A G→T splice site mutation of CRYBA1/A3 associated with autosomal dominant suture cataracts in a Chinese family

Zhenfei Yang,1 Qian Li,2,3 Zicheng Ma,1 Yuanyuan Guo,1 Siquan Zhu,1 Xu Ma2,3,4

1Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing Ophthalmology & Visual Sciences Key Lab, Beijing, China; 2National Research Institute for Family Planning, Beijing, China; 3Peking Union Medical College, Beijing, China; 4World Health Organization Collaborating Center for Research in Human Reproduction, Beijing, China

Purpose: To identify the genetic defect in a five-generation Chinese family with congenital Y-suture cataracts.

Methods: A five-generation Chinese family with inherited Y-suture cataract phenotype was recruited. Detailed family history and clinical data of the family were recorded. Candidate genes sequencing was performed to screen out the disease-causing mutation.

Results: The congenital cataract phenotype of the family was identified as Y-suture cataract type by using slit-lamp photography. Direct sequencing revealed a G→T splice site mutation in crystallin, beta A1 (CRYBA1/A3). This mutation co-segregated with all affected individuals in the family and was not found in unaffected family members or 100 unrelated controls.

Conclusions: Our study identified a novel type of a splice site mutation in CRYBA1/A3. The mutation was responsible for the congenital Y-suture cataracts in the family. This is the first report relating a G→T mutation of CRYBA1/A3 to congenital Y-suture cataract.

Congenital cataracts, characterized by opacification of all or part of the eye's crystalline lens within the first year of life, are a leading cause of visual impairment or blindness in children [1]. The prevalence of congenital cataracts is 1 to 6 per 10,000 live births [2]. Cataracts can be isolated or occur in association with a large number of metabolic diseases and genetic syndromes. Congenital cataracts are most frequently inherited as autosomal dominant traits, but can also be inherited in an autosomal recessive or X-linked fashion [3]. According to morphology, congenital cataracts can be classified into several subtypes: whole lens, nuclear, lamellar, cortical, polar, sutural, pulverulent, cerulean, coralliform, and other minor subtypes [4].

Approximately half of all cataract families have crystallin mutations, including crystalline, alpha A (CRYAA), crystallin, alpha B (CRYAB), crystallin, beta A1 (CRYBA1/A3), crystallin, beta A4 (CRYBA4), crystallin, beta B1 (CRYBB1), crystallin, beta B2 (CRYBB2), crystallin, gamma C (CRYGC), crystallin, gamma D (CRYGD), crystallin, gamma S (CRYGS). About one quarter have connexin mutations in gap junctional proteins, including gap junction protein, alpha 3, 46kDa (GJA3), and gap junction protein, alpha 8, 50kDa (GJA8), with the remainder divided among the genes for heat shock transcription factor-4 (HSF4), aquaporin-0 (AQP0, MIP), and beaded filament structural protein-2 (BFSP2) [5].

We applied a functional candidate approach testing the known cataract-causing genes in a Chinese family. A G→T splice mutation in CRYBA1/A3 was identified to be responsible for cataracts in the family. This is the first report to relate this mutation site to Y-suture cataracts also involving opacities of the nucleus.

METHODS

Family data: A five-generation Chinese family from Shandong Province with a history of cataracts was recruited from Beijing Tongren Hospital, Capital Medical University, Beijing, China. The research was approved by the ethics committee of Capital Medical University. Informed consent was obtained from all participants of the family. The study protocol followed the principles of the Declaration of Helsinki.

Detailed family medical history was recorded by interviewing the family members. All participating members underwent ophthalmic examination, including visual acuity, slit-lamp examination, intraocular pressure measurement, ultrasonography, and fundus examination of the dilated pupil. Slit-lamp photography was performed to document the phenotype of the cataracts in the patients. One hundred unrelated subjects without cataracts were recruited from the Ophthalmology Clinic of Beijing Tongren Hospital as normal controls and were given complete ophthalmologic examinations. None of the controls exhibited eye diseases except mild myopia.

Genomic DNA preparation: About 2 ml of peripheral blood was collected from the family members who took part in the
study. Genomic DNA was extracted from blood using the QIAamp Blood kit (Qiagen, Valencia, CA).

Mutation screening: We used the functional candidate gene analysis approach, including CRYAA (GenBank NM_000394), CRYAB (GenBank NM_001885), CRYBA1 (GenBank NM_050208), CRYBB1 (GenBank NM_001887), CRYBB2 (GenBank NM_000496), CRYGC (GenBank NM_020989), CRYGD (GenBank NM_006891), CRYGS (GenBank NM_005208), CRYBB1 (GenBank NM_001887), CRYBB2 (GenBank NM_000496), CRYGC (GenBank NM_020989), and CRYGD (GenBank NM_006891).
(GenBank NM_017541), GJA3 (GenBank NM_021954), GJA8 (GenBank NM_005267), MIP (GenBank NM_012064.3), HSF4 (GenBank NM_001040667.2), and BFSP2 (GenBank NM_003571). Each exon and intron-exon junction of the genes were amplified by polymerase chain reaction (PCR) using previously published primer sequences (Table 1) [6]. 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 2.5 U of Taq DNA polymerase (Qiagen). A PCR program was performed for DNA amplifying: 95 °C for 5 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 30 s; and a final extension at 72 °C for 10 min. 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 CRYBA1/A3 from the sample of the family members and 100 ethnically matched controls to confirm the mutation.

RESULTS

Clinical evaluation: Thirteen family members of a five-generation Chinese family with a history of cataracts participated in the study (six affected and seven unaffected individuals; Figure 1). All patients in this family had bilateral cataracts. Most patients experienced decreased visual acuity at 3–4 years old, and then their visual acuity decreased gradually until surgery was required. The proband, who was a 3-year-old girl, experienced a decrease in vision at 1.5 years old and had been diagnosed with bilateral cataracts at age 3.
Slit-lamp examination revealed opacification of Y-suture cataracts with opacities involving nucleus. The girl's best corrected visual acuity was 0.3/0.3. Her clinical features were similar to those of her uncle (IV:6) with peripheral cortical opacity (Figure 2). His best corrected visual acuity was 0.3/0.4. The affected member IV:3, who was the father of the proband, had undergone cataract removal at age 8.

**Mutation analysis:** Through direct gene sequencing of the coding regions of the candidate genes, we identified an IVS3+1 G→T substitution in the donor splice site of intron 3 in CRYBA1/A3 in all affected individuals (Figure 3). However, we did not find this mutation in any unaffected family members or in the 100 unrelated controls. We did not find any other mutations in this family except for a few non-pathogenic single nucleotide polymorphisms (SNPs).
In this study we identified a splice site mutation of CRYBA1/A3 in a five-generation Chinese family with Y-suture opacities of the lens involving embryonic and fetal nuclei. Sutural cataracts affect the sutural regions of the nucleus, at which the ends of the lens fiber cells meet. Sutural cataracts may occur in isolation or be associated with opacities involving other lens regions. There is some correlation between the pattern of expression of the mutant gene and the morphology of the resulting cataract.

To date, seven genes have been identified to be associated with sutural cataracts, including BFSP2, CRYBA1/A3, CRYBB1, CRYBB2, GJA8, FTL, CRYGA. Among these genes, almost all the mutations of BFSP2 are associated with sutural cataract phenotype. CRYBA1/A3 has great correlation with sutural cataracts (Table 2).

So far, in the CRYBA1/A3 gene, three types of mutations have been associated with autosomal dominant cataracts. Our report of IVS3+1 G→T will be the fourth type of CRYBA1/A3 mutation. The first one is the IVS3+1 G→A mutation. Regarding IVS3+1 G→A, in 1998 Kannabiran et al. [21] reported an Indian family with zonular cataracts with sutural opacities. In 2008, Devi et al. [22] reported another two Indian families with zonular lamellar cataracts. In 2010, Gu et al. [23] identified a Chinese family with posterior polar cataracts, which was the first time this mutation was found in the Chinese population. Also in 2010, Zhu et al. [18] reported a Chinese family with progressive childhood cataracts characterized by opacities in the fetal nucleus and peripheral cortex. The second type of mutation is IVS3+1 G→C. In 2000, Bateman et al. [16] reported a Brazilian family with varied clinical characteristics among the affected members. The affected individuals who were examined had pulverulent opacities in the embryonal nucleus and sutures and star-shaped, shieldlike, or radial opacities in the posterior embryonal nucleus. The third type of mutation is a 3-bp deletion at positions 276–281 in exon 4, which causes an in-frame deletion of a glycine residue at position 91 (ΔG91). In 2004, Qi et al. [24] identified a Chinese family with nuclear cataracts. In 2007, Lu et al. [25] reported two Chinese families with pulverulent congenital cataracts (Table 3).

### Table 2. Summary of mutations responsible for sutural cataract.

| Gene     | Position       | Sequence change | Lens phenotype                                      | Reference |
|----------|----------------|-----------------|----------------------------------------------------|-----------|
| CRYGA    | 2q33-q35       | Unknown         | Sutural cataract                                    | [7]       |
| FTL      | 1q13.3         | 32 G>A          | Y-suture congenital cataract                        | [8]       |
| GJA8     | 1q21           | 235G>C          | Full moon with Y-suture cataract                    | [9]       |
| GJA8     | 1q21           | 262C>A          | Y-suture cataract                                    | [10]      |
| BFSP2    | 3q12.3-q27.2   | 697–699delGAA   | Congenital nuclear and sutural cataract             | [12]      |
| BFSP2    | 3q12.3-q27.2   | 697–699delGAA*  | Progressive sutural congenital cataract             | [13]      |
| BFSP2    | 3q12.3-q27.2   | 696–698delGAA   | Progressive congenital cataract with suture and     | [14]      |
| BFSP2    | 3q12.3-q27.2   | 696–698delGAA   | cortex opacity                                      |           |
| CRYBA1   | 14q13-q21      | IVS3+1G>A       | Sutural, nuclear, and peripheral cortical opacity   | [15]      |
| CRYBA1   | 4q13-q21       | IVS3+1G>C       | Zonular and sutural cataract                        | [16]      |
| CRYBA1   | 4q13-q21       | IVS3+1G>C       | Y-shaped sutural cataract                           | [17]      |
| CRYBA1   | 4q13-q21       | IVS3+1G>A       | Progressive childhood cataract with Y-suture        | [18]      |
| CRYBB2   | 22q11.23       | 483C>T          | opacities with suture and cerulean                  | [19]      |
| CRYBB1   | 22q12.1        | 658G>T          | Ustlike cataract with the anterior and posterior Y- | [20]      |
|          |                |                 | suture opacities                                    |           |

### Table 3. Summary of mutations in CRYBA1/A3 responsible for congenital cataract.

| Exon | Nucleotide   | Amino acid | Phenotype                                                                 | Reference |
|------|--------------|------------|---------------------------------------------------------------------------|-----------|
| IVS3 | IVS3+1G>A    | Splice site mutation | Zonular cataract with sutural opacity                                       | [21]      |
| IVS3 | IVS3+1G>A    | Splice site mutation | Zonular lamellar cataract                                                   | [22]      |
| IVS3 | IVS3+1G>A    | Splice site mutation | Y-suture,mild nucleus and cortical dot cataract                             | [15]      |
| IVS3 | IVS3+1G>A    | Splice site mutation | Posterior polar cataract                                                    | [23]      |
| IVS3 | IVS3+1G>A    | Splice site mutation | Progressive childhood nucleus and peripheral cortex cataract                | [18]      |
| IVS3 | IVS3+1G>C    | Splice site mutation | Pulverulent, star-shaped, shieldlike and radial cataract                    | [16]      |
| EX4  | 278–280delGGA| P.91Glydel   | Pulverulent cataracts                                                       | [24]      |
| EX4  | 279–281delGGA| P.91Glydel   | Pulverulent congenital cataracts                                             | [25]      |
| EX4  | 279–281delGGA| P.91Glydel   | Congenital nuclear cataract                                                 | [26]      |

DISCUSSION

In this study we identified a splice site mutation of CRYBA1/A3 in a five-generation Chinese family with Y-suture opacities of the lens involving embryonic and fetal nuclei.

Sutural cataracts affect the sutural regions of the nucleus, at which the ends of the lens fiber cells meet. Sutural cataracts may occur in isolation or be associated with opacities involving other lens regions. There is some correlation between the pattern of expression of the mutant gene and the morphology of the resulting cataract.

To date, seven genes have been identified to be associated with sutural cataracts, including BFSP2, CRYBA1/A3, CRYBB1, CRYBB2, GJA8, FTL, CRYGA. Among these genes, almost all the mutations of BFSP2 are associated with sutural cataract phenotype. CRYBA1/A3 has great correlation with sutural cataracts (Table 2).

So far, in the CRYBA1/A3 gene, three types of mutations have been associated with autosomal dominant cataracts. Our report of IVS3+1 G→T will be the fourth type of CRYBA1/A3 mutation. The first one is the IVS3+1 G→A mutation. Regarding IVS3+1 G→A, in 1998 Kannabiran et al. [21] reported an Indian family with zonular cataracts with sutural opacities. In 2008, Devi et al. [22] reported another two Indian families with zonular lamellar cataracts. In 2004, Burdon et al. [15] reported an Australian family with Y-sutural cataracts. In 2010, Gu et al. [23] identified a Chinese family with posterior polar cataracts, which was the first time this mutation was found in the Chinese population. Also in 2010, Zhu et al. [18] reported a Chinese family with progressive childhood cataracts characterized by opacities in the fetal nucleus and peripheral cortex. The second type of mutation is IVS3+1 G→C. In 2000, Bateman et al. [16] reported a Brazilian family with varied clinical characteristics among the affected members. The affected individuals who were examined had pulverulent opacities in the embryonal nucleus and sutures and star-shaped, shieldlike, or radial opacities in the posterior embryonal nucleus. The third type of mutation is a 3-bp deletion at positions 276–281 in exon 4, which causes an in-frame deletion of a glycine residue at position 91 (ΔG91). In 2004, Qi et al. [24] identified a Chinese family with nuclear cataracts. In 2007, Lu et al. [25] reported two Chinese families with pulverulent congenital cataracts (Table 3).
CRYBA1/A3 consists of six exons encoding two proteins (βA3-crystallin and βA1-crystallin) by using an alternative translation initiation site. βA1/3-crystallin consists of seven protein regions: four homologous (Greek key) motifs, a connecting peptide, and NH2- and COOH-terminal extensions.

In the CRYBA1/A3 gene, the first two exons encode the sequence of the N-terminal arm, and exons 3–6 encode the Greek key motifs 1–4 [27]. The G at position +1 of the 5′ (donor) splice site is highly conserved, and mutation of this base can be expected to disrupt the splice site [28]. In this study the mutation at IVS3+1 G→T can be expected to skip the donor splice junction, which may cause the wrong junction of the exons in CRYBA1/A3. This may result in premature termination of the polypeptide. In this condition, it would cause structural instability and disrupt the folding of the protein [21].

In conclusion, we have identified a new type IVS3+1 G→T mutation of the CRYBA1/A3 gene associated with Y-sutural congenital cataracts in a Chinese family. This mutation supports the role of the CRYBA1/A3 gene in human cataract formation and provides more evidence of genetic heterogeneity of congenital cataracts.

ACKNOWLEDGMENTS

We thank the family members for participation in the project. This work was supported by the National Science & Technology Pillar Program of China (No.2008BAH24B05), the National Infrastructure Program of Chinese Genetic Resources (2006DKA21300), and the National Natural Science Foundation of China (30471864). Professors Xu Ma (genetic@263.net.cn) and Siqian Zhu contributed equally to the research project and can be considered co-corresponding authors.

REFERENCES

1. Rahi JS, Sripathi S, Gilbert CE, Foster A. Childhood blindness in India: causes in 1318 blind school students in nine states. Eye (Lond) 1995; 9:545-50. [PMID: 8543070]
2. Holmes JM, Leske DA, Burke JP, Hodge DO. Birth prevalence of visually significant infantile cataract in a defined U.S. population. Ophthalmic Epidemiol 2003; 10:67-74. [PMID: 12660855]
3. Wirth MG, Russell-Eggitt IM, Craig JE, Elder JE, Mackay DA. Aetiology of congenital and paediatric cataract in an Australian population. Br J Ophthalmol 2002; 86:782-6. [PMID: 12084750]
4. Reddy MA, Francis PJ, Berry V, Bhattacharya SS, Moore AT. Molecular genetic basis of inherited cataract and associated phenotypes. Surv Ophthalmol 2004; 49:300-15. [PMID: 15110667]
5. Hejtmancik JF. Congenital cataracts and their molecular genetics. Semin Cell Dev Biol 2008; 19:134-49. [PMID: 18035564]
6. Wang KJ, Li SS, Yun B, Ma WX, Jiang TG, Zhu SQ. A novel mutation in MIP associated with congenital nuclear cataract in a Chinese family. Mol Vis 2011; 17:70-7. [PMID: 21245956]
7. Klopp N, Héon E, Billingsley G, Illig T, Wjst M, Rudolph G, Graw J. Further genetic heterogeneity for autosomal dominant human sutural cataracts. Ophthalmic Res 2003; 35:71-7. [PMID: 12646746]
8. Vanita V, Hejtmancik JF, Hennies HC, Guleria K, Nürnberg P, Singh D, Sperling K, Singh JR. Sutural cataract associated with a mutation in the ferritin light chain gene (FTL) in a family of Indian origin. Mol Vis 2006; 12:93-9. [PMID: 16518306]
9. Vanita V, Hennies HC, Singh D, Nürnberg P, Sperling K, Singh JR. A novel mutation in GJA8 associated with autosomal dominant congenital cataract in a family of Indian origin. Mol Vis 2006; 12:1217-22. [PMID: 17110920]
10. Vanita V, Singh JR, Singh D, Varon R, Sperling K. A mutation in GJA8 (p.P88Q) is associated with “balloon-like” cataract with Y-sutural opacities in a family of Indian origin. Mol Vis 2008; 14:1171-5. [PMID: 18587493]
11. Zhang Q, Guo X, Xiao X, Yi J, Jia X, Hejtmancik JF. Clinical description and genome wide linkage study of Y-sutural cataract and myopia in a Chinese family. Mol Vis 2004; 10:890-900. [PMID: 15570218]
12. Jakobs PM, Hess JF, FitzGerald PG, Kramer P, Weleber RG, Litt M. Autosomal-dominant congenital cataract associated with a deletion mutation in the human beaded filament protein gene BFSP2. Am J Hum Genet 2000; 66:1432-6. [PMID: 10739768]
13. Zhang L, Gao L, Li Z, Qin W, Gao W, Cui X, Feng G, Fu S, He L, Liu P. Progressive sutural cataract associated with a BFSP2 mutation in a Chinese family. Mol Vis 2006; 12:1626-31. [PMID: 17200662]
14. Cui X, Gao L, Jin Y, Zhang Y, Bai J, Feng G, Gao W, Liu P, He L, Fu S. The E233del mutation in BFSP2 causes a progressive autosomal dominant congenital cataract in a Chinese family. Mol Vis 2007; 13:2023-9. [PMID: 17982427]
15. Burdon KP, Wirth MG, Mackey DA, Russell-Eggitt IM, Craig JE, Elder JE, Dickinson JL, Sale MM. Investigation of crystallin genes in familial cataract, and report of two disease associated mutations. Br J Ophthalmol 2004; 88:79-83. [PMID: 14693780]
16. Bateman JB, Geyer DD, Flodman P, Johannes M, Sikela J, Walter N, Moreira AT, Clancy K, Spence MA. A new betaA1-crystalline splice junction mutation in autosomal dominant cataract. Invest Ophthalmol Vis Sci 2000; 41:3278-85. [PMID: 11006214]
17. Boyadjiev SA, Justice CM, Eyaid W, McKusick VA, Lachman RS, Chowdry AB, Jobak M, Zwaan J, Wilson AF, Jacobs E. A novel dysmorphic syndrome with open calvarial sutures and sutural cataracts maps to chromosome 14q13-q21. Hum Genet 2003; 113:1-9. [PMID: 12677423]
18. Zhu Y, Shentu X, Wang W, Li J, Jin C, Yao K. A Chinese family with progressive childhood cataracts and IVS3+1G→A CRYBA3/A1 mutations. Mol Vis 2010; 16:2347-53. [PMID: 21139983]
19. Vanita, Reis A, Jung M, Singh D, Sperling K, Singh JR, Bürger J. A unique form of autosomal dominant cataract explained by gene conversion between beta-crystallin B2 and its
pseudogene. J Med Genet 2001; 38:392-6. [PMID: 11424921]

20. Mackay DS, Boskovska OB, Knopf HL, Lampi KJ, Shiels A. A nonsense mutation in CRYBB1 associated with autosomal dominant cataract linked to human chromosome 22q. Am J Hum Genet 2002; 71:1216-21. [PMID: 12360425]

21. Kannabiran C, Rogan PK, Olmos L, Basti S, Rao GN, Kaiser-Kupfer M, Hejtmancik JF. Autosomal dominant zonular cataract with sutural opacities is associated with a splice mutation in the betaA3/A1-crystallin gene. Mol Vis 1998; 4:21. [PMID: 9788845]

22. Devi RR, Yao W, Vijayalakshmi P, Sergeev YV, Sundaresan P, Hejtmancik JF. Crystallin gene mutations in Indian families with inherited pediatric cataract. Mol Vis 2008; 14:1157-70. [PMID: 18587492]

23. Gu Z, Ji B, Wan C, He G, Zhang J, Zhang M, Feng G, He L, Gao L. A splice site mutation in CRYBA1/A3 causing autosomal dominant posterior polar cataract in a Chinese pedigree. Mol Vis 2010; 16:154-60. [PMID: 20142846]

24. Qi Y, Jia H, Huang S, Lin H, Gu J, Su H, Zhang T, Gao Y, Qu L, Li D, Li Y. A deletion mutation in the betaA1/A3 crystallin gene (CRYBA1/A3) is associated with autosomal dominant congenital nuclear cataract in a Chinese family. Hum Genet 2004; 114:192-7. [PMID: 14598164]

25. Lu S, Zhao C, Jiao H, Kere J, Tang X, Zhao F, Zhang X, Zhao K, Larsson C. Two Chinese families with pulverulent congenital cataracts and deltaG91 CRYBA1 mutations. Mol Vis 2007; 13:1154-60. [PMID: 17653060]

26. Ferrini W, Schorderet DF, Othenin-Girard P, Uffer S, Héon E, Munier FL. CRYBA3/A1 gene mutation associated with suture-sparing autosomal dominant congenital nuclear cataract: a novel phenotype. Invest Ophthalmol Vis Sci 2004; 45:1436-41. [PMID: 15111599]

27. Hogg D, Tsui LC, Gorin M, Breitman ML. Characterization of the human beta-crystallin gene Hu beta A3/A1 reveals ancestral relationships among the beta gamma-crystallin superfamily. J Biol Chem 1986; 261:12420-7. [PMID: 3745196]

28. Krawczak M, Reiss J, Cooper DN. The mutational spectrum of single base-pair substitutions in mRNA splice junctions of human genes: causes and consequences. Hum Genet 1992; 90:41-54. [PMID: 1427786]