinical and genetics (locus and allelic) heterogeneity. This study also expands the mutation spectrum of the genes associated with congenital cataract. Other genes might be involved in the growth, development, differentiation, and maintenance of lens transparency.

The study of genes related to congenital cataract and knowledge about the molecular mechanisms of their origin, in the near future, could be extended to age-related cataracts, which remain the leading cause of blindness worldwide. The accumulation of information about the physiology of the lens and the factors associated with the formation of senile cataracts acquired through genetic studies of congenital hereditary form could lead to new treatments and techniques to prevent different forms of cataract.

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REFERENCES

1. Reddy MA, Francis PJ, Berry V, Bhattacharya SS, Moore AT. Molecular genetic basis of inherited cataract and associated phenotypes. Surv Ophthalmol 2004; 49:300-15. [PMID: 15110667].
2. Gilbert C, Foster A. Childhood blindness in the context of VISION 2020–the right to sight. Bull World Health Organ 2001; 79:227-32. [PMID: 11285667].
3. Rahi JS, Sripathi S, Gilbert CE, Foster A. Childhood blindness in India: causes in 1318 blind school students in nine states. Eye (Lond) 1995; 9:545-50. [PMID: 8543070].
4. Apple DJ, Ram J, Foster A, Peng Q. Elimination of cataract blindness: a global perspective entering the new millenium. Surv Ophthalmol 2000; 45:S1-196. [PMID: 11291895].
5. Francis PJ, Berry V, Bhattacharya SS, Moore AT. The genetics of childhood cataract. J Med Genet 2000; 37:481-8. [PMID: 10882749].
6. Gill D, Klose R, Munier FL, McFadden M, Priston M, Billingsley G, Ducrey N, Schorderet DF, Heon E. Genetic heterogeneity of the Coppock-like cataract: a mutation in CRYBB2 on chromosome 22q11.2. Invest Ophthalmol Vis Sci 2000; 41:159-65. [PMID: 10634616].
7. Scott MH, Hejtmancik JF, Wozencraft LA, Reuter LM, Parks MM, Kaiser-Kupfer MI. Autosomal dominant congenital cataract. Interocular phenotypic variability. Ophthalmology 1994; 101:866-71. [PMID: 8190472].
8. Héon E, Priston M, Schorderet DF, Billingsley GD, Girard PO, Lubsen N, Munier FL. The gamma-crystallins and human cataracts: a puzzle made clearer. Am J Hum Genet 1999; 65:1261-7. [PMID: 10521291].
9. Hejtmancik JF. Congenital cataracts and their molecular genetics. Semin Cell Dev Biol 2008; 19:134-49. [PMID: 18035564].
10. Hejtmancik JF, Smaoui N. Molecular genetics of cataract. Dev Ophthalmol 2003; 37:67-82. [PMID: 12876830].
11. Bhat SP. Crystallins, genes and cataract. Prog Drug Res 2003; 60:205-62. [PMID: 12790344].
12. Goodenough DA. The crystalline lens. A system networked by gap junctional intercellular communication. Semin Cell Biol 1992; 3:49-58. [PMID: 1320431].
13. Hansen L, Yao W, Eiberg H, Kjaer KW, Baggesen K, Hejtmancik JF, Rosenberg T. Genetic heterogeneity in microcornea-cataract: five novel mutations in CRYAA, CRYGD, and GJA8. Invest Ophthalmol Vis Sci 2007; 48:3937-44. [PMID: 17724170].
14. Jakobs PM, Hess JF, FitzGerald PG, Kramer P, Weleber RG, Litt M. Autosomal-dominant congenital cataract associated with a deletion mutation in the human beaded filament protein gene BFSP2. Am J Hum Genet 2000; 66:1432-6. [PMID: 10739768].
15. Forshew T, Johnson CA, Khaliq S, Pasha S, Willis C, Abbasi R, Tee L, Smith U, Trembath RC, Mehdi SQ, Moore AT, Maher ER. Locus heterogeneity in autosomal recessive congenital cataracts: linkage to 9q and germline HSF4 mutations. Hum Genet 2005; 117:452-9. [PMID: 15959809].
16. Graw J. Cataract mutations and lens development. Prog Retin Eye Res 1999; 18:235-67. [PMID: 9932285].
17. Beby F, Morle L, Michon L. M B, Edery P, Burillon C, Denis P. J Fr Ophtalmol 2003; 26:400-8. The genetics of hereditary cataract.[PMID: 12843900].
18. Amaya L, Taylor D, Russell-Eggitt I, Nischal KK, Lengyel D. The morphology and natural history of childhood cataracts. Surv Ophthalmol 2003; 48:125-44. [PMID: 12686301].
19. Forster JE, Abadi RV, Muldoon M, Lloyd IC. Grading infantile cataracts. Ophthalmic Physiol Opt 2006; 26:372-9. [PMID: 16792736].
20. Somasundaram T, Bhat SP. Developmentally dictated expression of heat shock factors: exclusive expression of HSF4 in the postnatal lens and its specific interaction with alphaB-crystallin heat shock promoter. J Biol Chem 2004; 279:44497-503. [PMID: 15308659].
21. Berry V, Francis P, Reddy MA, Collyer D, Vithana E, MacKay I, Dawson G, Carey AH, Moore A, Bhattacharya SS, Quinlan RA. Alpha-B crystallin gene (CRYAB) mutation causes dominant congenital posterior polar cataract in humans. Am J Hum Genet 2001; 69:1141-5. [PMID: 11577372].
22. Sambrook J, Russell DW. Purification of nucleic acids by extraction with phenol:chloroform. CSH Protoc. 2006.
23. Schwarz JM, Rodelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods 2010; 7:575-6. [PMID: 20676075].
24. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res 2003; 31:3812-4. [PMID: 12824425].
25. Sunyaev S, Ramensky V, Koch I, Lathe W 3rd, Kondrashov AS, Bork P. Prediction of deleterious human alleles. Hum Mol Genet 2001; 10:591-7. [PMID: 11230178].
26. Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. Nucleic Acids Res 2002; 30:3894-900. [PMID: 12202775].
27. Inc AS. Discovery Studio Modeling Environment. San Diego. 2007.
28. Slingsby C, Clout NJ. Structure of the crystallins. Eye (Lond) 1999; 13:Pt 3b395-402. [PMID: 10627816].
29. Billingsley G, Santhiya ST, Paterson AD, Ogata K, Wodak S, Hosseini SM, Manisastry SM, Vijayalakshmi P, Gopinath PM, Graw J, Heon E. CRYBA4, a novel human cataract gene, is also involved in microphthalmia. Am J Hum Genet 2006; 79:702-9. [PMID: 16960806].
30. Shiels A, Bennett TM, Hejtmancik JF. Cat-Map: putting cataract on the map. Mol Vis 2010; 16:2007-15. [PMID: 21042563].
31. Srivastava K, Gupta R, Chaves JM, Srivastava OP. Truncated human betaB1-crystallin shows altered structural properties and interaction with human betaA3-crystallin. Biochemistry 2009; 48:7179-89. [PMID: 19548648].

32. Mackay DS, Boskovska OB, Knopf HL, Lampi KJ, Shiels A. A nonsense mutation in CRYBB1 associated with autosomal dominant cataract linked to human chromosome 22q. Am J Hum Genet 2002; 71:1216-21. [PMID: 12360425].

33. Willoughby CE, Shafiq A, Ferrini W, Chan LL, Billingsley G, Priston M, Mok C, Chandna A, Kaye S, Heon E. CRYBB1 mutation associated with congenital cataract and microcornea. Mol Vis 2005; 11:587-93. [PMID: 16110300].

34. Van Montfort RL, Bateman OA, Lubsen NH, Slingsby C. Crystal structure of truncated human betaB1-crystallin. Protein Sci 2003; 12:2606-12. [PMID: 14573871].

35. Kar R, Batra N, Riquelme MA, Jiang JX. Biological role of connexin intercellular channels and hemichannels. Arch Biochem Biophys 2012; 524:2-15. [PMID: 22430362].

36. Molina SA, Takemoto DJ. The role of Connexin 46 promoter in lens and other hypoxic tissues. Commun Integr Biol 2012; 5:114-7. [PMID: 22808311].

37. Pfenniger A, Wohlwend A, Kwak BR. Mutations in connexin genes and disease. Eur J Clin Invest 2011; 41:103-16. [PMID: 20840374].

38. Mathias RT, White TW, Gong X. Lens gap junctions in growth, differentiation, and homeostasis. Physiol Rev 2010; 90:179-206. [PMID: 20086076].

39. Song S, Hanson MJ, Liu BF, Chylack LT, Liang JJ. Protein-protein interactions between lens vimentin and alphaB-crystallin using FRET acceptor photobleaching. Mol Vis 2008; 14:1282-7. [PMID: 18618007].

40. Ramachandran RD, Perumalsamy V, Hejtmancik JF. Autosomal recessive juvenile onset cataract associated with mutation in BFSP1. Hum Genet 2007; 121:475-82. [PMID: 17225135].

41. Devi RR, Yao W, Vijayalakshmi P, Sergeev YV, Sundaresan P, Hejtmancik JF. Crystallin gene mutations in Indian families with inherited pediatric cataract. Mol Vis 2008; 14:1157-70. [PMID: 18587492].

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