Identification of a *De Novo* 3bp Deletion in *CRYBA1/A3* Gene in Autosomal Dominant Congenital Cataract

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Abstract- Autosomal dominant congenital cataract (ADCC) is the most common form of inherited cataracts and accounts for one-third of congenital cataracts. Heterozygous null mutations in the crystallin genes are the major cause of the ADCC. This study aims to detect the mutational spectrum of four crystallin genes, *CRYBA1/A3*, *CRYBB1*, *CRYBB2* and CRYGD in an Iranian family. Genomic DNA was isolated from whole blood cells from theproband and other family members. The coding regions and flanking intronicsequences of crystalline genes were analyzed by Sanger sequencing in aproband with ADCC. The identified mutation was further evaluated in available family members. To predict the potential protein partners of *CRYBA1/A3*, we also used an *in-silico* analysis. A *de novo* heterozygous deletion (c.272-274delGAG, p.G91del) in exon 4 of *CRYBA1/A3* gene, leading to a deletion of Glycine at codon 91 was found. This genetic variation did not change the reading frame of *CRYBA1* protein. In conclusion, we identified a *de novo* in-frame 3-bp deletion in the proband with an autosomal dominant congenital cataract, but not in her parents, in an Iranian family. This mutation has occurred *de novo* on a paternal gamete during spermatogenesis. The *in-silico* results predicted the interaction of *CRYBA1* protein with the other CRY as well as proteins responsible for eye cell signaling.

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Keywords: Cataract; *CRYBA1/3*; Directsequencing; Frameshift mutation; Truncated protein

Introduction

Congenital cataracts are a major abnormality of the eye and are the most common cause of childhood blindness in developing countries (1). These groups of ocular problems cover a wide spectrum of symptoms; while some lens opacities do not progress and are visually irrelevant, others can create profound visual impairment. In infants with cataract, vision can only be restored by surgery. Studies estimated a significant reduction might occur in the global prevalence of childhood blindness from the current level of 0.75/1000 to 0.4/1000 children by the year 2020 (2,3). It has been well-documented that congenital cataracts are genetically and clinically heterogeneous. These diseases can occur isolated or be associated with other ocular and/or systemic abnormalities and are thus known as "nonsyndromic" or "syndromic" forms (4,5). Approximately one-third of congenital cataracts show a positive family history and genetic alteration is a main cause of the disease. In spite of the fact that autosomal dominant is the most common mode of inheritance, it can be transmitted as autosomal recessive, autosomal dominant and X-linked traits (6-8).

So far, mutations in more than 35 genes associated with isolated cataracts have been mapped to different chromosomes (Cat-Map; http://cat-map.wustl.edu/) (9). The most recent studies determined that mutations in genes encoding crystallins, connexins, and NHS are responsible for about 60% of families with cataract (8). Crystallins are a diverse group of proteins that constitute more than 95% of the water-soluble cytoplasmic

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proteins in the lens. *CRYBA1/A3* is a member of the β-crystallin family and encodes two proteins (crystallinbeta A3 and crystallin beta A1) from a single mRNA. Although crystallin beta A1 and beta A3 are identical, the beta A1 is 17 a shorter than other (8,9). Studies reported that these biomolecules are crucial for conferring and maintaining lens transparency (7-9).

In the present work, we performed mutation screening of coding sequences of crystallin genes including *CRYBA1/A3*, *CRYBB1*, *CRYBB2* and *CRYGD* in an Iranian family with congenital autosomal dominant cataracts (CADC).

Materials and Methods

Patients and clinical investigations

A 10-year-old girl was admitted to our center (Farabi Eye Hospital, Tehran, Iran). A written informed consent was obtained from her parents before mutation analyses were conducted. In this study, ADCC was diagnosed based on the following criteria: (1) bilateral congenital cataracts that have been approved by detailed ophthalmologist's examination; (2) no other ocular or systemic disease; (3) no other congenital and syndrome-related malformation; (4) no history of any teratogenic drug usage during pregnancy; (5) compatible family pedigree with autosomal dominant pattern of the disease. Ocular examination using Slit-lamp photographs of the proband eyes was also utilized forthecharacterizing disease.

Mutation screening and DNA sequencing

Genomic DNA was isolated from peripheral blood cells oftheproband and her parents using QIAamp DNA Mini Kit (Qiagen, USA) according to manufacturer's instructions. PCR amplification was conducted (10,11) using specific primers (Table 1) corresponding to coding regions and exon-intron boundaries of four candidate genes (*CRYBA1/A3*, *CRYBB1*, *CRYBB2*, and *CRYGD*).

Table 1. Primer sequences used in this study

	Specific primers	PCR product (bp)	Sequence (5'-3')
Crystallin alpha A (CRYAA)	**AA1 F**	441 bp	AGCAGCCTTCTTCATGAGC
	AA1 R		CAAGACCAGAGTCCATCG
	AA2 F	338 bp	GGCAGGTGACCGAAGCATC
	AA2 R		GAAGCCATGGTGCAGGTG
	AA3 F	376 bp	GCAGCTTCTCTGGCATGG
	AA3 R		GGGAAGCAAAGGAAGACAGA
	A8-1 F	399 bp	CCGCGTTAGCAAAAACAGAT
	A8-1 R		CCTCCATGCGGACGTAGT
	A8-2 F	400 bp	GCAGATCATCTTCGTCTCCA
	A8-2 R		TCGAGGAGAAGATCAGCACA
	A8-3 F	378 bp	CCACGGAGAAAACCATCTTC
	A8-3 R		GAGCGTAGGAAGGCAGTGTC
	A8-4 F	375 pb	TCGAGGAGAAGATCAGCACA
	A8-4 R		GGCTGCTGGCTTTGCTTAG
Crystallin beta A1 (CRYBA1)	**BA1-1 F**	207 bp	GGCAGAGGGAGAGCAGAGTG
	BA1-1 R		CACTAGGCAGGAGAACTGGG
	BA1-2 F	293 bp	AGTGAGCAGCAGAGCCAGAA
	BA1-2 R		GGTCAGTCACTGCCTTATGG
	BA1-3 F	269 bp	AAGCACAGAGTCAGACTGAAGT
	BA1-3 R		CCCCTGTCTGAAGGGACCTG
	BA1-4 F	358 bp	GTACAGCTCTACTGGGATTG
	BA1-4 R		ACTGATGATAAATAGCATGAACG
	BA1-5 F	291 bp	CAATGATAGCCATAGCACTAG
	BA1-5 R		TACCGATACGTATGAAATCTGA
	BA1-6 F	295 bp	CATCTCATACCATTGTGTTGAG
	BA1-6 R		GCAAGGTCTCATGCTTGAGG
Crystallin gamma D (CRYGD)	**GC-1 F**	556 bp	TGCATAAAATCCCCTTACCG
	GC-1 R		CCTCCCTGTAACCCACATTG
	GC-2 F	491 bp	TGGTGTGACAAATTCTGGAAG
	GC-2 R		CCCACCCCATTCACTTCTTA
	GD-1 F	484 bp	CAGCAGCCCTCCTGCTAT
	GD-1 R		GGGTCCTGACTTGAGGATGT
	GD-2 F	395 bp	GCTTTTCTTCTCTTTTTATTTCTGG
	GD-2 R		AAGAAAGACACAAGCAAATCAGT
Crystallin alpha B (CRYAB)	**AB-1 F**	352 bp	AACCCCTGACATCACCATTC
	AB-1 R		AAGGACTCTCCCGTCCTAGC
	AB-2 F	237 bp	CCATCCCATTCCCTTACCTT
	AB-2 R		GCCTCCAAAGCTGATAGCAC
	AB-3 F	477 bp	TCTCTCTGCCTCTTTCCTCA
	AB-3 R		CCTTGGAGCCCTCTAAATCA

This was done in a 25 µl reaction volume using 100 ng of genomic DNA as template, 10 pmol of each primer, 2.5 µl of 10X PCR buffer (Roche, Germany), 1.5 mM $MgCl_2$, 0.2 mMdNTPs and 0.2 U Taq polymerase (Roche, Germany). The samples were heated at 95° C for 5 min followed by 32 cycles (45 s at 94° C, 45 s at 55° C, and 50 s at 72° C) and a final extension period of 5 min at 72° C. PCR products were separated by electrophoresis. Subsequently, to determine any mutation the PCR product was subjected to direct sequencing (Gene Fanavaran, Iran). Sequence data searches were performed in non-redundant nucleotide and protein databases BLAST (http://www.ncbi.nlm.nih.gov/BLAST).

In-silico analysis

The hydrophobic change between the mutant and wild type was evaluated by the ExPASy-ProtScale tool (www.expasy.org/cgi-bin/pro- tscale.pl). In order to predict protein-protein interaction network of CRYBA1/A3, we used STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) (http://string.embl.de).

Results

Ophthalmological evaluation

The proband was a 10-year-old girl who had a bilateral nuclear cataract. She and her parents were clinically examined by an ophthalmologist to diagnosis the ADCC. The opacification in both eyes was symmetrical and homogeneous and with a radial diameter of 4.35 mm and a depth of 1.9 mm. Pathological records of one affected family member with earlier surgery confirmed that the cataract was present at the first decade of life. Nystagmus and other ocular anomalies were not observed in the family members (Figure 1).

CRYBA1/A3 analysis

The whole coding sequence and splicing junctions of the crystallin genes were systematically sequenced. An independent DNA sample evaluated and controlled the mutations after a computer analysis using the Chromas software. Direct PCR sequencing revealed a novel de novo mutation duplication (c.272-274 delGAG, p.(G91del) in exon 4 of the CRYBA1/A3 gene, resulting in a deletion of Glycine of codon91, which presumably occurred in the paternal gamete. This heterozygous mutation was found in the proband, but not in any of the unaffected family members including the parents (Figure 1C).

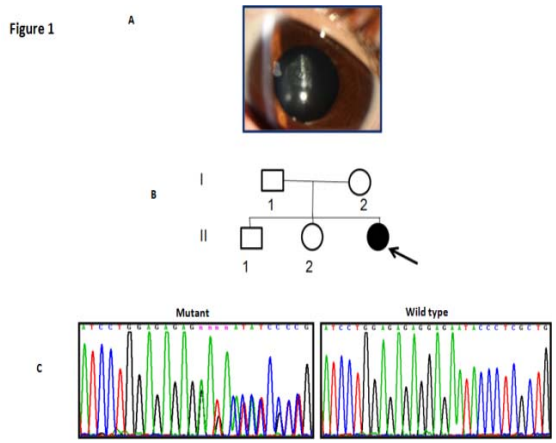

Figure 1. Pedigree, clinical feature and molecular study of affected patients with ADCC. A: Ocular examination using Slit-lamp photographs of the eyes oftheproband. Slit lamp examination revealed the opacity of nuclear cataracts. B: Two generation pedigree of the family affected with congenital cataract. The arrow indicates the proband. C: Chromatogram has shown 3-bp duplication in exon 4 of *CRYBA1* gene, which is marked on the sequence. Vertical arrow corresponds to the mutation point, andthe horizontal arrow shows the frame shift. The topology of *CRYBA1* deletion mutation showing the novel in-frame 3-bp deletion (p.G91del) identified in the Iranian family with ADCC.

In-silico analysis

To further investigatethe role of the Glycine amino acid, an online tool ExPASy-ProtScale was employed. This strategy was determined by a score value assigned to predict the hydrophobicity or hydrophilicity scales based on different chemical and physical properties of the amino acids. The hydropathicity of the Glycine residue at the position 91 in the wild type was slightly different from the mutant type, and the deletion may, in turn, results in modification of the protein structure (Figure 2).

Finally, to predict the *CRYBA1/A3*-protein interaction networks, we used the STRING online tool to identify a core network of interacting proteins by submitting the *CRYBA1*. The in-silico findings showed some members of crystallinfamily, as well as other functional proteins including GJA3, GJA8, GALK1, NT5C and LIM2, might be associated with *CRYBA1/A3* (Figure 3).

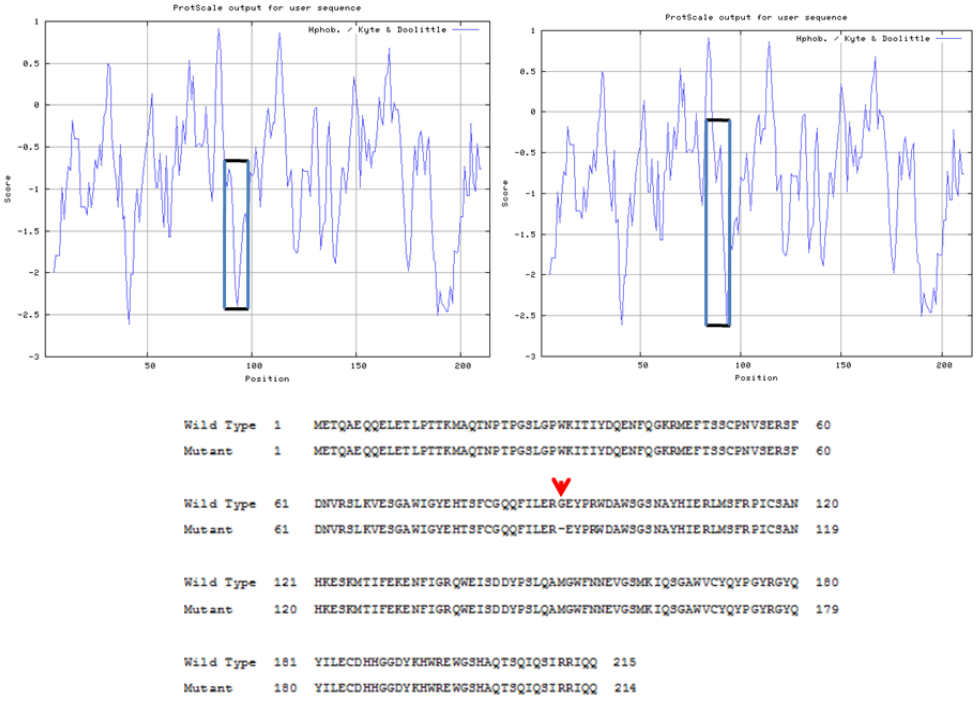

Figure 2. Changed hydrophobicity in *CRYBA1/A3* protein. The hydrophobicity of wild-type and mutant *CRYBA1/A3* were predicted using the ProtScale program on the Expasy. (A) Hydrophobicity of wild-type *CRYBA1/A3*. (B) Hydrophobicity of mutant-type *CRYBA1/A3*. The mutant exhibits increased hydrophobicity, which is shown by the rectangles.

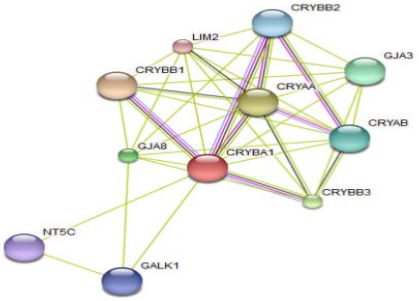

Figure 3. The figure shows *CRYBA1/A3* interaction with other proteins with evidence score ranging (medium to high confidence) predicted by STRING tool

Discussion

In the present study, we first reported a *de novo* heterozygous deletion mutation (c.272-274delGAG, in exon 4 of the *CRYBA1* gene, in an Iranian family. Deletion of the GAG codon removes the Glycine amino acid, but does not change the reading frame, and the other amino acids remain unchanged. However, whilst the reading frame is maintained, in-frame deletions give risk to a protein that lacks one amino acid. This could disturb the tertiary structure and affect the normal biological function of the protein. Therefore, the clinical features we observed, in this case, confirm the relevance of *CRYBA1/3* haploinsufficiency in human for normal lens developmental perturbations. It is well-known that haploinsufficiency of *CRYBA1/3* genes is not uncommon in human eye normal function and structure. The clinical relevance of the mutation described in this study that leads to a Glycine deletion in codon 91 in a premature termination of a truncated protein of *CRYBA1/3* has not been described so far (8).

The *CRYBA1/3* gene contains sixexons separated by five introns. Exon 1 and 2 encode the N-terminal arm, while the subsequent four exons encode the Greek key

motifs (8). This type of structure forms easily during the protein folding. Two polypeptides encoded by this gene, βA1-crystallin, and βA3-crystallin, most have been reported to have diverse cellular functions and roles as structural elements in the lens (8,9). Various studies have reported a number of eye diseases-relatedCRYBA1mutationsin multiple families and ethnic backgrounds (12-14).

A previous study analyzed the crystallin composition of rat's lens and showed that βA1-crystallin and βA3-crystallin, inthe aggregate, constitute 25-30% of the total crystalline complement. Furthermore, by using a genetically engineered mouse model it has shown that βA3-and βA1-crystallins are expressed in retinal astrocytes and retinal pigment epithelial (RPE) cells. It has been hypothesized that accumulation of these proteins could result in some damages and suggested an imperative regulatory function of βA1/A3-crystallin in these cells (12). Ferrini W and colleagues using sequencing of CRYBA1/A3 gene identified an in-frame 3-bp deletion in exon 4 (279delGAG). They revealed *CRYBA1/A3* gene mutation is associated with suture-sparing autosomal dominant congenital nuclear cataract. Data showed mutation responsible for the deletion of Glycine-91 co-segregated in all affected individuals by same ethnic background (13). Qi and colleagues using haplotype analysis for a dominant congenital nuclear cataract locus characterized a specific gene harboring the gene coding for *CRYBA1/A3*. Mutation analysis by others, in agreement with our findings, revealed a *de novo* 3-bp deletion in exon 4 in cataract patients (14-16). This mutation results in the deletion of a Glycine at codon 91 (DeltaG91) and could be related to an incorrect folding of βA1/A3 crystallin. These evidences emphasize the physiological significance of crystalline and confirm the role of CRYBA1/A3 in cataract development (18,19).

Taken together, these findings indicate phenotypic heterogeneity related to mutations in this gene. Despite the fact that our study did not focus on the biological function of the observed duplication, we believe that the reported mutation here is possible to possess a significant biological impact on the normal function of *CRYBA1/3* protein. According to our findings as well as those have published so far (14-17), two main questions needed to be addressed, namely: (1) which parts of this protein are implicated in the cellular function? And (2) how can we functionally define the cellular and molecular characterization of the *CRYBA1/A3* protein?

The first question was somewhat addressed by distinct studies on the nature of crystalline protein properties, suggesting that crystallin family is crucial for conferring and maintaining lens transparency. In order to address the second question, we performed an*in-silico* study to predict *CRYBA1/A3* protein interaction network. The *in-silico* findings showed some members of crystallinfamily, as well as other functional proteins, including GJA3, GJA8, GALK1, NT5C and LIM2, might be associated with *CRYBA1/A3*. Mutations in some of these genes includingLIM2 (18) GJA (21,22) and GALK1 (21) have been associated with the autosomal recessive cataracts. Here, we would like to discuss a key protein that its functions could potentially affect the cell signaling pathways in eye cells. GALK1 or galactokinase play critical roles in chemical signaling, cellular structures and transporting molecules. Deficiency of this protein was first recognized in a cataract patient. Mutations of GALK1 have been identified in families with cataracts and cause autosomal-recessive as well as age-related cataracts as a result of galactitol accumulation (23,24). Altogether, *CRYBA1/A3* cell signaling as a part of this signaling network could indirectly involve in cataract development.

We also used an online tool ExPASy-ProtScale to predict the hydrophobicity or hydrophilicity scales for Glycine. Our findings showed that the hydropathicity of Glycine residue at the position 91 in the wild type was slightly different from the mutant type and this deletion may, in turn, result in modification of the protein structure. On the other hand, the pathogenic mutation identified in exon 4 of *CRYBA1/A3* gene is associated with Glycine hydropathicity which finally results in cataract. However, further studies are required for better understanding of the molecular pathogenesis of this mutation and can provide insights into the structure-function relationships of the *CRYBA1/3* protein. For example, the examinations such as phase behavior and X-ray structure analysis could resolve the physicochemical properties and conformational changes resulting from *CRYBA1/3* mutation and mutant proteins.

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