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Hôpital ophtalmique Jules Gonin – Lausanne
Chef de service: Professeur Leonidas Zografos

CRYBA3/A1 Gene Mutation Associated with Suture-Sparing Autosomal Dominant Congenital Nuclear Cataract: A Novel Phenotype

THESE

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Walter FERRINI

Médecin diplômé de la Confédération Suisse
Originaire d'Italie

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Mutation du gène *CRYBA3/A1* associée à une cataracte congénitale autosomique dominante de type nucléaire avec épargne suturale: un nouveau phénotype.

La transparence du cristallin est un facteur essentiel au développement du système visuel. Son opacification, ou cataracte, est une cause fréquente de déficit visuel héréditaire chez les enfants. Les opacifications du cristallin sont étiologiquement associées à des mutations de gènes spécifiques au cristallin, à des anomalies chromosomiques, à des désordres métaboliques ou à des traumatismes pendant la vie intra-utérine. Dans le cas de mutations génétiques, leur mode de transmission est fréquemment de type autosomique dominant et à forte pénétrance. Les anomalies génétiques peuvent n'affecter que le cristallin (cataracte isolée), ou s'associer à d'autres anomalies systémiques. A ce jour, plus de 15 gènes associés à une cataracte isolée ont été identifiés. Ces gènes incluent les gènes de la famille des crystallines. Les crystallines constituent plus de 95% des protéines cytoplasmiques hydrosolubles du cristallin. Ces protéines confèrent sa transparence au cristallin.

Nous avons étudié une famille suisse non-cosanguine de cinq générations, originaire du Valais, atteinte d'une forme autosomique dominante de cataracte congénitale bilatérale de type purement nucléaire et épargnant les sutures du cristallin. Cette famille comprenant 15 patients affectés a été examinée et le phénotype a été documenté. Une analyse de liaison a été effectuée en utilisant 396 marqueurs couvrant l'entier du génome. Une liaison a été observée sur le locus du chromosome 17 avec le marqueur D17S1857 (lod score: 3.44 à $\theta = 0$). Le gène *CRYBA3/A1* dans ce locus se présentait comme un excellent candidat. Ce gène est un membre de la superfamille des crystallines. Il est composé de 6 exons. Le séquençage de ce gène a permis d'identifier la délétion de 3 nucléotides dans l'exon 4 (279delGAG). Cette délétion induit la perte d'une glycine en position 91 (ΔG91) au sein de la protéine. Cette mutation co-ségrégue avec tous les membres affectés de la famille et n'a pas été observée dans la population normale ($n = 250$).

Ce travail a permis d'identifier une nouvelle mutation du gène *CRYBA3/A1* associée à une nouvelle forme de cataracte congénitale autosomique dominante.

CRYBA3/A1 Gene Mutation Associated with Suture-Sparing Autosomal Dominant Congenital Nuclear Cataract: A Novel Phenotype

Walter Ferrini,[1,2] *Daniel F. Schorderet,*[1,2,3,4] *Philippe Othenin-Girard,*[2,4] *Sylvie Uffer,*[2,4] *Elise Héon,*[5] *and Francis L. Munier*[2,3,4]

Purpose. To identify the genetic defect leading to the congenital nuclear cataract affecting a large five-generation Swiss family.

Methods. Family history and clinical data were recorded. The phenotype was documented by both slit lamp and Scheimpflug photography. One cortical lens was evaluated by electron microscopy after cataract extraction. Lenticular phenotyping and genotyping were performed independently with short tandem repeat polymorphism. Linkage analysis was performed, and candidate genes were PCR amplified and screened for mutations on both strands using direct sequencing.

Results. Affected individuals had a congenital nuclear lactescent cataract in both eyes. Linkage was observed on chromosome 17 for DNA marker D17S1857 (lod score: 3.44 at $\theta = 0$). Direct sequencing of CRYBA3/A1, which maps to the vicinity, revealed an in-frame 3-bp deletion in exon 4 (279delGAG). This mutation involved a deletion of glycine-91, cosegregated in all affected individuals, and was not observed in unaffected individuals or in 250 normal control subjects from the same ethnic background. Electron microscopy showed that cortical lens fiber morphology was normal.

Conclusions. The ΔG91 mutation in CRYBA3/A1 is associated with an autosomal dominant congenital nuclear lactescent cataract. A splice mutation (IVS3+1G/A) in this gene has been reported in a zonular cataract with sutural opacities. These results indicate phenotypic heterogeneity related to mutations in this gene. (*Invest Ophthalmol Vis Sci.* 2004;45:1436–1441) DOI:10.1167/iovs.03-0760

Congenital cataracts are a frequent cause of hereditary visual impairment in infants.[1] Without prompt treatment, these diseases can interfere with the development of normal visual cortical synaptic connections, which may result in irreversible visual loss. Cataracts are genetically heterogeneous, most often transmitted as an autosomal dominant trait,[2] showing high penetrance and considerable inter- and intrafamilial clinical variations. Identical mutations have been reported to cause different phenotypes[3] and changes in more than one gene can give a similar phenotype.[4,5] Lens opacification may have different causes, including mutations in lens-specific genes, chromosomal abnormalities, metabolic disorders, and intrauterine insults, or it can occur as part of a complex developmental disorder of the eye. To date, more than 15 independent gene loci associated with isolated cataracts have been mapped to different chromosomes. These include: 7 crystallin genes: CRYAA mapped to 21q22.3 (MIM 123580),[6] CRYAB to 11q22.3-q23.1 (MIM 123590),[7] CRYBA3/A1 to 17q11.1-q12 (MIM 123610),[8] CRYBB1 to 22q11.2-q12.1 (MIM 600929),[9] CRYBB2 to 22q11.2-q12.2 (MIM 123620),[3] CRYGC to 2q33-q35 (MIM 123680),[4,10,11] and CRYGD to 2q33-q35 (MIM 123690)[4,11–13]); two gap junction protein genes: GJA3 mapped to 13q11 (MIM 121015)[14,15] and GJA8 to 1q21.1 (MIM 116200)[16–18]); a beaded filament structural protein, BFSP2 mapped to 3q21-q25 (MIM 603212)[19,20]; and the major intrinsic protein, MIP mapped to 12q13 (MIM 154050).[21] Three autosomal recessive forms of hereditary cataracts have been mapped to the CAAR locus on 9q13-q22 (MIM 605749),[22] to the CRYAA gene,[23] and to the lens intrinsic membrane protein LIM2 on 19q13.4 (MIM 154045).[24]

Crystallins constitute more than 95% of the water-soluble cytoplasmic proteins in the lens. They are essential for conferring and maintaining lens transparency. At least 13 functional crystallin genes have been mapped in the human genome, and 11 have been isolated from the young human lens[25]: two α-crystallins (αA and αB) and nine β/γ-crystallins (βA1, βA3, βA4, βB1, βB2, βB3, γS, γC, and γD). γA and γB are poorly expressed in the lens.

The crystallins are highly conserved during evolution. The tertiary structures of the β- and γ-crystallins are very similar.[26–28] The main structural difference between these two families is that the β-crystallin–connecting peptide is extended, and dimerization of two β-crystallin monomers is necessary for interdomain interaction. In γ-crystallins, the flexible connecting peptide folds back on itself, allowing the amino and carboxyl domains to participate in close interactions. Long N-terminal sequence extensions in β-crystallin further distinguish these two families. The basic β-crystallin also have a C-terminal extension.

A five-generation family affected with a congenital nuclear cataract with various degrees of suture-sparing was studied to identify the genetic defect associated with this phenotype.

Methods

Patients and Clinical Data

This study was approved by the Ethics Committee of the Faculty of Medicine at the University of Lausanne, Switzerland, and was conducted according to the tenets of the Declaration of Helsinki. All participants gave informed consent to the study protocol.

The family comprised 15 affected patients and 10 unaffected individuals from a five-generation pedigree (Fig. 1) originating from the canton of Valais, Switzerland. Genomic DNA was isolated from peripheral blood leukocytes as described elsewhere.[29] Detailed clinical and ophthalmic examination included Snellen visual acuity testing, intraocular pressure measurement, and fundus examination. Lens phenotype was documented by both slit-lamp and Scheimpflug photography.

From the [1]Institute of Research in Ophthalmology, Sion, Switzerland; the [2]Department of Ophthalmology, University of Lausanne, Lausanne, Switzerland; the [3]Unit of Oculogenetics and the [4]Jules Gonin Eye Hospital, Lausanne, Switzerland; and the [5]Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, Toronto, Ontario, Canada.

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Corresponding author: Francis L. Munier, Hôpital Jules Gonin, Unité d'Oculogénétique, Avenue de France 15, CH-1004 Lausanne, Switzerland; francis.munier@ophtal.vd.ch.

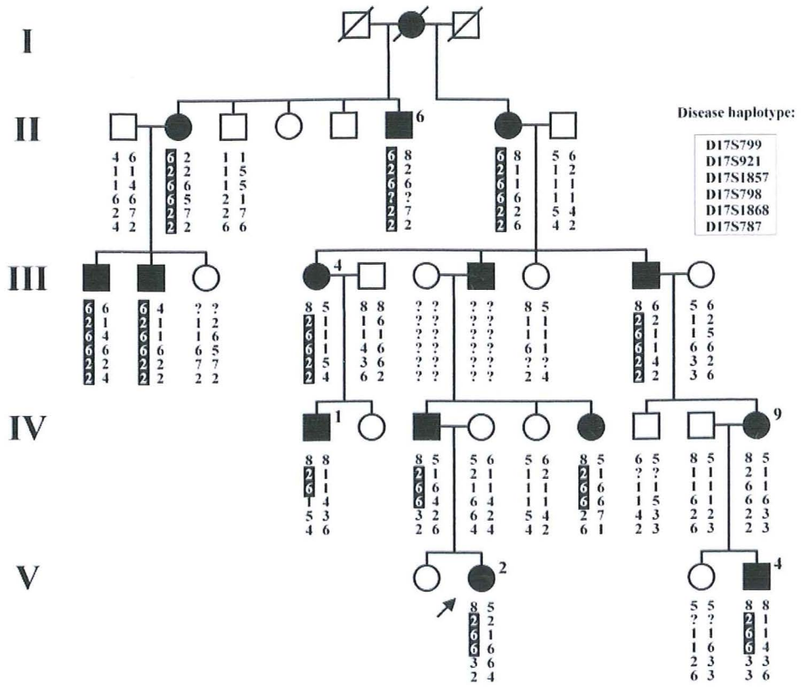

FIGURE 1. Family with congenital nuclear cataract. *Squares*: males; *circles*: females; *filled symbols*: affected individuals; *arrow*: the proband.

Genotype and Linkage Analysis

Genotyping was performed by using 396 fluorescent dye-labeled dinucleotide repeat markers −FAM, −HEX or −NED (Prism Linkage Mapping Set, ver. 2.5; Applied Biosystems, Zürich, Switzerland) according to instructions from the manufacturer. PCR products of the panels were pooled and denatured at 90°C for 2 minutes. Markers were electrophoresed on denaturing polyacrylamide gels gel (Performance Optimized Polymer 4; Applied Biosystems) on an automatic DNA sequencer (Prism 310 Genetic Analyser; Applied Biosystems). Data were collected and analyzed by commercial software (Genescan and Genotyper; Applied Biosystems). Markers associated with candidate loci related to dominant congenital cataracts, and in particular the crystallin gene loci, were initially screened. Linkage analysis was performed with MLINK using equal allele frequency and a new mutation rate of 10^{-4} (a program of the LINKAGE (ver. 5.1) package (http: www.hgmp.mrc.ac.uk/ provided in the public domain by the Human Genome Mapping Project Resources Center, Cambridge, UK).

Amplification of CRYBA3/A1 Exons 1–6

PCR amplification conditions were as follows: 200 ng of DNA, 1.25 μM of each exon primer (Table 1), 2% formamide, and 15 μL of PCR mix (Master Mix; Qiagen, Basel, Switzerland) in a final reaction volume of 30 μL, performed for 35 cycles (1 minute at 94°C, 1 minute at 52°C, and 1 minute at 72°C) after an initial denaturation step at 95°C for 5 minutes and a final 10-minute 72°C extension.

DNA Sequencing

PCR products were purified with a PCR product purification kit (High Pure; Roche, Rotkreuz, Switzerland). The sequencing reaction, which included 1 μL template, 1 pM sequencing primer, and dye termination chemistry (Big Dye Terminator, ver. 1.0; Applied Biosystems) for a 10-μL final volume, was performed for 25 cycles (10 seconds at 96°C, 5 seconds at 55°C, and 4 minutes at 60°C) after an initial denaturation step (96°C for 10 seconds). The product was purified on a separation column (Sephadex G-50; Millipore, Geneva, Switzerland), and templates were sequenced bidirectionally with a gene analyzer (Prism 310 genetic analyzer; Applied Biosystems) and primers shown in Table 1.

Denaturing HPLC

Human genomic DNA from 250 normal control subjects was screened by denaturing (D)HPLC for exon 4 of CRYBA3/A1 gene using a commercial system (Wave DHPLC; Transgenomic, Crewe, UK). The DNA polymerase in the amplified PCR products was inactivated using 5 mM EDTA, 60 mM NaCl, and 10 mM Tris HCl (pH 8.0). DHPLC was performed as follows: initial concentration at 45% of buffer A (0.1 M triethylammonium acetate-TEAA; Transgenomic), and 55% of buffer B (0.1 M TEAA containing 25% acetonitrile; Transgenomic) at 58.5°C. Data were analyzed by comparison of the chromatograms.

TABLE 1. Primers Used for Amplification of the 6 Exons of Human βA1/A3-Crystallin

Exon		Primer Sequences	Product Size (bp)
1	Fwd	5′-GGCAGAGGGAGAGCAGAGTG-3′	207
	Rev	5′-CACTAGGCAGGAGAACTGGG-3′	
2	Fwd	5′-AGTGAGCAGCAGAGCCAGAA-3′	293
	Rev	5′-GGTCAGTCACTGCCTTATGG-3′	
3	Fwd	5′-AAGCACAGAGTCAGACTGAAGT-3′	269
	Rev	5′-CCCCTGTCTGAAGGGACCTG-3′	
4	Fwd	5′-GTACAGCTCTACTGGGATTG-3′	357
	Rev	5′-ACTGATGATAAATAGCATGAACT-3′	
5	Fwd	5′-GAATGATAGCCATAGCACTAG-3′	290
	Rev	5′-TACCGATACGTATGAAATCTGA-3′	
6	Fwd	5′-CATCTCATACCATTGTGTTGAG-3′	295
	Rev	5′-GCAAGGTCTCATGCTTGAGG-3′	

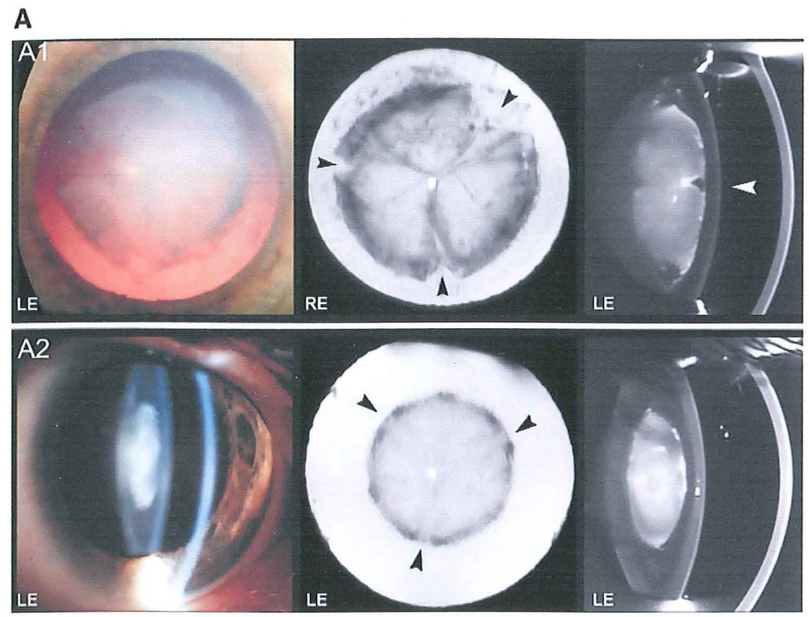

FIGURE 2. **(A)** Slit lamp and Scheimpflug photography showing nuclear lactescent cataract. **(A1)** A 1.5-year-old girl: the opacification is symmetrical, homogenous, and of the same density. Anterior and posterior sutures are not involved (*arrowheads*). **(A2)** A 3-year-old boy: similar phenotype but with discrete sparing of the sutures (*arrowheads*). **(B)** Sequencing analysis of CRYBA3/A1 exon 4. Normal DNA sequence showing the GAG short tandem repeat (nucleotides 276-281). Heterozygous affected DNA showing forward and reverse sequences and the in-frame 3-bp deletion at position 279-281. This mutation deletes a glycine in position 91. Superposed nucleotides are denoted according to the IUB code.

Electron Microscopy

During cataract extraction from the index patient, cortical fragments of lens were removed and fixed in a 1% paraformaldehyde solution at pH 7.3, postfixed with 2% osmium tetroxide and dehydrated with acetone. For transmission electron microscopy (TEM), the fragments were embedded in epoxy resin. Ultrathin sections were poststained with uranyl acetate and lead citrate and inspected in a microscope (EM10; Carl Zeiss Meditec, Jena, Germany).

RESULTS

Clinical Data

The proband was a 1.5 year-old girl (Fig. 1, V-2) who had a bilateral nuclear lactescent cataract (Fig. 2A1). The opacification was symmetrical, homogeneous, and of the same density in both eyes, with a radial diameter of 5.20 mm and a depth of 2.18 mm. Anterior and posterior Y-sutures were not involved.

TABLE 2. Visual Outcome for Nuclear Lactescent Cataract without Early or Late Complications after Cataract Extraction

Subjects	BCV (%) OD/OS	Nystagmus	Strabismus	Age at Operation (y)
V-2	0.5/0.6	Yes	Yes	2 years
V-4	0.6/0.5	No	No	3 years
IV-1	0.7/0.9	No	Yes	3 years
IV-9	0.9/0.9	No	No	5 years
III-4	0.6/0.8	No	No	6 years
II-6	0.8/0.8	No	No	25 years

No family histories of other associated ocular or systemic disorders were recorded. Late age at operation was probably a result of changing practice and access to health care over several decades. BCV, best corrected postoperative visual acuity.

A similar phenotype with discrete sparing of the sutures (Fig. 2A2) was observed in a 3-year-old male (Fig. 1, V-4). All other affected family members had undergone surgery, but hospital records confirmed that the cataract was present either at birth, or diagnosed in the first decade of life. Best corrected postoperative visual acuity was better than 0.5 in both eyes in all patients without visually impairing complications of surgery such as bullous keratopathy or glaucoma (Table 2). Nystagmus was observed in only one of the six patients. No other ocular anomalies were observed.

Linkage Analysis

A genome-wide scan was performed, starting with the panels containing cataract loci. Significant linkage was found with microsatellite marker D17S1857 with pair-wise lod score exceeding 3.0 (Table 3). A max lod score of 3.44 was obtained for D17S1857 at $\theta = 0$. This marker strongly mapped to the vicinity of CRYBA3/A1.

CRYBA3/A1 Analysis

Direct cycle sequencing of crystallin βA3/A1 exons 1 to 6 in an affected individual identified an in-frame 3-bp deletion at positions 279-281 in exon 4, the first nucleotide of the initiation codon is set at +1. By convention for deletions in short tandem repeats,[30] the mutation was designated as 279delGAG (Fig. 2B). This deletion induced the loss of glycine in position 91(ΔG91). This change cosegregated with the disease phenotype and was not seen in 250 normal control subjects of the same ethnic background. The remainder of the coding sequence did not show any sequence change.

TABLE 3. Linkage Data for Markers of the CRYBA3/A1 Region

Markers	IMD (cM)	Recombination Fraction (θ) 0.00	0.10	0.20	0.30	0.40
D17S799		−∞	1.45	1.17	0.76	0.33
	5.0					
D17S921		2.34	1.81	1.26	0.71	0.25
	7.0					
D17S1857		3.44	2.84	2.18	1.44	0.66
	12.0					
D17S798		−∞	1.55	1.35	0.97	0.51
	15.0					
D17S1868		−∞	−0.95	−0.23	0.03	0.09
	10.0					
D17S787		−∞	−2.00	−0.86	−0.35	−0.12

IMD, intermarker distances.

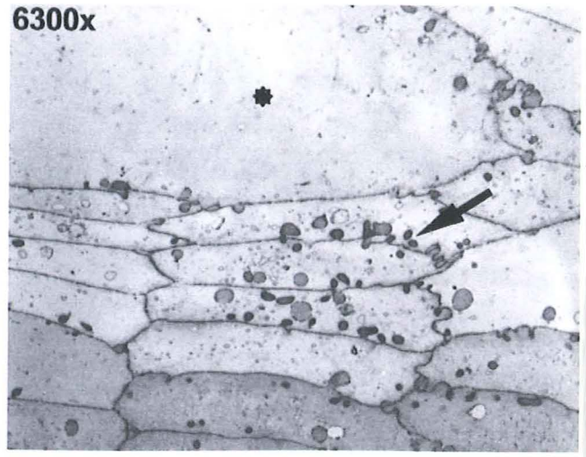

FIGURE 3. Transmission electron micrograph of the anterior cortex showing occasional swollen fibers (*) and numerous projections (*arrow*).

Electron Microscopy

The hexagonal shape of the anterior cortical lens fibers was apparent on TEM (Fig. 3). The cell membranes were well defined, exhibited classic ball-and-socket interdigitations common to the cortex, and were found between the long sides of the hexagons. Some cells had complex profile borders with numerous interdigitations occurring at the short sides of the cells. The cytoplasm consisted of a fine homogeneous granular substance that was uniformly distributed within the cell. Membranous organelles were absent in this field. Circular membrane profiles were examined inside the cells. The majority of cortical cells were similar in size; however, a few were swollen. Their cytoplasms were homogeneous and no "watery" vacuoles were found. No eosinophilic material, cell fragments, or morgagnian globules were observed.

DISCUSSION

This study identified a novel mutation in the CRYBA3/A1 gene, associated with a novel autosomal dominant congenital cataract characterized by a lactescent nuclear lens opacification sparing the sutures. This cataract is phenotypically different from the previously described lens phenotype associated with a CRYBA3/A1 mutation (IVS3+1G/A), which was zonular and sutural in nature.[8,31] Despite the fact that the lactescent nucleus was dense and lying close to the visual axis, this phenotype seemed to be only slightly amblyogenic, with postoperative vision ranging from 0.5 to 0.9. This good visual prognosis may be attributed to the persistence of clear sutures minimizing the degree of central visual deprivation in the early period of life.

The morphologic examination of the anterior lens cortex showed that its overall structure was intact, although slight variations of the texture and size of the fibers were noted. These observations were consistent with the preservation of cortical lens transparency seen by slit lamp and Scheimpflug photography at the macroscopic level.

Crystallin proteins are synthesized during differentiation and are retained throughout life, making them extremely stable and long-lived proteins. The nucleus of the lens contains the oldest synthesized crystallins, whereas the cortex contains the most recent ones. Because the crystallins are differentially synthesized during development, they are not distributed uniformly throughout the lens. RNA extraction from rat lenses at various fetal and postnatal development stages showed that the expression of the CRYBA3/A1 gene had virtually ceased in the mature lens.[32] The central location of nuclear lactescent cataract is consistent with early expression of crystallin βA3/A1 in the lens and CRYBA3/A1 gene mutations reported in human[8,31] and mouse.[33] Crystallin proteins are the most abundant transcripts of the adult human lens, and CRYβA1 mRNA ranked among the top 10 in the expressed sequence tag (EST) analysis.[34] Beside their functions in lens transparency, some lens crystallins have biochemical or enzymatic activity. The only enzymatic activity identified to date in a member of the β/γ-crystallin superfamily is the detergent-activated proteolytic activity of the βA3/A1.[35] However, the two cataracts associated with mutations in these proteins are more likely to result from disruption of their roles as structural lens proteins than from loss of a particular enzymatic function.

Crystallin βA3/A1 consists of six exons.[36] Seven protein regions exist in β-crystallin: four homologous motifs, a connecting peptide, and N- and C-terminal extensions. Each motif is a Greek key of four β-strands and consists of approximately 40 amino acid residues. In β-crystallin genes the four Greek key motifs are approximately encoded by exons 3 to 6. The extensions as well as the intramolecular domain interfaces are thought to play a role in the ability of β-crystallin to self-associate to form homo- or heterodimers with other β-crystallins. The βA1-crystallin aggregates range from dimers to octamers,[37] and further complexity is related to temporal and spatial regulation of expression as well as posttranslational modifications.[38] The sequence of the connecting peptide is not critical for the association of βA3-crystallin monomers into dimers.[39]

The ΔG91 mutation observed in CRYBA3/A1 exon 4 deletes a highly conserved amino acid in the β/γ-crystallin gene. Glycine 91 (G91) is located in the c_2 β-strand (i.e., the third strand of the second motif, two residues apart from a conserved tyrosine). Because of the structural similarity between the β- and γ-crystallins, the recent knowledge derived from human γD (HGD) crystallography is of great interest. An x-ray crystal structure analysis has shown that both wild-type HGD and the mutant proteins R36S and R58H are similar in their secondary and tertiary structures,[40] and that R58H and HGD are of an identical space group and very similar lattice contacts. These important findings suggested that protein misfolding of γ-crystallin is not required for cataract formation. Additional work on the three γD-crystallin mutant proteins (R14C, R36S, R58H) has shown that these mutants are less soluble and crystallize more readily than the wild type.[40,41] Further investigations, such as phase behavior and x-ray structure analysis will determine the physicochemical properties and conformational changes associated with CRYBA3/A1 protein mutants.

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