Novel Mutations in the Crystallin Gene in Age-Related Cataract Patients from a North Indian Population

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Key Words

Cataract · Crystallin · India · Lens · Mutation

Abstract

Cataract is the most prevalent leading cause of visual impairment and blindness worldwide. In comparison to congenital cataract, which affects relatively few individuals, age-related cataract is responsible for slightly half of all cases of blindness worldwide. Although significant work has been done, the genetic aspect of age-related cataract is still in its infancy. The current study was performed to analyze the mutations and polymorphisms in the *CRYAA*, *CRYAB*, *CRYBB1*, and *GJA8* genes in 40 unrelated age-related cataract patients. Mutational analysis of the above-mentioned genes in 40 cataract cases revealed 14 different substitutions of which 8 variants were novel and 6 were reported SNPs. Two disease-causing mutations, g.44590631G>A (p.R65Q) and g.44592224G>A (p.R119H), were also observed in the *CRYAA* gene. The disease-causing variants mildly affect the stability, functionality, and localization of crystallin, and, with progressing age, a small change in the microenvironment of the crystallin lens occurs. This change in combination with a mutation may significantly alter the functionality of the crystallin protein, leading to age-related cataract.

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Cataract formation is one of the leading causes behind visual impairment and blindness worldwide characterized by opacity of the lens that affects vision [Brian and Taylor, 2001]. Cataracts can be congenital in origin or can develop in advanced age (age-related cataract). According to a WHO report, age-related cataract with reduced vision of 3/60 or worse accounts for about 20 million people (48% of world blindness), and it is predicted to reach 50 million by the year 2020 [Asbell et al., 2005; Murthy et al., 2008]. Both congenital and age-related cataract have been associated with genetic and extrinsic factors [McCarty and Taylor, 2001; Hejtmancik and Kantorow, 2004; Iyengar et al., 2004]. Several factors such as UV-B exposure, certain medications, cigarette smoking, diabetes, gout as well as family history are known to be risk factors in the development of age-related cataract [McCarty and Taylor, 2001; Hejtmancik and Kantorow, 2004]. Cataract can be classified morphologically into nuclear, cortical, posterior subcapsular, and mixed types [Hejtmancik and Kantorow, 2004]. Congenital cataract can appear as an isolated abnormality or as part of a syndrome. There are approximately 44 independent loci and 33 genes reported to be involved in nonsyndromic autosomal dominant congenital cataract and can be divided into subgroups of genes such as crystallin, beaded filament structural pro-

tein 1 gene (*BFSP1*), lens intrinsic membrane proteins, heat shock factor proteins, transferases, and gap junction protein alpha 8 gene (*GJA8*) [Tsai et al., 2003; Iyengar et al., 2004]. In contrast to congenital cataract, only a few loci and genes have been reported for age-related cataract probably due to its complex inheritance pattern [Shiels and Hejtmancik, 2016]. The first gene variant reported as a risk for age-related cataract was galactokinase-1 (*GALK1*) in a Japanese cohort [Okano et al., 2001]. Apart from this, several other gene variants were also reported as a risk for age-related cataract, namely polymorphisms in EPH receptor A2 (*EPHA2*), *CRYAA*, *SLC16A12* (monocarboxylate transporter), *GJA8*, and crystallin β B2 (*CRYBB2*) [Shiels et al., 2008; Bhagyalaxmi et al., 2009, 2010; Liu et al., 2011; Zhou et al., 2011; Sundaresan et al., 2012; Abplanalp et al., 2013; Liao et al., 2014]. Some previous studies were also carried out to establish the association of *GSTM1*, *GSTT1*, DNA repair genes (*WRN*, *XPD*, and *XRCC1*), *HSF4*, and the kinesin light chain 1 gene (*KLC1*) with age-related cataract but resulted into inconsistent or inconclusive association [Sun et al., 2010; Padma et al., 2011; Jiang J et al., 2013; Jiang S et al., 2013; Su et al., 2013; Liao et al., 2015].

Lens crystallin has been known to undergo a wide variety of alterations with age, and many of these alterations are accelerated in the presence of oxidative, osmotic, or other stresses [McCarty and Taylor, 2001; Hejtmancik and Kantorow, 2004]. The alterations include proteolysis, an increase in disulfide bridges, deamidation of asparagine and glutamine residues, racemization of aspartic acid residues, phosphorylation, nonenzymatic glycosylation, and carbamylation [Hejtmancik and Kantorow, 2004]. Some mutations in crystallin cause protein aggregation which lead to congenital cataract; however, some mutations make them susceptible to environmental insults such as light and hyperglycemic or oxidative damage, possibly contributing to age-related cataract [Brian and Taylor, 2001; Congdon et al., 2003; Shiels et al., 2010].

Lens crystallins form a target for accumulated damage over the lifetime of an individual, and α A-crystallin's association with age-related cataract has already been established. Therefore, for the present study both α -crystallin genes were selected, i.e., *CRYAA* and *CRYAB* encoding α A- and α B-crystallins, respectively, as both proteins share 57% sequence similarity and similar phosphorylation patterns. *CRYBB1* is a major subunit of the β -crystallins (9% of the total soluble crystallin), which plays an important role in protein aggregation and orientation. Studies also revealed that the loss of the terminal arm of *CRYBB1* can hamper dimerization of the

Table 1. Grading of age-related cataract patients according to LOCS-III grading system

Patient ID	Age, years	Sex	LOCS-III grading
P1	58	M	N3
P2	54	F	P3N2
P3	62	F	N3
P4	40	M	P2N2
P5	48	M	C2N1
P6	55	M	N2C2
P7	62	F	P3N3
P8	60	M	N3
P9	65	M	P2C3
P10	50	M	C3
P11	50	F	P2C1
P12	50	F	P3
P13	60	F	N3
P14	60	M	N2C1
P15	70	F	P2N3C2
P16	40	F	P2N1
P17	92	M	N4
P18	40	M	N3C2
P19	74	M	P2N3C1
P20	66	F	P3N3
P21	65	F	P3C3
P22	70	M	N4
P23	70	F	N3C2
P24	42	M	P2N2
P25	45	M	P3N1
P26	65	F	C2N1
P27	70	F	C3N3
P28	50	M	P2N2
P29	52	M	C2N2
P30	75	F	N3
P31	80	M	P4
P32	70	M	N5
P33	58	F	P2C3
P34	47	F	P1N2
P35	60	F	N3
P36	65	F	C2N2
P37	65	F	N2
P38	54	M	C3N2
P39	40	F	P2N2
P40	46	F	C2N2

LOCS III, Lens Opacities Classification System III.

β -crystallins, which causes cataract [Yang et al., 2008]. *GJA8* and *CRYBB2* have already been reported as susceptible genes for age-related cataracts. In the light of the above-mentioned facts, 4 genes – *CRYAA*, *CRYAB*, *CRYBB 1*, and *GJA8* – were selected to screen age-related cataract patients from a northern Indian cohort. The study revealed 14 variants of which 2 were suspected to be pathogenic.

Fig. 1. Electropherograms of all 14 variants observed during screening of the *CRYAA*, *CRYBB1*, *CRYAB*, and *GJA8* genes in cataract patients.

Materials and Methods

A total of 40 patients, ≥ 40 years of age, with late-onset cataract were recruited from the eastern Uttar Pradesh and western Bihar states of India. Only patients with primary cataract who had visual impairment due to cataract were included in the study. Patients with secondary cataract, i.e., acquired cataract, due to trauma and toxins as well as complicated cataract, due to inflammatory and degenerative ocular diseases, were excluded from the study. In addition, patients with associated conditions such as diabetes, hypertension, myopia, glaucoma, and patients under medications known to be associated with development of cataract (e.g., steroids) were not considered. Controls were also selected from the same population by ruling out the possibility of having cataract, diabetes or any other eye defect in the individuals and their families.

Diagnosis of different types of cataract was done based on the slit-lamp examination following the Lens Opacities Classification System III (LOCS-III). The LOCS-III grading of all patients are shown in Table 1.

Blood samples and clinical photographs were collected from each patient after receiving written informed consent. Genomic DNA was isolated from 3 to 5 mL peripheral blood according to standard protocol. All the exons as well as exon-intron boundaries of the *CRYAA*, *CRYBB1*, *CRYAB*, and *GJA8* genes were PCR amplified and directly resequenced using the genetic analyzer-3130 (Applied Biosystems, Carlsbad, CA, USA) following the manufacturer's protocol. Primer sequences used in this study were

taken from Guo et al. [2012]. Two disease-causing variants (g.44590631G>A; p.R65Q and g.44592224G>A; p.R119H) were screened in 160 control individuals from the same population using ARMS PCR. The primer sets used to screen g.44590631G>A were 5'-GGCAGGTGACCGAAGCATC-3' (forward primer) and 5'-GAAGGCATGGTGCAGGTG-3' (reverse primer) along with either wild-type allele-specific primer, 5'-CACGTTTGGATTTCAGGTTTCG-3', or mutant allele-specific primer 5'-CACGTTTGCATTTCAGGTTTCA-3'. Primer sets used to screen g.44592224G>A were 5'-GCAGCTTCTCTGGCATGG-3' (forward primer) and 5'-GGGAAGCAAAGGAAGACAGA-3' (reverse primer) along with either wild-type allele-specific primer, 5'-AGTTCCACCGC-CGCTACCG-3', or mutant allele-specific primer 5'-AGTTCC-ACCGCCGCTACCA-3'.

Sequencing data were analyzed by several in silico analysis tools as mentioned below. The effect of each SNP was predicted by in silico analysis using MutationTaster and other prediction tools such as SIFT, Panther, SNAP, Polyphen-1, Polyphen-2, PhD-SNP, MAPP, and PredictSNP. The Exome Variant Server (<http://evs.gs.washington.edu/EVS/>), dbSNP (www.ncbi.nlm.nih.gov/SNP), 1000 Genomes (<http://www.internationalgenome.org/>), and ExAC (<http://exac.broadinstitute.org/>) were used to determine the frequency of sequence alteration in the population.

The comparison of the protein sequence with the genomic DNA sequence was performed using GeneWise (<http://www.ebi.ac.uk/Tools/psa/genewise/>) to reveal the altered amino acid due to nucleotide substitution. In silico nucleotide to protein translation

Table 2. Single nucleotide substitutions observed in age-related cataract patients while screening of *CRYAA*, *CRYAB*, *CRYBB1*, and *GJA8* genes

No.	Patient ID	Gene	Genomic coordinate	Amino acid change	Database status	In silico prediction
1	P4, P6, P7, P11, P12, P13, P14, P19, P25, P29, P38, P40	<i>CRYAA</i>	g.44589215C>T	p.D2D	reported, rs872331	polymorphism
2	P27	<i>CRYAA</i>	g.44590631G>A	p.R65Q	reported, rs199640007	disease causing
3	P19	<i>CRYAA</i>	g.44592224G>A	p.R119H	novel	disease causing
4	P4, P13, P20, P34	<i>CRYAB</i>	g.111782284C>T	p.L55L	reported, rs2228387	polymorphism
5	P16, P29, P32	<i>CRYAB</i>	g.111782389G>A	p.P20P	reported, rs4252582	polymorphism
6	P2, P3, P5	<i>CRYAB</i>	g.111782227C>T	intron	novel	polymorphism
7	P9, P10, P12, P15, P17, P18, P21, P22, P23, P24, P30, P31	<i>CRYAB</i>	g.111782227C>A	intron	novel	polymorphism
8	P5	<i>CRYBB1</i>	g.27013913C>A	intron	novel	polymorphism
9	P10	<i>CRYBB1</i>	g.27013784C>T	intron	reported, rs77926469	polymorphism
10	P9	<i>CRYBB1</i>	g.27013998C>A	5'UTR	novel	polymorphism
11	P11, P24, P25, P26, P13, P15, P40	<i>CRYBB1</i>	g.27012465A>T	intron	novel	polymorphism
12	P3	<i>CRYBB1</i>	g.27007974C>A	intron	novel	polymorphism
13	P9	<i>CRYBB1</i>	g.27004089A>C	intron	reported, rs5761626	polymorphism
14	P25	<i>GJA8</i>	g.147380435C>A	p.A118E	novel	polymorphism

(comparing normal and mutant protein) was done using the online tool EMBOSS Sixpack (http://www.ebi.ac.uk/Tools/st/emboss_sixpack/). The tertiary structure of protein was downloaded from the Protein Data Bank (<http://www.pdb.org>) and visualized on the standalone tool DeepView – Swiss-PdbViewer (<http://sp-dbv.vital-it.ch/>).

Results and Discussion

Resequencing of the *CRYAA*, *CRYBB1*, *CRYAB*, and *GJA8* genes identified 14 single nucleotide substitutions of which 6 were reported and 8 were novel (Fig. 1; Table 2). In *CRYAA*, 3 single nucleotide substitutions were identified, of which 2 were predicted to be disease causing (g.44590631G>A; p.R65Q in patient P27 and g.44592224G>A; p.R119H in patient P19) by Mutation-Taster as well as other prediction tools. These 2 disease-causing variants were not found in 160 unrelated control individuals from the same population.

Clinical History and in silico Analysis of Mutational Effects

CRYAA, g.44590631G>A (p.R65Q, rs199640007)

The 70-year-old female patient (P27) presented with bilateral cataract (LOCS III grading C3N3; Fig. 2A). The age of onset was 55 years as stated by the patient. Her mother was also affected by late-onset cataract, but her 2 brothers and their children were not affected. She has 2 children, 42 and 45 years old, but they were not available for testing.

CRYAA screening detected the nonsynonymous variant g.44590631G>A. The allele frequency of the variant analyzed from 1000 Genomes and ExAC revealed that the

Fig. 2. Clinical photographs of cataract patients. **A** Slit-lamp image of patient P27 showing C3N3 cataract by LOCS-III grading. **B** Slit-lamp image of patient P19 with P2N3C1 cataract.

frequency of the mutant allele A was very low (0.0006 and 0.0005453, respectively) and was present only in the South Asian population. Phylogenetic analysis of the variant across species revealed that the arginine at position 65 was evolutionarily conserved (Fig. 3A, C). Other properties of the protein such as hydrophobicity and helix formation were measured using ProtScale, which revealed a mild alteration in the protein microenvironment due to mutation. This mildly altered microenvironment might have a severe effect later in life due to change in the lens microenvironment that happens with ageing (Fig. 4). Furthermore, the tertiary structure of *CRYAA* was predicted by ModBase to study the effect of amino acid alteration on its protein structure. The amino acid substitution of R with Q results in a break of the hydrogen bond (H-bond) Glu63 and formation of a new H-bond with Asp67 in the *CRYAA* protein as predicted in silico (Fig. 5A, B).

Fig. 3. A The multiple sequence alignment of the p.R65Q mutant protein reveals the conservation of arginine at this position throughout evolution. **B** Multiple sequence alignment of the p.R119H mutant CRYAA protein reveals the conservation of R119 across species. **C** In silico prediction of both variants using prediction tools: SIFT, SNAP, Polyphen-1, Polyphen-2, PhD-SNP, MAPP, and PredictSNP.

CRYAA, g.44592224G>A (p.R119H)

The 74-year-old male patient (P19) was clinically diagnosed with bilateral cataract (LOCS III grading P2N3C1; Fig. 2B). The age of onset was 58 years as stated by the patient. He has no family history of cataract, and his 3 children were also unaffected. This could be a case of sporadic age-related cataract, although his children could manifest cataract later in life regarding they are all younger than 50 years.

The nonsynonymous novel single nucleotide substitution in exon 3 of the CRYAA gene causes p.R119H in the patient. Arginine at position 119 was also almost conserved across species except in a few (Fig. 3B, C). The analysis of alteration in hydrophobicity and helix-form-

ing properties of the protein due to this mutation revealed minor changes (Fig. 4). Despite this, in silico analysis of the tertiary structure of the protein revealed major changes in the intramolecular H-bonding pattern due to the p.R119H shift. Wild-type R119H bonded with Glu91 and Asp92, while mutant H119 devastated both H-bonding and created an additional bond with a ligand (Fig. 5C, D). The p.R119H not only disturbed intramolecular H-bonding, but also interfered with the protein-ligand interaction which may create a disturbance in the functionality of the protein. An independent study on congenital nuclear cataract by Li et al. [2010] revealed a heterozygous p.R116C mutation, while Zhang et al. [2011] revealed another mutation, p.R116H, in the CRYAA gene as a poten-

Fig. 4. Hydrophobicity profile of wild-type and mutant (p.R65Q and p.R119H) CRYAA proteins are shown predicted by the ProtScale program at the ExPASy server. **A** The circles represent the hydrophobicity around R65 and R119 in the wild-type protein. **B** The circles represent the hydrophobicity around Q65 and H119

in the mutant protein showing a mild shift in hydrophobicity compared to the wild type. **C** Secondary structure formation (alpha helix in this case) property of wild-type and mutant protein predicted by ProtScale program using Chou-Fasman algorithm. **D** Alpha helix-forming property of each amino acid in the wild-type protein.

tial cause for congenital anterior polar cataract. Although the identified p.R119H variant lies very close to these reported mutations and did not exert its effect immediately, it is probable that it will generate a similar abnormal microenvironment in advanced age, leading to age-related cataract [Pang et al., 2010].

α -Crystallins are molecular chaperones thought to allow the lens to tolerate aging-induced deterioration of the lens proteins. Besides the chaperone's activity, its other

functions include remodeling and protection of the cytoskeleton, inhibition of apoptosis, and enhancing the resistance of cells to stress [Andley, 2007]. The fact that the 2 alterations, p.R65Q and p.R119H, probably will interfere with the protein stability and solubility may lead to the development of age-related cataract, not evident in early age of the patients.

The SNP g.44589215C>T (rs872331) was one of the most commonly (in 12 patients) observed variations in pa-

Fig. 5. The tertiary structure of the CRYAA protein predicted by ModBase and visualized on the standalone tool DeepView – Swiss-PdbViewer is shown. **A** The wild-type CRYAA protein with R65 hydrogen (H)-bonded with Glu63. **B** The mutant Q65 CRYAA protein formation of a new H-bond occurs with Asp67; the previ-

ous H-bonding with Glu63 was diminished. **C** The wild-type CRYAA protein with R119 has 2 H-bonds with Glu91 and Asp92. **D** The mutant CRYAA protein with H119 results in a breakage of both H-bonds with Glu91 and Asp92 and creates an additional bond with a ligand.

Table 3. Minor allele frequency of nonsynonymous variants in different populations reported on ExAC browser

Population	rs872331	rs199640007	rs2228387	rs4252582
South Asian	0.3605	0.0005453	0.0315	0.04843
African	0.7359	0	0.003963	0
East Asian	0.1993	0	0.0002774	0
European (Finnish)	0.5787	0	0.02085	0.0003249
European (non-Finnish)	0.608	0	0.02323	0.0001922
Latino	0.4638	0	0.005371	0.00164
Other	0.5509	0	0.0176	0.009091
Total minor allele frequency	0.5404	7.46e-05	0.01935	0.008093

tients from the present study as well as in other reported populations (Table 3). In *CRYBB1*, 6 different substitutions were identified in either UTR or intronic regions, which are predicted to be polymorphic. In the *CRYAB* gene, 4 different substitutions, g.111782284C>T (rs2228387),

g.111782389G>A (rs4252582), g.111782227C>T and g.111782227C>A, were identified. The frequency of the 2 *CRYAB* variants, i.e., rs2228387 and rs4252582, were very low in various reported populations. In the future, it will be of interest to understand the role of the reported variants

(rs4252582 and rs2228387) and novel variants (*GJA8*; g.147380435C>A) in age-related cataract because the current study did not cover the case-control association study.

Here, we reported 2 disease-causing mutations and 12 polymorphic variants in 4 genes in age-related cataract cases from a north Indian cohort.

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Statement of Ethics

The study was approved by the institutional ethical committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

Disclosure Statement

The authors have no conflicts of interest to declare.

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