A novel mutation in CRYAA is associated with autosomal dominant suture cataracts in a Chinese family

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Purpose: To identify the genetic defect in a three-generation Chinese family with congenital cataracts.
Methods: The phenotype of a three-generation Chinese family with congenital cataracts was recruited. Detailed family history and clinical data of the family were recorded. Candidate gene sequencing was performed to screen out the disease-causing mutation. Bioinformatics analysis was performed to predict the function of the mutant gene.
Results: The phenotype of the family was identified as Y-suture cataract by using slit-lamp photography. Direct sequencing revealed a c.161G>C transversion in exon 1 of crystallin, alpha A (CRYAA). This mutation cosegregated with all affected individuals in the family and was not found in unaffected family members or in the 100 unrelated controls. Bioinformatics analysis indicated that the 54th amino acid position was highly conserved and the mutation R54P caused an increase in local hydrophobicity around the substitution site.
Conclusions: This study identified a novel disease-causing mutation c.161G>C (p.R54P) in CRYAA in a Chinese family with autosomal dominant Y-suture cataracts. This is the first report relating a G→C mutation in CRYAA leading to congenital Y-suture cataract.

Congenital cataract is the most common cause of treatable childhood blindness. Worldwide, more than 1 million blind children in Asia suffer from cataracts [1]. The cataract may be isolated, may be associated with other developmental abnormalities of the eye, or may form part of an inherited multisystem disorder. Approximately one-quarter to one-third of congenital cataracts is inherited and has been reported with all three types of Mendelian inheritance, including autosomal dominant, autosomal recessive, and X-linked. Most inherited cataracts manifest as an autosomal dominant trait in which penetrance is almost complete but expressivity is highly variable [2]. According to morphology, congenital cataracts can be classified into several subtypes: sutural, pulverulent, whole lens, nuclear, amellar, cortical, polar, cerulean, coralliform, and other minor subtypes [3]. These subtypes of cataracts can result from mutations at different genetic loci and can have different inheritance patterns.

Approximately half of all families with cataract have crystalline mutations, including crystallin, alpha A (CRYAA), crystallin, alpha B (CRYAB), crystallin, beta A1 (CRYBA1/A3), crystallin, beta B1 (CRYBB1) crystallin, beta B2 (CRYBB2), crystallin, gamma C (CRYGC), crystallin, gamma D (CRYGD), and crystallin, gamma S (CRYGS). About one-quarter have connexin mutations in gap junctional proteins, including gap junction protein alpha 3 (GJA3) and gap junction protein alpha 8 (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) [4].

In this study, we applied a functional candidate approach to test the known genes in a Chinese family. A novel missense mutation in CRYAA was identified as responsible for the cataracts in the family.

METHODS

Clinical examination and isolation of genomic DNA: A three-generation Chinese family from Hebei Province with autosomal dominant congenital cataract was examined at Beijing Tongren Hospital (Figure 1). One hundred unrelated subjects without eye diseases except mild myopia were also recruited from the Ophthalmology Clinic of Beijing Tongren Hospital as normal controls. The ethics committee of Capital Medical University approved the research, and all participants from the family gave informed consent. The study protocol followed the principles of the Declaration of Helsinki.
Six family members who were from Hebei Province (II:5, female, 45 years; II:7, male, 43 years; II:8, female, 43 years; III:4, male, 21 years; III:5, female, 16 years; III:6, male, 13 years) participated in the study. All participating members were healthy when they were recruited, all of them underwent ophthalmologic examination in Beijing Tongren Hospital. A history of cataract extraction or ophthalmologic examination was used to determine those whose status was considered to be affected; six family members (II:5, II:7, II:8, III:4, III:5, III:6) participated in the study. All participating members underwent ophthalmic examination, including visual acuity, slit-lamp examination, intraocular pressure measurement, ultrasonography, and fundus examination of the dilated pupil. The phenotypes were documented with slit-lamp photography, and 5 ml of venous blood was collected in BD Vacutainers (BD, San Jose, CA) containing EDTA from participating family members and controls. Genomic DNA was extracted with QIAamp DNA Blood Mini Kits (Qiagen Science, Germantown, MD).

Mutation detection: All coding exons and intron-exon junction of the candidate genes known to be associated with congenital cataract, including CRYAA, CRYAB, CRYBA1, CRYBB1, CRYBB2, CRYGC, CRYGD, CRYGS, GJA3, GJA8, MIP, HSF4, and BFSP2, were amplified with PCR with the primers listed in Table 1. Each reaction mix (25 μl) contained 20 ng of genomic DNA, 1× PCR buffer, 1.5 mM MgCl2, 0.2 mM mixture of deoxyribonucleoside-5′-triphosphates (dNTPs), 0.5 μM each of forward and reverse primers, and 2.5 U of Taq DNA polymerase (TianGen, Beijing, China). 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 to 63 °C for 30 s (annealing temperature depending on different primer), and 72 °C for 30 s, and a final extension at 72 °C for 10 min. The PCR products were sequenced using ABI 3730 Automated Sequencer (PE Biosystems, Foster City, CA). The sequencing results were analyzed using Chromas 2.33 and compared to the reference sequence in the NCBI database.

Bioinformatics analysis: The amino acid sequences of CRYAA from various species (humans, rats, and chickens, Xenopus laevis, and zebrafish) were obtained from the NCBI GenBank, and conservation analysis was performed with CLC Main Workbench Software (Aarhus, Denmark). The function impact of the mutation was predicted with PolyPhen. The hydrophobicity change was predicted with ProtScale.

RESULTS

Clinical evaluation: All individuals in this family had bilateral cataracts. Slit-lamp examination of the left eye of the proband (III:5), who had been diagnosed with bilateral cataracts at the age of 6 months, revealed slight opacification of Y-suture cataracts, with opacities involving the nucleus (Figure 2A,B). Her right eye had undergone cataract removal when she was aged 14 months, and the best-corrected visual acuity was 0.02/0.6. Slit-lamp examination of both eyes of the proband’s father (II:7) showed Y-suture opacities of the lens, involving the nucleus (Figure 2C,D). The best-corrected visual acuity of affected member III:6 was 0.8/0.8. According to the medical records, affected member III:2 underwent cataract removal at the age of 3 years.

Mutation analysis: We identified a transversion of G>C at c.161 in exon 1 of crystallin, alpha A (CRYAA) in all affected individuals, via direct gene sequencing of the coding regions of the candidate genes (Figure 3). However, we did not find this mutation in any unaffected family members, or in the 100 unrelated control individuals. We found no further gene mutations in individuals from the studied family, except a few nonpathogenic single nucleotide polymorphisms.

DISCUSSION

A sutural cataract is defined as an opacity affecting whole or part of the anterior or posterior suture of one or both eyes. Most sutural cataracts are congenital without progression [5], and have been described in association with nuclear, pulverulent, cerylent, and lamellar cataracts [3]. Seven genes have been identified as being associated with sutural cataracts to
| Name       | Forward (5′-3′)                      | Reverse (5′-3′)                      |
|------------|-------------------------------------|-------------------------------------|
| CRYAA-1    | AGCAGCCTTCTTTATGAGC                 | CAAGACCAGAGTCACAG                  |
| CRYAA-2    | GGCAGGTGACCGAAGACATC                | GAAGGCAAGGTGTCAGG                  |
| CRYAA-3    | GCAGCTCTTCGGCATG                    | GGGAAGCAAAGGAAGACAGA               |
| CRYAB-1    | AACCCCTGACATCACAATTC                | AAGGACTCTCCGGTCCTAGC               |
| CRYAB-2    | CCATCCCATTTCCCTACCTTT              | GCCTCCAAAGTCATAGCAC                |
| CRYAB-3    | TCTCTCTGCGCTTTTCTCT                | CCTGGGACCCCTTAAATCA                |
| CRYBA1-1   | GGCAGAGGGAGAGCACAG                  | CACAGGCAGGAGAACCTGG                |
| CRYBA1-2   | AGTGAGCAGACGGACGCAA                 | GGTCACTCATGGCCTTATGG               |
| CRYBA1-3   | GAGCAACAGAGTCAGCTGACAG             | TCCCTAGATGCCATAGCAATG              |
| CRYBA1-4   | AACCCCTGACATCACAATTC                | AAGGACTCTCCGGTCCTAGC               |
| CRYBB1-1   | CATCCCATACCATTTGGTTGAGG             | GCAAGGTGCTCATGTCGCTTAGG            |
| CRYBB1-2   | CCATCCCATTTCCCTACCTTT              | GCCTCTACATGGCAGCTTATGG             |
| CRYBB1-3   | CCTGCCACTCTGCTTTTATTTA             | TCTCCAGAGCCCAGAACCAG              |
| CRYBB1-4   | CCACTCTGACAGGAGCAACAGCATA          | CTCCTCATGGCTCTTCTCT                |
| CRYBB1-5   | CAGACGAGGAGTCAGCTGACAG             | AGCAGTGAGGAGACTGAGGAGGAG          |
| CRYBB1-6   | CCTAGAAGAGAAACCGAGGCC              | AGGAGGAGAAGCAGCTACCCCTAGA          |
| CRYBB2-1   | GTTTGGCGAGGGAGGAGGGTGTG            | TGGGCTGGGAGGAGGCTCTCAGTA           |
| CRYBB2-2   | CCTCCATACCTTTGGTTGCTCT             | GCAAGTCTTAGGAGTTCTTCTTAGCT         |
| CRYBB2-3   | GTAGCCGAGGATTTGCTGCTAGGGA          | GTGCCCTATTGGAGAGCTTTCTAGT          |
| CRYBB2-4   | GGCCTCCTACCCACTACA                 | CTGCCCTCTGCTCAAACCTAA             |
| CRYBB2-5   | CTACCCCTGCGAGGAGACCAGG            | TGAAGACCCAGACGACAGAAATG            |
| CRYGC-1    | TGCAATAAATCCTACCCATTCCG            | CTCCTCATTACCACATCAG                |
| CRYGC-2    | TGTTGGACAAATCTGGGAGA               | CTCCTCCTCACCCTTCTCTA               |
| CRYGD-1    | CAGAGGAGGACGGCTGAGA                | GGTCACTGGGAGATGAG                 |
| CRYGD-2    | GCATGGGCTCTGACGGCTGATTCTCAT        | AAGAAGACACAAAGCGAATCAG             |
| CRYGS-2    | GAAACCATAACATGGGCTCAAATG           | TGAAGAACGGGTAGGCAA                 |
| CRYGS-3    | AATTAAGCCACCCAGACTCT              | GGTCACTGGGAGATGAG                 |
| CRYGS-4    | GACCCTGCTGGTGATTCTCAT              | GATCTGGGAGGAGCAGTGT               |
| GJA3–1     | CGGTGTTCTAGGACAGCTTCT              | CCTTCCATGCGAGCTTAG                |
| GJA3–2     | GAGGAGGAGGACGGCTGAGA               | AGGAGGAGGAGCAGTGT                 |
| GJA3–3     | TGGGTTCCACACCTACTAT               | TATCTGCTGTTGAGAGTGC               |
| GJA8–1     | CCGCGTATGGCAAACAGAGAT             | CTCCTGATAGGGAGTAT                 |
| GJA8–2     | GCAGATCATCTGCTCTGCT                | GGCACAGGAGACCATGGA                |
| GJA8–3     | CCAGGGGAGGAGACACATCTCT             | GAGCGTAGGAGGAGCAGTGT              |
| GJA8–4     | TCCAGGAGAAGAGATCGACCA              | GGTGCTGGCTTTTCTGATG               |
| MIP-1      | GTGAAAGGGGTGTAAGG                  | GGATGACGGCTGAGTG                  |
| MIP-2,3    | CGGGGAGGAGGCTTTGTGAGG              | CACGCAGAAGGAAAGCGA                 |
| MIP-4      | CACATAAGG TGGCGTGAAG               | CTCATGGCCAAAATCTCA                |
| HSF4–1     | CATCCCATCCAGCCAGCTTCTTC            | GGCGATGGGCTTCTGCTGAGC             |
| HSF4–2     | CCTCGACCCATATCCCCGTAAG            | GCGAGGAGGAGGAGCAGTC               |
date (Table2) [5-19], including BFSP2, CRYBA1/A3, CRYBB1, CRYBB2, GJA8, and FTL. In this study, we identified a novel mutation, c.161G>C, in CRYAA, which was associated with isolated Y-suture cataracts in a Chinese family. CRYAA is the major protein of the eye lens in vertebrates, and plays a structural role in maintaining lens transparency and a proper refractive index. CRYAA is also a member of the small heat-shock-protein (sHSP) family, which are stress-induced proteins, and has chaperone activity [20,21]. CRYAA contains a conserved α-crystallin domain (about 90 amino acids), which is flanked on either side by a hydrophobic NH2-terminal domain (about 60 amino acids) and a hydrophilic unstructured COOH-terminal (about 30 amino acids) [22,23]. Genetic and biochemical studies have suggested that some mutant forms of lens opacities or cataracts are associated with decreased chaperone-like activities of α-crystallins. Several mutations in CRYAA have been reported to date, and the Arg at the 54th locus of the peptide is a mutation hotspot. In 2007, Arif et al. [24] reported an autosomal recessive congenital total cataract

| Name         | Forward (5′-3′)                     | Reverse (5′-3′)                     |
|--------------|------------------------------------|------------------------------------|
| HSF4–3       | GCGGGAATGAGCAAAGAGGAGG             | GCCAAAGCAGGAGGAGGAGGAAGG           |
| HSF4–4       | TCCCCAGCCTCGCCATTCT                | CCCCAGGAGGAGGAGTTCCAGAG            |
| HSF4–5       | GCTGGGCTCTGAGGGAGG                | GGCTTCCATCTCTCTCTCTCTCTTT          |
| BFSP2 (1a)   | AATGCACAAACCCAAATGGT              | AGGCCCTGGSACACT                    |
| BFSP2 (1b)   | GAGAGGCGAGTGGTAGGTGGA             | GGCCTCAGCCTACTCAAC                 |
| BFSP2 (2)    | TGCAAGACAGACATTTCCAC             | GAGGGGTGTGAGCTGGAATA               |
| BFSP2 (3)    | GCTGCAATTGCTTCTTCTTT             | GGGAACCTGACCCACCTTCA               |
| BFSP2 (4)    | TCTTGGAAGGCTGTGCTG                | CCCGGCTCATTATTTCTTT                |
| BFSP2 (5)    | ACCCAAGGAGGAGGAGGTGTG             | GGGAAATCCCTGGAACACTA               |
| BFSP2 (6)    | GGGAATAGTCAGGGCTACC              | ATGGGTTCCTATGTGAGGGAAGG            |
| BFSP2 (7)    | TTGTCCCAAAGGGCAGATT              | CACTCAAGGAAATCCTTCA                |
with microcornea caused by a missense mutation of CRYAA (R54C). Furthermore, Gong et al. [25] reported that a R54C mutation in CRYAA leads to recessive cataracts in humans and mice. In 2008, Devi et al. [26] showed that a CRYAA R54C mutation was associated with autosomal dominant congenital nuclear cataracts associated with microcornea, and a missense CRYAA mutation (R54P) was identified in an autosomal dominant family with Y-suture cataract in this study. The 54th amino acid is in the NH2-terminal region of CRYAA, which is an important determinant of α-crystallin aggregate size, and plays a role in the resistance of α-crystallin to environmental stress [27]. In the present study, protein analysis with ProtScale clearly showed an increase in local hydrophobicity around the Arg–Pro substitution site in CRYAA. In addition, PolyPhen indicated that R54P is possibly damaging. As exhibited in other lens proteins, hydrophobicity is associated with crystallin activities; increased hydrophobic interaction could reduce their solubility or lead to abnormal folding [28-30]. For CRYAA, changes in hydrophobicity also affect the protein structure and function. Sharma et al. [31] showed that the hydrophobic NH2-terminal domain of the CRYAA protein is involved in chaperone-like activity. Shroff et al. [32] showed that the alpha A-R116C mutant CRYAA led to the generation of a highly oligomerized crystallin, alpha A and decreased chaperone-like function. Thus, we speculate that the increase in local hydrophobicity around the Arg–Pro substitution site in CRYAA may affect its oligomerization or chaperoning activity. Furthermore, a knock-in mouse model has demonstrated that the CRYAA R49C mutation enhances protein insolubility and cell death [33]. The native protein is an active polypeptide that has antiapoptotic properties that are important for maintaining the survival of lens epithelial cells. Since the 54th amino acid is near the 49th amino acid, the R54P may also have a similar impact; the mutant may be less resistant to environment stress, and enhance cell death. All these changes may have resulted in the cataracts that formed in the studied family.

In conclusion, we are the first to identify that a G→C mutation of CRYAA resulted in congenital autosomal dominant Y-suture cataracts. This mutation supports the role of the CRYAA gene in human cataract formation and provides further evidence of the genetic heterogeneity of congenital cataracts.
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