Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jasi

Case Report

A novel p.Glu75Lys mutation in CRYBB1 with low penetrance in an Indian family with nuclear form of congenital cataract

Manoj Kumar [a], *Sudarshan Khokhar* [c], *Punit Kaur* [b], *Tushar Agarwal* [c], *Rima Dada* [a,*]

[a] *Molecular Reproduction and Genetics Laboratory, Department of Anatomy, All India Institute of Medical Sciences, New Delhi 110029, India*

[b] *Department of Biophysics, All India Institute of Medical Sciences, New Delhi 110029, India*

[c] *Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi 110029, India*

ARTICLE INFO

Article history:
Received 9 September 2013
Accepted 6 November 2014
Available online 4 December 2014

Keywords:
Congenital cataract
Beta Crystallin B1
Autosomal dominant

ABSTRACT

Introduction: To screen *CRYAA*, *CRYAB*, *CRYGC*, *CRYGD*, *CRYGS*, *CRYBA1*, *CRYBA4*, *CRYBB1*, *CRYBB2*, *CRYBB3*, *BFSP1*, *GJA3*, *GJA8* and *HSF4* gene in an Indian family having congenital cataract.

Methods: The congenital cataract family presented at Dr. R.P. Centre for Ophthalmic Sciences, a tertiary research and referral hospital (AIIMS, New Delhi, India). *CRYAA*, *CRYAB*, *CRYGC*, *CRYGD*, *CRYGS*, *CRYBA1*, *CRYBA4*, *CRYBB1*, *CRYBB2*, *CRYBB3*, *BFSP1*, *GJA3*, *GJA8* and *HSF4* genes were amplified. Protein structure differences analysis was performed using Discovery Studio (DS) 2.0.

Results: A three-generation Indian family with diagnosis of congenital cataract presented with bilateral dense nuclear opacities in the affected individuals. Direct sequencing analysis of 14 genes identified a novel and pathogenic nucleotide variation p.E75K in *CRYBB1* gene. The mutation p.Glu75Lys alters the environment and charge on the protein surface, disrupting ionic interaction between Glu75 and Arg60. Thus p.Glu75Lys changes the electrostatic potential of the protein surface which could affect the interactions with other interacting partner proteins.

Discussions: In this case a novel heterozygous p.Glu75Lys mutation in *CRYBB1* was detected in an Indian family with nuclear cataract. The mutation was inherited in autosomal dominant pattern and showed reduced penetrance. Identification and characterization of this mutation further confirm the importance of protein interactions in maintaining lens transparency, and provides insight into the molecular mechanism underlying the pathogenesis of human congenital cataract.

* *Corresponding author*. Tel.: +91 11 26593517; fax: +91 11 26588663.
E-mail address: rima_dada@rediffmail.com (R. Dada).

http://dx.doi.org/10.1016/j.jasi.2014.11.005

1. 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 visual impairment and blindness during childhood.[1] Its prevalence is 0.6–6 per 10,000 live births with an incidence of 2.2–2.49 per 10,000 live births.[2] It is estimated 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.[3] The population of India in 2001 was estimated to be 1.03 billion, approximately 420 million of whom are children under 16 years of age (40.9%). Congenital cataract is responsible for about 12% of childhood blindness in India.[4,5]

Till date more than 20 genes of 40 genetic loci (mapped for congenital cataract) have been identified with different types of mutations.[6] More than 50% of congenital cataract families harbor mutations in crystallin genes (crystallin alpha A (*CRYAA*), crystallin alpha B (*CRYAB*), crystallin beta B1 (*CRYBB1*), crystallin beta B2 (*CRYBB2*), crystallin beta B3 (*CRYBB3*), crystallin beta A1 (*CRYBA1*), crystallin beta A4 (*CRYBA4*), crystallin gamma C (*CRYGC*), crystallin gamma D (*CRYGD*) and crystallin gamma S (*CRYGS*). It has been reported that approximately 25% of affected families have disease causing mutation in gap junction protein alpha 8 (*GJA8*), gap junction protein alpha 3 (*GJA3*).[1] The other genes encoding cytoskeletal protein (*BFSP1*: beaded filament structural protein 1[7]) have been reported to cause congenital cataract in patients associated with other anterior segment anomalies.[8] Crystallins are the major cytoplasmic proteins of the lens and their stability and appropriate interactions are critical for lens transparency. Crystallin genes encode more than 95% of the water soluble structural proteins and their encoded proteins account for more than 30% of its mass. In the mature human lens, α-crystallin makes up roughly 40%, β-crystallin 35%, and γ-crystallin 25% of the total crystallin protein[8] and are good candidate genes for screening incongenital cataract patients. α-crystallin is essential for lens transparency and accounts for nearly 50% of the protein mass in human lens. αA-crystallin/HSPB4 is a member of the small heat shock protein family, which also includes αB-crystallin/HSPB5 and Hsp27/HSPB1.[9]

Mutations of *CRYBB1* are common genetic lesions causing different types of congenital cataract. *CRYBB1* is a major subunit of the β-crystallins and comprises 9% of the total soluble crystallin in the human lens.[9] In this study, we detected a novel E75K mutation in CRYBB1 gene in an Indian family in which three members having nuclear cataract harbor heterozygous mutation.

2. Materials and methods

2.1. Patient ascertainment and clinical examination

The proband was clinically diagnosed with nuclear form of congenital cataract, at the Dr. R.P. Centre for Ophthalmic Sciences (AIIMS, New Delhi, India). Affected status was determined by a history of cataract extraction or ophthalmologic examination. On pedigree analysis three member of the family were found to be affected with congenital cataract. All other factors responsible for congenital cataract were ruled out with negative history of intrauterine infections such as rubella, TORCHES (TOxoplasmosis, Rubella, Cytomegalovirus, HErpes simplex, Syphilis), and traumatic cataract. Informed consent in accordance with the Declaration of Helsinki was obtained from all participants or their parents and ethical approval was taken from the institutional review board (IRB#00006862; All India Institute of Medical Sciences, Delhi, India).

2.2. Polymerase chain reaction and DNA sequencing

Genomic DNA was extracted from blood samples of congenital cataract patients and controls, using organic method.[10] The exon-intron and promoter regions of the 14 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.[1] Amplified PCR products were purified using a gel/PCR DNA fragments extraction kit. Purified PCR products were sequenced (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) (Table 2), using ClustalW2 (multiple sequence alignment program for DNA; European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK).

2.3. Bioinformatics analysis

MutationTaster,[11] SIFT (Sorting Intolerant From Tolerant)[12] and PolyPhen-2[13] were used for rapid evaluation of the disease causing potential ofDNA sequence alterations. The variations were considered as pathogenic only when the outcome of two out of three applications suggested them to be disease causing.

2.4. Protein modeling

Prediction of structure differences between wild and mutant were performed using Discovery Studio (DS) 2.0 (Accelrys Inc., San Diego, CA, U.S.A.).[14] The model structure of the three mutant proteins were developed and refined by minimization programs in the presence of CHARM force field in a manner similar to the structure of A4 protein.

3. Results

3.1. Clinical features of the family

A three-generation Indian family (Fig. 1a) with diagnosis of bilateral dense nuclear opacities (Fig. 1b) presented to Dr. R.P. Centre for Ophthalmic Sciences. The average corneal diameter was 10.4 mm. The proband was a six year old female. Clinical features of cataract were asymmetric in two eyes of affected individuals. According to the history and medical records, all affected individuals showed cataract within the first year after birth. None of the affected members had

Table 1 – Primers of *CRYAA*, *CRYAB*, *CRYBA1*, *CRYBA4*, *CRYBB1*, *CRYBB2*, *CRYBB3*, *CRYGC*, *CRYGD*, *CRYGS*, *BFSP1*, *GJA3*, *GJA8* and *HSF4* genes and annealing temperatures.

Gene name	Primer name	Sequence	Anne temp	Product size (bp)
CRYAA	Ex-1-F	5′-CTCCAGGTCCCCGTGGTACCA-3′	62 °C	254
	Ex-1-R	5′-GCGAGGAGAGGCCAGCACCAC-3′		
	Ex-2-F	5′-CTGTCTCTGCCAACCCCAGCAG-3′	65 °C	223
	Ex-2-R	5′-CCCCTCTCCCACCTCTCAGTG-3′		
	Ex-3-F	5′-GGCAGCTTCTCTGGCATGGGG-3′	65 °C	312
	Ex-3-R	5′-GGGGAGCCAGCCGAGGCAATG-3′		
CRYAB	Ex-1-F	5′-CCTGACATCACCATTCCAGA-3′	57 °C	399
	Ex-1-R	5′-AGGCAGGGTAGGAAAGGAAA-3′		
	Ex-2-F	5′-TGCAGAATAAGACAGCACCTG-3′	57 °C	289
	Ex-2-R	5′-CCAGCCTCCAAAGCTGATAG-3′		
	Ex-3-F	5′-TGTTGTCATGGCATTTGGTC-3′	57 °C	376
	Ex-3-R	5′-TCATTCACTGGTGGGGAAA-3′		
CRYBA1	Ex-1-F	5′-GGTCTTAGGAAGATCCCAAG-3′	54 °C	394
	Ex-1-R	5′-AAGGAGAGGAAGGGCAAGGG-3′		
	Ex-2-F	5′-CGTGTGTGCTCTGTCTTCC-3′	58 °C	179
	Ex-2-R	5′-GGTCAGTCACTGCCTTATGG-3′		
	Ex-3-F	5′-TCCTTCCTTCAGCTTTGG-3′	54 °C	520
	Ex-3-R	5′-TCCTTCCTTCAGCTTTGG-3′		
	Ex-4-F	5′-CAAACACTACATGTCTTTGG-3′	48 °C	210
	Ex-4-R	5′-CTTGCTACCCTCATATGC-3′		
	Ex-5-F	5′-TGCTTCCTTGTATAATCC-3′	52 °C	306
	Ex-5-R	5′-ACTATTGATGCAACCTCAGG-3′		
	Ex-6-F	5′-GGTTTGCTACCATTATCTTGG-3′	54 °C	183
	Ex-6-R	5′-CATGCTTGAGGAATTATCG-3′		
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 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′		
CRYBB3	Ex-2-F	5′-AACACCTGGCTTCTCCCGGGTGG-3′	57 °C	282
	Ex-2-R	5′-TGCTGCTTGGCTGGGAAGATGACCC-3′		
	Ex-3-F	5′-GTACCTCCCTCTAATGCCCAAAGG-3′	57 °C	328
	Ex-3-R	5′-AACAGATCTAGAGCTCAGACTGGGGC-3′		
	Ex-4-F	5′-AAGGTTCAAGGTCAGCAGCTCTTGG-3′	57 °C	359
	Ex-4-R	5′-AGCTTGTGCAATGAGCTGCCTGACCAGC-3′		
	Ex-5-F	5′-TGATTCTTTCCGGCATCTGGAGCCTC-3′	57 °C	337
	Ex-5-R	5′-AGAAACTATGGGCACTGATTCTGTTTGG-3′		
	Ex-6-F	5′-AGGAATGTAGGCAGGCAGAGTGCATGG-3′	58 °C	347
	Ex-6-R	5′-GCCCTCGCCCCAACCCTGGAGCCTCC-3′		

Table 1 – (*continued*)

Gene name	Primer name	Sequence	Anne temp	Product size (bp)
CRYGC	Ex-1-F	5′-TTTCCAGTGAATGCAGGATG-3′	58 °C	300
	Ex-1-R	5′-GAAGGCCCTGTCCTCATAGA-3′		
	Ex-2-F	5′-GGAAGGTGAGCAGAACACAA-3′	58 °C	476
	Ex-2-R	5′-TGGCTTATTCAGGTCTCTGATC-3′		
	Ex-3-F	5′-ATGACAATTCCATGCCACAA-3′	58 °C	595
	Ex-3-R	5′-CAACGRCTGAGGCTTGTTCA-3′		
CRYGD	Ex-1-F	5′-AGAACACGAAAATGCCCTTG-3′	58 °C	248
	Ex-1-R	5′-GTCTCACAGGCCTGCTCCT-3′		
	Ex-2-F	5′-AGCCATGGGGAAGGTGAG-3′	58 °C	486
	Ex-2-R	5′-TTCATCTTTTGTCCACTCTCAGTT-3′		
	Ex-3-F	5′-GCTGGACTGCCTAACAATGC-3′	58 °C	498
	Ex-3-R	5′-CACATCTTGGTTGCCATTTFG-3′		
CRYGS	Ex-1-F	5′-TTGACTGAAACCAGCCCATA-3′	58 °C	247
	Ex-1-R	5′-TCTCAATCCCAGTTCTCTAACCA-3′		
	Ex-2-F	5′-CTCAAAATTGAGGTGAAAGGAA-3′	58 °C	478
	Ex-2-R	5′-AGCAGCCAACAAGCAGCTA-3′		
	Ex-3-F	5′-CATGGTGAGATGGGAGTTCA-3′	58 °C	499
	Ex-3-R	5′-GGATGCATGCCAACTGTTTT-3′		
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′		
GJA8	Ex-1A-F	5′-TATGGGCGACTGGAGTTTCCT-3′	60 °C	310
	Ex-1A-R	5′-CTCCATGCGGACGTAGTGCAC-3′		
	Ex-1B–F	5′-CTCTGGGTGCTGCAGATCATC-3′	60 °C	419
	Ex-1B-R	5′-CACAGAGGCCACAGACAACAT-3′		
	Ex-1C–F	5′-CACTACTTCCTGTACGGGTTC-3′	60 °C	450
	Ex-1C-R	5′-CTCTTGGTAGCCCCGGGACAA-3′		
	Ex-1D-F	5′-GTCTCCTCCATCCAGAAAGCC-3′	60 °C	534
	Ex-1D-R	5′-TCATACGGTTAGATCGTCTGA-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′		
	Ex-9-F	5′-CTCTCCTGAGTCACCCAAGC-3′	58 °C	688
	Ex-9-R	5′-CTCATGAAGCTGACCCACCT-3′		
HSF4	Ex-1-F	5′-CCTCCACTCCACTCCACTCCACAC-3′	60 °C	647
	Ex-1-R	5′-GACCAGCCCTTCCCATCTCC-3′		
	Ex-2,3,4-F	5′-TGGTCTAGCTCAGGGTCACATTG-3′	57 °C	984
	Ex-2,3,4-R	5′-TCCCAGAGATCGCCCATAAGTC-3′		
	Ex-5,6-F	5′CAGTCAGCGGGGTGGTCTC-3′	58 °C	454
	Ex-5,6-R	5′-GGGGGTCTTGGAAGTGGGTGTG-3′		
	Ex-7,8-F	5′-GGTTCCCTCCCTCCCCTCTC-3′	60 °C	766
	Ex-7,8-R	5′-CTCCCTCTACCCCTACAGCCATCT-3′		
	Ex-9-F	5′-GGCTGTAGGGGTAGAGGGAGAAGT-3′	55 °C	405
	Ex-9-R	5′-ATGCTGGGGCCAAGAAATCA-3′		
	Ex-10,11-F	5′-GGGAACTTAATGCGGGGTGGACA-3′	58 °C	456
	Ex-10,11-R	5′-GAGGGGGACAGCTGGGAAATCA-3′		
	Ex-12,13-F	5′-TGGGTCGGGGAGGGCAGAG-3′	60 °C	811
	Ex-12,13-R	5′-GCAGGGATAGTCGGGGTAGTGGAG-3′		

Table 2 – Genomic information of all the genes referred in this study.

Gene	Genomic DNA	mRNA	Protein
CRYAA	NC_000021.8	NM_000394.2	NP_000385.1
CRYAB	NC_000011.9	NM_001885.1	NP_001876.1
CRYBA1	NC_000017.10	NM_005208.4	NP_005199.2
CRYBA4	NC_000022.10	NM_001886.2	NP_001877.1
CRYBB1	NC_000022.10	NM_001887.3	NP_001878.1
CRYBB2	NC_000022.10	NM_000496.2	NP_000487.1
CRYBB3	NC_000022.10	NM_004076.3	NP_004067.1
CRYGC	NC_000002.11	NM_020989.3	NP_066269.1
CRYGD	NC_000002.11	NM_006891.3	NP_008822.2
CRYGS	NC_000003.11	NM_017541.2	NP_060011.1
GJA3	NC_000013.10	NM_021954.3	NP_068773.2
GJA8	NC_000001.10	NM_005267.4	NP_005258.2
HSF4	NC_000016.9	NM_001538.3	NP_001529.2
BFSP1	NC_000020.10	NM_001195.3	NP_001186.1

nystagmus or amblyopia. There was no family history of other ocular or systemic abnormalities. All the affected members (III.1, III.4 and I.4) of the family were heterozygous for the mutation with nuclear form of cataract whereas the siblings (III.2 and III.3) and father (II.8) of the proband showed no mutation. Although the mother (II.9) of the proband was not affected but on sequence analysis she was found to be heterozygous for the mutation and the mutant allele showed incomplete or reduced penetrance. The family showed autosomal dominant pattern of inheritance.

3.2. *Molecular analysis*

Direct sequencing analysis of 14 genes identified a novel and pathogenic variation in *CRYBB1* gene. The heterozygous non synonymous mutation at nucleotide codon c.223 resulted in change of Guanine to Adenine (Fig. 1c) which leads to the replacement of glutamic acid (E) amino acid with lysine (K) at position 75. The mutation was found in both the affected (III.1 and III.4) siblings, father (II.8) showed wild genotype, the mother was heterozygous for this change but did not show lens opacification. Multiple sequence alignment of different orthologs showed that the mutation occurred in a highly conserved functionally important region of the *CRYBB1* protein which is critical for protein function (Fig. 1d).

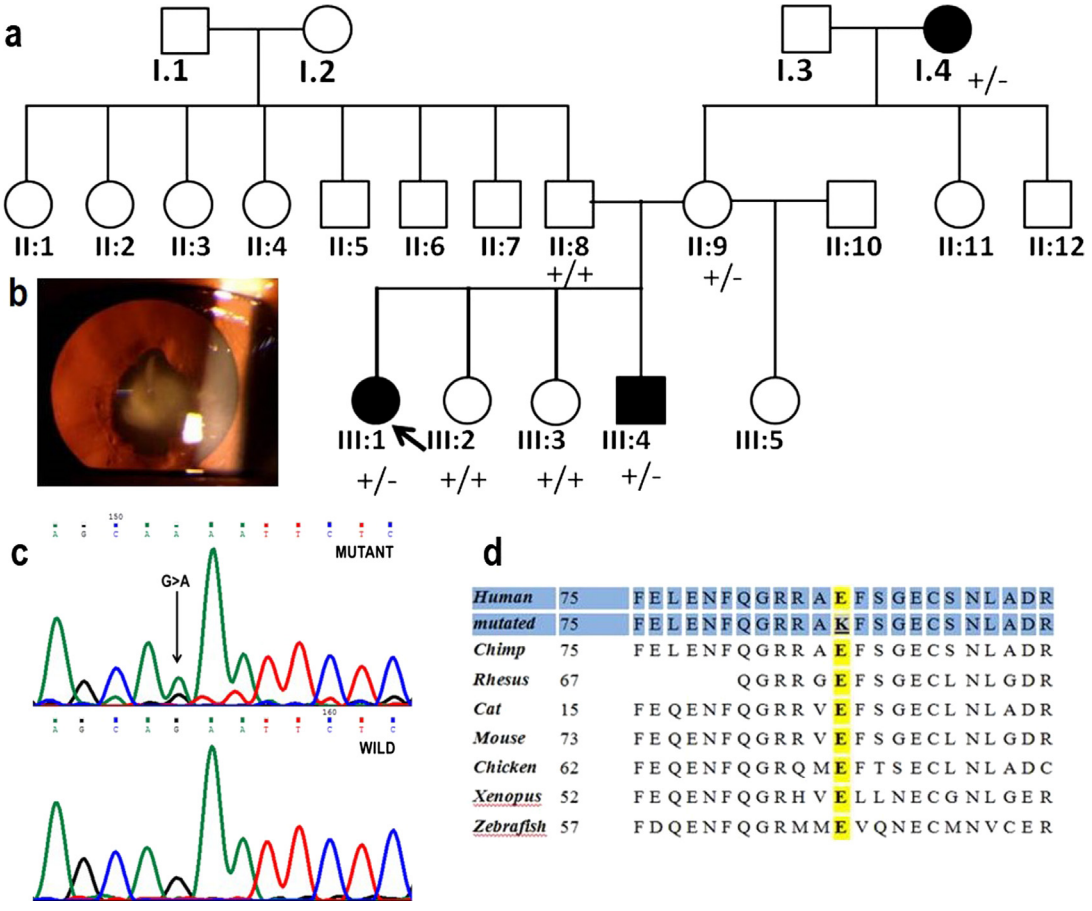

Fig. 1 – a) Pedigree of ADCC families with a mutation in the CRYBB1 (p.E75K) gene. In the pedigree, the square symbol represents males while the circle symbol represents females. A blackened symbol denotes an affected individual and the arrow denotes the proband. Family shows a heterozygous c.223G >A resulting in nuclear cataract phenotype. The +/− indicated heterozygous mutation and the +/+ indicated wild type. b) Nuclear cataract phenotype observed in proband. c) Electropherogram of ADCC family with a mutation in the CRYBB1 (p.E75K) gene shows a heterozygous c.223G >A. d) Conservation alignments of protein orthologs for novel p.E75K mutation. The region with p.E75K mutation CRYBB1 (crystalline beta B1) gene was highly conserved.

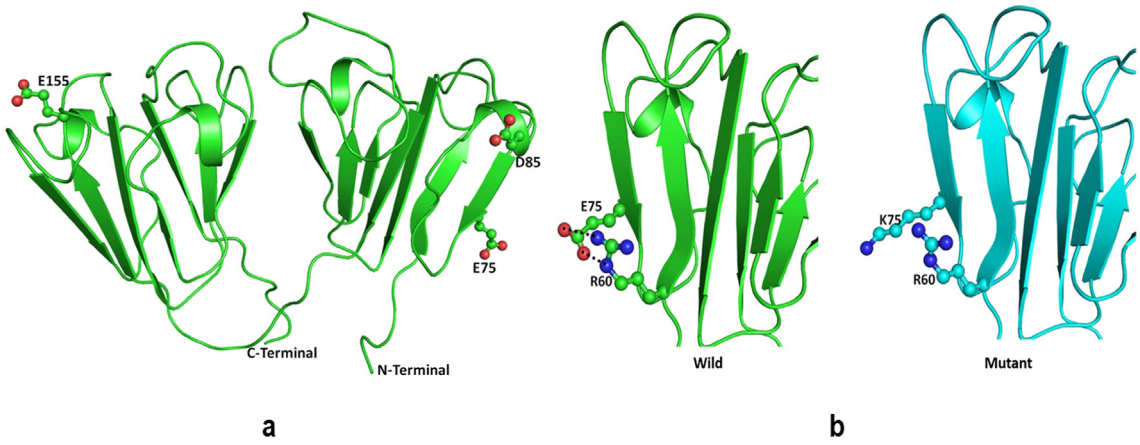

Fig. 2 – a) Cartoon representation of the crystal structure of wild type beta crystallin B1 protein. The residues at mutation site have been shown in ball and stick rendering. b) Cartoon representation of the model structure of wild and mutant (Glu75Lys) beta crystallin B1 protein showing the important residues (ball and stick) and hydrogen bonds (black dotted lines). The contacts are lost in the mutant.

3.3. *Protein modeling analysis*

The crystal structure of human *CRYBB1* protein (PDB ID: 1OKI) was used as template. The N-terminal domain of *CRYBB1* protein harbor p.Glu75Lys mutation. The p.Glu75Lys mutation is present on the surface of protein (Fig. 2) and thus exposed to solvents and would be engaged in protein–protein interactions. The Glu75 (an acidic residue), a component of the β strand and is positioned to make two hydrogen bonds with the guanidino group of Arg60 present on the adjacent anti-parallel β strand in the wild type protein (Fig. 2a). The mutation p.Glu75Lys alters the environment and charge on the protein surface, disrupting ionic interaction between Glu75 and Arg60 (Fig. 2b). Thus p.Glu75Lys changes the electrostatic potential of the protein surface which could affect the interactions with other interacting partner proteins.

4. Discussion

We identified a novel pathogenic mutation p.Glu75Lys in *CRYBB1* in an Indian family with Autosomal dominant form of congenital cataract (ADCC). Mutations in more than 40 genetic loci have been linked to congenital cataract and of these mutations, majority involve crystallins, one-quarter involve connexins, and the remaining involve other genes.[8] βB1-crystallin is a major subunit of the β-crystallins and comprises 9% of the total soluble crystallin in the human lens.[15] Apart from the mutation p.Glu75Lys, other mutations in *CRYBB1* have been reported to cause autosomal dominant congenital cataract (ADCC), some of which are p.X253ArgextX27, p. Gly220X, p.Gln223X and p.Ser228Pro.[1] Interestingly, all above sequence changes reported were located in exon 6, encoding the Greek key IV and the C-terminal extension. These mutations were predicted to result in an abnormally elongated or truncated COOH-terminus and production of a mutant protein. In addition, clinical phenotypes show some variations among these families but all involved nuclear opacification to a variable extent. The mutant allele showed reduced penetrance as mother did not develop cataract though she was heterozygous for the pathogenic mutation.

The possible molecular mechanism underlying congenital cataract caused by the p.Glu75Lys mutation was investigated by comparing the crystal structure of wild and mutant human *CRYBB1* protein. Many disease-causing mutations of congenital cataract have been characterized to destabilize the corresponding proteins. In this study the p.Glu75Lys mutation alters the environment and charge on the protein surface, disrupting ionic interaction between Glu75 and Arg60 (Fig. 2b). The p.Glu75Lys mutation changes the electrostatic potential of the protein surface which may adversely affect the interactions with other interacting partner proteins and thus may cause protein precipitation and opacification of lens.

In summary, the novel heterozygous *CRYBB1*:p.Glu75Lys mutation was detected in an Indian family with nuclear form of congenital cataract, It was inherited in autosomal dominant pattern and showed reduced penetrance. Protein modeling studies indicate that the mutation may modify the structure of βB1-crystallin by disrupting the normal protein–protein interactions, particularly of the interactions of βB1-crystallin with the acidic β-crystallins, which decrease the stability of the β-crystallin heteromers. Identification and characterization of this mutation further confirm the importance of protein interactions in maintaining lens transparency, and provides insight into the molecular mechanisms underlying the pathogenesis of human congenital cataract.

Conflicts of interest

All authors have none to declare.

Acknowledgments

We are thankful to the patient and their family members for their co-operation. This study was financially supported by

ICMR (Indian Council of Medical Research (45/19/2009-Hum/BMS), New Delhi, India). Manoj Kumar is an SRF (senior research fellow awarded by ICMR) gratefully acknowledges the financial support for this work.

REFERENCES

1. Kumar M, Agarwal T, Khokhar S, et al. Mutation screening and genotype phenotype correlation of α-crystallin, γ-crystallin and GJA8 gene in congenital cataract. *Mol Vis*. 2011;17:693–707.
2. Reddy MA, Francis PJ, Berry V, Bhattacharya SS, Moore AT. Molecular genetic basis of inherited cataract and associated phenotypes. *Surv Ophthalmol*. 2004;49:300–315.
3. Johnson GJ, ed. *The Epidemiology of Eye Disease*. 2nd ed. London: Arnold; 2003.
4. Bhattacharjee H, Das K, Borah RR, et al. Causes of childhood blindness in the northeastern states of India. *Indian J Ophthalmol*. 2008;56:495–499.
5. Santhiya ST, Kumar GS, Sudhakar P, et al. Molecular analysis of cataract families in India: new mutations in the CRYBB2 and GJA3 genes and rare polymorphisms. *Mol Vis*. 2010;16:1837–1847.
6. Shiels A, Hejtmancik JF. Genetic origins of cataract. *Arch Ophthalmol*. 2007;125:165–173.
7. Ramachandran RD, Perumalsamy V, Hejtmancik JF. Autosomal recessive juvenile onset cataract associated with mutation in BFSP1. *Hum Genet*. 2007;121:475–482.
8. Hejtmancik JF. Congenital cataracts and their molecular genetics. *Semin Cell Dev Biol*. 2008;19:134–149.
9. Srivastava K, Chaves JM, Srivastava OP, Kirk M. Multi-crystallin complexes exist in the water-soluble high molecular weight protein fractions of aging normal and cataractous human lenses. *Exp Eye Res*. 2008t;87:356–366.
10. Sambrook J, Russell DW. Purification of nucleic acids by extraction with phenol:chloroform. *CSH Protoc*. 2006;2006.
11. Schwarz JM, Rodelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods*. 2010;7:575–576.
12. Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. *Nucleic Acids Res*. 2003;31:3812–3814.
13. Sunyaev S, Ramensky V, Koch I, Lathe 3rd W, Kondrashov AS, Bork P. Prediction of deleterious human alleles. *Hum Mol Genet*. 2001;10:591–597.
14. *Discovery Studio Modeling Environment. Release*. 2.2 ed. San Diego: Inc. AS; 2007.
15. Lampi KJ, Ma Z, Shih M, et al. Sequence analysis of betaA3, betaB3, and betaA4 crystallins completes the identification of the major proteins in young human lens. *J Biol Chem*. 1997;272:2268–2275.