Clinical characteristics of congenital lamellar cataract and myopia in a Chinese family

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Abstract

To investigate the clinical characteristics and the genetic defect in a Chinese family with congenital lamellar cataract with myopia. Three generations of a single family were recruited in this study. A detailed family history and clinical data were recorded. A total of 100 unrelated ethnically matched controls without family history of congenital cataracts and myopia were also recruited. Genomic DNA was extracted from peripheral blood leukocytes. The sequencing of candidate genes was performed to screen out the disease-causing mutation. The effects of amino acid changes on the structure of proteins were predicted by bioinformatics analysis. Affected individuals presented lamellar lens opacities and myopia. Direct sequencing revealed a heterozygous c. 34 C>T variation in the αA-crystallin protein (CRYAA) gene, which resulted in the replacement of a highly conserved arginine by cystine at codon 12 (p.R12C). This mutation co-segregated with all affected individuals and was not observed in unaffected members or the 100 normal controls. Bioinformatic analysis showed that a highly conserved region was located around Arg12, an increase in local hydrophobicity was shown around the substitution site and the secondary structure of the mutant CRYAA protein has been changed. This is the case of a congenital lamellar cataract phenotype with myopia associated with the mutation of Arg12Cys (p.R12C) in CRYAA. Our finding confirms the high rate of mutations at this dinucleotide. In addition, these results demonstrate a myopia susceptibility locus in this region, which might also be associated with the mutation in CRYAA.

Keywords: congenital lamellar cataract; myopia; Chinese family
Introduction

Congenital cataract is a clinically and genetically heterogeneous lens disease characterized by significant visual impairment and blindness in childhood [1,2]. The incidence is 0.6/10,000 to 6/10,000 [3]. Cataracts can be isolated or occur in association with metabolic diseases or genetic syndromes. About one-third cataracts are genetic [3]. A majority of congenital cataracts are single gene disorders. Autosomal dominant inheritance is the most common mode of congenital cataract [3]. Autosomal recessive and X-linked fashion have also been reported [4]. According to morphology, cataracts can be classified into different categories, including nuclear, lamellar, cortical, polar, sutural, pulverulent, cerulean, coralliform, and whole lens, as well as other minor subtypes [3].

Up to date, more than 34 loci and 18 genes on different chromosomes have been identified to be associated with autosomal dominant congenital cataract (ADCC) [5,6], moreover, about half of them have mutations in crystallins, a quarter have mutations in connexins, and the remainder is evenly divided into intrinsic membrane proteins, intermediate filament proteins, transcription factors and other genes [7]. Currently, genes with mutations associated with mixed lamellar cataracts include four groups. The first group is crystalline genes (CRYAA, CRYAB, CRYBA1, CRYBB2, CRYGC, CRYGD). The second group is encoding membrane transport proteins, including GJA3 and GJA8. The third group is beaded filament structural protein 2 (BFSP2), encoding cytoskeletal protein. The last group is heat shock transcription
factor 4 (HSF4), encoding transcription factors [3,7]. Therefore, it is rational to consider these genes as the top list of candidate genes for screening studies in congenital lamellar cataracts.

In this study, we applied a function candidate testing approach to the known lamellar cataract-causing genes in a Chinese family. A R→C mutation in CRYAA that co-segregated with the disease phenotype was identified to be responsible for ADCC, which provides a possible mechanism of action for the mutant gene. This mutation has previously been described in Danish and Hong Kong families, which were both shown microcornea-cataract [8, 9]. However, isolated lamellar cataract such as those reported here has not been identified in the previous reports.

Materials and Methods

Clinical evaluation and DNA specimens

A three-generation family with autosomal dominant lamellar cataract and myopia from Henan province, China, were recruited at Beijing Tongren Hospital, the Capital Medical University. Both affected and unaffected individuals of the family underwent detailed ophthalmic examinations including visual acuity, slit lamp examination, ultrasonography, fundus examination, and intraocular pressure measurement. The phenotypes were
documented by slit lamp photography. A total of 100 unrelated ethnically matched controls with no family history of congenital cataracts and myopia were also recruited. They were given complete ophthalmologic examinations as the study subjects of the cataract family and confirmed without eye diseases except for senile cataracts. Individuals with one of the following three criteria were considered to be affected with myopia, (1) cycloplegic refraction of -1.00 D spherical equivalent or lower in individuals <30 years old; (2) manifest refraction of -1.00 D spherical equivalent or lower in individuals ≥30 years old, or (3) axial length >26 mm (An extension of 1 mm would generally result in myopia of -3.00 D, the normal range of axial length in Chinese is 23.5-24.5 mm). With the consent of all participants, the peripheral venous blood of all participants was collected and genomic DNA was extracted using a QIAamp DNA kit (Qiagen, Valencia, CA) in accordance with the manufacturer’s instructions. All patients had provided informed consents before participating in this study. This study was conducted in accordance with the tenets of the Declaration of Helsinki and approved by the ethics committees for medical research at the Capital Medical University in Beijing, China.

**Mutation screening**

Eleven candidate genes, including *CRYBA1* (GenBank NM_005208), *CRYBB2* (GenBank
NM_000496), MIP (GenBank NM_012064.3), and BFSP2 (GenBank NM_003571), are highly expressed in the lens and have been considered as candidate genes for hereditary lamellar cataracts [3,7]. Mutation screening was performed in these candidate genes. We amplified each exon and intron-exon junction of the genes with previously published primer sequences (Table 1) [10] by polymerase chain reaction (PCR). Each reaction mix (25 μl) contained 20 ng of genomic DNA, 1× PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 0.5 μM each of forward and reverse primers and 2.5U of Taq DNA polymerase (Qiagen). A PCR program was performed for DNA amplifying: 95 °C for 3 min; followed by 35 cycles at 95 °C for 30 s, 57 °C-63 °C for 30 s (annealing temperature depending on different primer); 72 °C for 45 s; and a final extension at 72 °C for 7min. The PCR products of the proband and one unaffected member were sequenced using an ABI3730 Automated Sequencer (PE Biosystems, Foster City, CA). The sequencing results were analyzed using Chromas 2.33 and compared with the reference sequence in the NCBI database. Then we screened the mutation in CRYAA from the sample of the family members and 100 ethnically matched controls to confirm the mutation.

**Bioinformatics analysis**

The CLC Free Workbench 5.0 software (CLC bio, Aarhus, Denmark) was used to align the
protein sequences from several different species. The comparison of hydrophobicity between wild type and mutant type was analyzed by ProtScale. Garnier-Osguthorpe-Robson (GOR) software was used to predict the effect of the mutation on the secondary structure of **CRYAA**.

**Results**

**Clinical characteristics**

A three-generation Chinese family with clear diagnosis of ADCC and myopia was identified (Figure 1). All affected individuals in this family had lamellar opacity cataracts (Figure 2) in total eleven family members (six affected and five unaffected) participated in the study (Table 2). The proband was a sixteen-year-old girl (III:7), whose vision had decreased since birth. She was diagnosed with bilateral cataract at the age of four and myopia at seven years old. An affected member (III:6), the 22-year-old sister of the proband, was diagnosed with bilateral cataract and myopia at the age of seven, and underwent right eye’s cataract extraction at the age of ten. Another affected member (II:9), whose phenotype was similar with the proband, had obvious lamellar and cortical opacity. According to the medical records, other affected individuals diagnosed with bilateral lamellar cataract and myopia, have been treated with cataract extraction in different years.

Myopia was present in all affected members, the ocular axial length was extended in the four
affected individuals for whom records are available (Table 2). Thus, the myopia is more likely to be of an axial nature rather than being secondary to lens. Most patients experience decreased visual acuity at birth, although it is unclear whether it is caused by lamellar cataract or the accompanying myopia. There was no family history of other ocular or systemic diseases.

**Mutation analysis**

Through direct sequencing of the coding regions of the candidate genes, we identified a heterozygous c. 34C >T variation of *CRYAA* in the affected individuals (Figure 3). It resulted in a substitution of arginine to cystine at codon 12 (p. R12C). The substitution was not found in any of the unaffected family members or in the 100 participants in the control group. We did not find any other mutations in the family except for a few nonpathogenic single nucleotide polymorphisms (SNPs).

**Bioinformatics analysis**

The place where the mutation occurred was located within a phylogenetically conserved region by multiple-sequence alignment (Figure 4). The comparison of hydrophobicity
between wild type and mutant type, and hydrophobicity of the mutant protein increased between 8 amino acids and 16 amino acids (Figure 5). Using the GOR method, the results for secondary structure prediction suggested that the mutant CRYAA 12C replaced one coil “C” with one turns “T” at amino acid 14 and a coil “C” with a turns “T” at position 19 (Figure 6).

Discussion

In this study, we identified a novel mutation (c. 34C>T) in CRYAA in a three-generation Chinese family with congenital cataract and myopia. This variation seemed to be the disease causative factor as it co-segregated with the disease phenotype in all affected individuals and was absent in unaffected family members and 100 control subjects.

Crystallins are the major structural proteins in lens accounting for nearly 90% of total soluble proteins [11]. The crystallin superfamily is composed of α, β and γ-crystallins which contribute to the maintenance of lens transparency and a proper refractive index of the lens [12]. CRYAA is a major structural protein of the lens, and is required for maintenance of lens transparency. It is also a member of the small heat-shock-protein (sHSP) family, which consists of stress-induced proteins, and has chaperone activity [13,14]. CRYAA contains a conserved α-crystallin domain, which is flanked on either side by a hydrophobic
NH2-terminal domain or a hydrophilic unstructured COOH-terminal [15,16]. The COOH-terminal domain was important for substrate-binding ability and chaperoning activity [17]. Previous research has also shown that CRYAA may play a possible role in stimulating epithelial cell differentiation in the lens [18]. Furthermore, a knockout mouse strain with an αA crystallin gene deletion shows microphthalmia and eventual opacity of the lens, which demonstrates the important role of alpha A crystallin in the development and maintenance of lens transparency [19].

In our study, the mutation led to an amino acid substitution, R12C, close to the NH2-terminus of CRYAA. The multiple-sequence alignments showed that Arg12 was a highly conserved residue. The NH2-terminus was reported to assist protein oligomerization, stability, and substrate binding [20]. Changes in the NH2-terminus, would greatly affect the function of the protein including reducing chaperoning activity. Furthermore, the bulky polar amino acid, arginine, plays an important role in maintaining the structural integrity of the CRYAA protein and oligomeric assembly [21]. Loss of arginine would cause a loss of positive charge and lead to abnormal folding. Functional studies have shown that loss of arginine destabilizes CRYAA, reduces its interactions with substrate proteins and chaperoning activity [22]. The phenotype of cataract is presumed to be caused by the reduced CRYAA molecular chaperoning ability on other lens proteins [23].

Up to now, a total of eleven mutations in CRYAA have been reported to cause inherited
cataract (Table 3). Among them, five mutations are associated with cataract-microcornea syndrome (CMCC), R12C [9], R21W [8], R54C [24], R116C [25], and R116H [8,26], which are all located in the highly conserved arginine residues in the two major functional domains of the αA-crystallin, the NH2-terminus and the COOH-terminus. In 2007, Hansen et al. [8] firstly reported the mutation of R12C, which was associated with cataract-microcornea syndrome in a Danish family, showing posterior polar opacity progressing to dense nuclear and laminar cataract. In 2009, Zhang et al. [9] found the same mutation causing non-progressive CMCC in a Hong Kong family from China, which exhibits an altered heat-shock response. This mutation nuclear phenotype, with or without microcornea has also been reported in other three families [20,27,28]. Interestingly, all of them were clinically different from our study, which was associated with isolated lamellar cataract with myopia, but without microcornea. These studies have demonstrated that Arg at the 12th locus of the peptide is a mutation hotspot, which is the essential role causes congenital cataracts.

Myopia is the most common visual problem in the world. The main causes are environment and genetics [29]. A number of evidence reveal the importance of genetic factors in the development of myopia, although environmental factors such as near work and a city lifestyle appear to have a great impact on prevalence of myopia. Recent genome wide linkage studies have provided evidence of susceptibility loci for mild, moderate, and high myopia [29-31]. In this family, myopia is not random. The diagnosis is through stringent
standards, and the affected members of this family live in the countryside where myopia is rare compared to urban populations [29,32-35]. Most myopia begins at school age except for hereditary high myopia [32].

The c.34C>T substitution observed in the present study caused the replacement of glycine to glutamic at codon 12 (p. R12C), localized in the first exon of CRYAA. The result of multiple-sequence alignments showed that Arg12 was a highly conserved residue. To investigate the effect of R12C substitution on CRYAA chaperoning activity, we use the GOR method, the secondary structure of the mutant type has been changed. Protein analysis by ProtScale clear showed that substitution in the CRYAA protein would increase the hydrophobicity between amino acids 8 and 16. This data indicates that the critical function of arginine. Mutation may have a detrimental physiological effect. As reported in other lens proteins, hydrophobicity is associated with crystallin activities, such as CRYBB2 and CRYGD [36-38]. For CRYAA, hydrophobicity is involved in protein oligomerization and chaperoning activity [39,40]. Thus, the predicted increase of hydrophobicity around Arg-Cys substitution site might change the local protein structure and function.

There were also some limitations in this study. First, the mutation has not been proved to be pathogenic in this study. Further research using animal models to confirm the pathogenicity of the newly discovered disease sites was needed. Second, only one family data was analyzed in this study. Further study with larger sample size was needed.
In conclusion, an autosomal dominant isolated lamellar cataract associated with myopia in a Chinese family was described. Studies of functional protein, especially in a larger group of patients with mutations of *CRYAA* gene, will be very valuable in confirming typical reasons of *CRYAA*-related cataract. Research on *CRYAA* gene variations in populations with or without myopia would be very helpful in illustrating the role of *CRYAA* alterations in multifactorial myopia. Our finding confirms the high rate of apparently independent mutations at this dinucleotide.
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Availability of data and materials

All data generated or analyzed during this study are included in this published article. The additional datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ Contributions

Qing Liu was dedicated to the literature research, clinical studies, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation and manuscript editing; Siquan Zhu carried out the integrity of the entire study, study concepts, study design and manuscript review. All authors have read and approved this article.

Ethics approval and consent to participate
This study was conducted in accordance with the tenets of the Declaration of Helsinki and approved by the ethics committees for medical research at the Capital Medical University in Beijing, China.

**Consent for publication**

N/A

**Competing interests**

The authors declare that they have no competing interests.
References

1. Pichi F, et al., *Genetics of Congenital Cataract*. Dev Ophthalmol, 2016. 57: p. 1-14.

2. Solebo AL, Teoh L, Rahi J., *Epidemiology of blindness in children*. Arch Dis Child, 2017. 102: p. 853-7.

3. Reddy MA, Francis PJ, BelTy V., *Molecular genetic basis of inherited cataract and associated phenotypes*. Surv Ophthalmol, 2004. 49: p. 300-15.

4. Francis PJ, Berry V, Bhattacharya SS, Moore AT., *Genetics of childhood cataract*. J Med Genet, 2000. 37: p. 481–8.

5. Shiels A, Hejtmancik JF., *Genetic origins of cataract*. Arch Ophthalmol, 2007. 125: p. 165-73.

6. Wang K, et al., *A novel GJA8 mutation (p.I31T) causing autosomal dominant congenital cataract in a Chinese family*. Mol Vis, 2009. 15: p. 2813-20.

7. Hejtmancik JF., *Congenital cataracts and their molecular genetics*. Semin Cell Dev Biol, 2008. 19: p. 134-49.

8. Hansen L, et al., *Genetic heterogeneity in microcornea cataract: five novel mutations in CRYAA, CRYGD, and GJA8*. Invest Ophthalmol Vis Sci, 2007. 48: p. 3937-44.

9. Zhang LY, et al., *An αA-crystallin gene mutation, Arg12Cys, causing inherited cataract-microcornea exhibits an altered heat-shock response*. Mol Vis, 2009. 15: p. 1127-38.
10. Wang KJ, et al., A novel mutation in MIP associated with congenital nuclear cataract in a Chinese family. Mol Vis, 2011. 17: p. 70-7.

11. Bloemendal H, et al., Ageing and vision: structure, stability and function of lens crystallins. Prog Biophys Mol Biol, 2004. 86: p. 407–85.

12. Horwitz J., Alpha-crystallin. Exp Eye Res, 2003. 76: p. 145–53.

13. Graw J., Genetics of crystallins: Cataract and beyond. Exp Eye Res, 2009. 88: p. 173-89.

14. Andley UP., Effects of alpha-crystallin on lens cell function and cataract pathology. Curr Mol Med, 2009. 9: p. 887-92.

15. Asomugha CO, Gupta R, Srivastava OP., Structural and functional properties of NH(2)-terminal domain, core domain, and COOH-terminal extension of alphaA- and alphaB-crystallins. Mol Vis, 2011. 17: p. 2356-67.

16. Augusteyn RC., alpha-crystallin: a review of its structure and function. Clin Exp Optom, 2004. 87: p. 356-66.

17. Berengian AR, Bova MP, Mchaourab HS., Structure and function of the conserved domain in alphaA-crystallin. Sitedirected spin labeling identifies a beta-strand located near a subunit interface. Biochemistry, 1997. 36: p. 9951-7.

18. Boyle DL, Takemoto L., A possible role for alpha-crystallins in lens epithelial cell differentiation. Mol Vis, 2000. 6: p. 63–71.
19. Brady JP, et al., Targeted disruption of the mouse alpha A-crystallin gene induces cataract and cytoplasmic inclusion bodies containing the small heat shock protein alpha B-crystallin. Proc Natl Acad Sci U S A, 1997. 94: p. 884–9.

20. Kundu M, Sen PC, Das KP., Structure, stability and chaperone function of αA-crystallin: Role of N-terminal region. Biopolymers, 2007. 86: p. 177-92.

21. Santhiya ST, et al., Identification of a novel, putative cataract – causing allele in CRYAA (G98R) in an Indian family. Mol Vis, 2006. 12: p. 768-73.

22. Fu L, Liang JJ., Alteration of protein-protein interactions of congenital cataract crystallin mutants. Invest Ophthalmo Vis Sci, 2003. 44: p. 1155-9.

23. Chang B, et al., Identification of a missense mutation in the alphaA-crystallin gene of the lop18 mouse. Mol Vis, 1999. 5: p. 2

24. Devi RR, et al., Sunda25-Hydroxycholecalciferolsan P and Hejtmancik JF. Crystallin gene mutations in Indian families with inherited pediatric cataract. Mol Vis, 2008. 14: p. 1157-70.

25. Litt M, et al., Autosomal dominant congenital cataract associated with a missense mutation in the human alpha crystallin gene CRYAA. Hum Mol Genet, 1998. 7: p. 471–4.

26. Richter L, et al., Clinical variability of autosomal dominant cataract, microcornea and corneal opacity and novel mutation in the alpha A crystallin gene (CRYAA). Am J Med
Genet A, 2008. 146: p. 833-42.

27. Sun W, Xiao X, Li S, Guo X, Zhang Q., *Mutational screening of six genes in Chinese patients with congenital cataract and microcornea*. Mol Vis, 2011. 17: p. 1508-13.

28. Santana A, et al., *Mutation analysis of CRYAA, CRYGC, and CRYGD associated with autosomal dominant congenital cataract in Brazilian families*. Mol Vis, 2009. 15: p. 793-800.

29. Feldkamper M, Schaeffel F., *Interactions of genes and environment in myopia*. Dev Ophthalmol, 2003. 37: p. 34-49.

30. Stambolian D, et al., *Genome wide linkage scan for myopia susceptibility loci among Ashkenazi Jewish families shows evidence of linkage on chromosome 22q12*. Am J Hum Genet, 2004. 75: p. 448-59.

31. Hammond CJ, et al., *A susceptibility locus for myopia in the normal population is linked to the PAX6 gene region on chromosome 11: a genome wide scan of dizygotic twins*. Am J Hum Genet, 2004. 75: p. 294-304.

32. Zhao J, et al., *Refractive Error Study in Children: results from Shunyi District, China*. Am J Ophthalmol, 2000. 129: p. 427-35.

33. Wickremasinghe S, et al., *Ocular biometry and refraction in Mongolian adults*. Invest Ophthalmol Vis Sci, 2004. 45: p. 776-83.

34. Kleinstein RN, et al., *Collaborative Longitudinal Evaluation of Ethnicity and Refractive...*
Error Study Group. Refractive error and ethnicity in children. Arch Ophthalmol, 2003. 121: p. 1141-7.

35. Zhan MZ, et al., Refractive errors in Singapore and Xiamen, China--a comparative study in school children aged 6 to 7 years. Optom Vis Sci, 2000. 77: p. 302-8.

36. Liu BF, Liang JJ., Interaction and biophysical properties of human lens Q155\(^*\) betaB2-crystallin mutant. Mol Vis, 2005. 11: p. 321-7.

37. Flaugh SL, Kosinski-Collins MS, King J., Contributions of hydrophobic domain interface interactions to the folding and stability of human gammaD-crystallin. Protein Sci, 2005. 14: p. 569-81.

38. Flaugh SL, Kosinski-Collins MS, King J., Interdomain sidechain interactions in human gammaD crystallin influencing folding and stability. Protein Sci, 2005. 14: p. 2030-43.

39. Sharma KK, Kumar RS, Kumar GS, Quinn PT., Synthesis and characterization of a peptide identified as a functional element in alphaA-crystallin. J Biol Chem, 2000. 275: p. 3767-71.

40. Shroff NP, Cherian-Shaw M, Bera S, Abraham EC., Mutation of R116C results in highly oligomerized alpha A-crystallin with modified structure and defective chaperone-like function. Biochemistry, 2000. 39: p. 1420-6.
Titles and legends to figures

Figure 1 A three-generation Chinese family with clear diagnosis of ADCC and myopia.

Figure 2 The patient with amellar opacity cataracts.

Figure 3 A heterozygous c. 34C >T variation of CRYAA in the affected individuals.

Figure 4 The place where the mutation occurred was located within a phylogenetically conserved region by multiple-sequence alignment. Arg12 was a highly conserved residue.

Figure 5 Substitution in the CRYAA protein increases the hydrophobicity between amino acids 8 and 16.

Figure 6 The effect of the mutation on the secondary structure of CRYAA. A. The structure of the wild type; B. The structure of mutant type. Using GOR method, the mutant CRYAA 12C replaced one coil “C” with “T” at amino acid 14 and a coil “C” with a turns “T” at position 19.
| Name | Forward (5'-3') | Reverse (3'-5') |
|------|----------------|----------------|
| CRYAA-1 | AGCAGCCTCTTTCTAGAC | CAAGACCAGAGTCCATCG |
| CRYAA-2 | GCCAGGTGACCGAAGCACT | GAAAGCTGGTGGCAGGTC |
| CRYAA-3 | GCAGCTCTCTTGGGATG | CAGGAAAGGAAAAGGAGC |
| CRYAB-1 | AACCCTGACATCCACCTTC | AAGGACTCTCCGCTCCTAG |
| CRYAB-2 | CCATCCATCCCTCTACCTT | GCCCTCAAAGGCTGATGGCC |
| CRYAB-3 | TTCCTCTGCTCTCTTCTCA | CTTTGAGGCCTCTAAATCA |
| CRYBA1-1 | GGCAGAGGGGAGGCAATGTG | CACTAGGCAGACAGGACTGG |
| CRYBA1-2 | AGTGAACAGCAGACGACAA | GGTCACTGACTGCTTTAG |
| CRYBA1-3 | AAGCACAGAGTCAAGTCAA | CCCCCTGCTCAGAGGACTG |
| CRYBA1-4 | GTTGGGACGAGAGGAGATG | TGGGCTGGGAGAGGACTTC |
| CRYBA1-5 | GAATGATAGAGCCATAGCAG | TACCCAGAATGATGAAAG |
| CRYBA1-6 | CATCTCAATACCTGTTGTGAG | GCAAGGCTCTGACTGCTAG |
| CRYBB2-1 | TTTTGAGGGGACGAGAGGATG | TGGGCTGGGAGAGGACTTC |
| CRYBB2-2 | CTTTCACATCTCTTTGGGTC | GCAAGTCTAAAAAGCTCTCA |
| CRYBB2-3 | TGAGCAGATTCTGGCATAG | GTGCCCTCTGAGCTTATTA |
| CRYBB2-4 | GGGCCTCTACCACACTACA | CCTCCCTCTGCTCTCAACCT |
| CRYBB2-5 | CCTTCCCTTGGGAGTTGGAAG | TCAAGACACACAGGACCA |
| CRYGC-1 | TGGTGGATACAAATCTGGAAG | CCCACCCATCTCCTCCTT |
| CRYGD-1 | CAGCAGCCCTCTCCTCCTT | GGTTCCTGAGCTTCTTAG |
| CRYGD-2 | GCTTCTTCTCTCTTCTCTT | AAAGAAGACACAGGACCA |
| GJA3-1 | CGGTGTTCAATGACATTTTC | CTCTTTGTGCTCTTCTC |
| GJA3-2 | GAGGAAGAGCAGCTGAAAG | AGCGGGTTGCGCATAGTG |
| GJA3-3 | TCCTGATCAGTACCCTCTCATT | TATCTGCTGGGGAGAAC |
| GJA8-1 | CCGGTTAGCAAAAACAGAT | CCTCCTATGCGACGTAAT |
| GJA8-2 | GCAGATCCTCTCTCTCTCCT | GGCACAGACACAGACAC |
| GJA8-3 | CCACGGAGAATAACCATCTT | GAGCGTAGGAAGGAGTTC |
| GJA8-4 | TCGAGGAAGAGTACAGACA | GGCTGCTGGCTTTCTTAG |
| BFS(2)1a | AATGACCAAAACCAATGGT | AGGCGCCCTGSGACACT |
| BFS(2)1a | GAGAGGGAGTGTTAGTGGAA | GGCTCATGCTACTCAAC |
| BFS(2)2 | TGCAAGACAGCATTCCAC | GAGGGGTGCTAGCTGATAA |
| BFS(2)3 | GCTGCAATGCTCTCTTCTT | GGTTAACCTGACCCACTT |
| BFS(2)4 | TCTGTGAAAGCTGTGTCCCTG | CCCGCGCCCTAAATTCTCTT |
| BFS(2)5 | ACCAGAGAGAGAAGGTGGGT | GGTTAACCTGACCCACTT |
| BFS(2)6 | GGGGAAATAGCTCAGGCTACC | ATGGGGTCTACTGAGAGG |
| BFS(2)7 | TTTTCCCAAGGCGCCAGATTC | CACTCAAGGGAACCCCTCA |
| HSF4-1 | CATCCATCACTCCAGGTCTT | GGGCATGGGTGTCATGAC |
| HSF4-2 | CCTCGACCCATATCCCGTAA | GCAAGGAGCAAGGAGGAGG |
| HSF4-3 | GGGGAATGAGCAAGAGGAGA | GCCAAGGAGCAAGGAGGAG |
| HSF4-4 | TCCCAAGCCCTCCATTCT | CCCGTTGAAGGAGTCTTCA |
| HSF4-5 | GCTGGGGCGCTGAGGAGG | GGTGCTCATCTTCTTCTCCTT |
Table 2  Clinical data of affected members in this family

| ID: | Gender | Age at first symptom | Visual acuity | Axial length |
|-----|--------|----------------------|---------------|-------------|
|     |        |                      | OD  | OS  | Phenotype                  | OD  | OS  |
| I:2 | female | NA                   | 0.08| 0.08| NA                         | NA  | NA  |
| II:2| female | at birth             | 0.2 | 0.3 | lamellar                   | NA  | NA  |
| II:7| female | at birth             | 0.3 | 0.3 | lamellar                   | 27.32| 26.82|
| II:9| male   | at birth             | 0.1 | 0.2 | lamellar, cortical         | 27.21| 27.10|
| III:6| female| at birth             | 0.4 | 0.3 | lamellar                   | 26.16| 25.53|
| III:7| female| at birth             | 0.3 | 0.4 | lamellar                   | 25.23| 25.21|
| III:9| female| at birth             | 0.4 | 0.5 | lamellar                   | NA  | NA  |

The visual acuity column(s) indicates the current visual acuity of the affected members, “NA” indicates not available.
Table 3 Summary of identified mutation in CRYAA

| Nucleotide Reference | Amino acid | Phenotype | Inherited mode |
|----------------------|------------|-----------|----------------|
| c.346C>T             | R116C      | congenital zonular central nuclear, some with microcornea | [20] |
| c.27G>A              | W9X        | congenital nuclear | AR [21] |
| c.145C>T             | R49C       | Sporadic nuclear, with fundus | AD [22] |
| c.62C>G              | R21L       | Hypoplasia | AD [23] |
| c.247G>A             | G98R       | lamellar to total progressing, nuclear, | AD [24] |
| c.34C>T              | R12C       | with or without microcornea | AD [24] |
| c.130C>T             | R21W       | central and laminar | AD [8] |
|                      |            | with varying anterior and posterior polar components | |
| c.347G>A             | R116H      | nuclear with polar and/or equatorial ramification | AD [8, 25] |
| c.230C>T             | R54C       | Nuclear, with microcornea | AD [26] |
| c.161G>C             | R54P       | Y-suture | AD [27] |