A Novel Mutation in CRYGC Mutation Associated with Autosomal Dominant Congenital Cataracts and Microcornea

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Purpose: Crystallin protein mutations are associated with congenital cataract (CC), and several disease-causing mutations in the CRYGC gene have been identified. We present the location of a new mutation in CRYGC in members of a Chinese family who presented with CCs with or without microcornea.

Design: Observational study.

Participants: A Chinese family diagnosed with autosomal dominant (AD) CCs with or without microphthalmia.

Methods: Because this was an observational study, it was not registered as a clinical trial. The proband and her 2 children were diagnosed with AD CCs and microcornea and were recruited for the study. Participants underwent complete ophthalmological examinations, and blood samples were used for genomic extraction.

Main Outcome Measures: We detected 1 disease-associated variant using Exomiser analysis by matching the proband’s phenotype and the inheritance pattern. The variant was determined to be pathogenic according to American College of Medical Genetics and Genomics (ACMG) guidelines.

Results: We detected 1 disease-associated variant using Exomiser analysis by matching the proband’s phenotype and the inheritance pattern. The variant was determined to be pathogenic according to the American College of Medical Genetics and Genomics guidelines. Next-generation sequencing was verified using Sanger sequencing, and we confirmed that the proband and her children carried the same mutation. We identified the heterozygous variant c.389_390insGCTG (p.C130fs), which includes a frameshift mutation. The residues in p.C130fs are all highly conserved across species. This disease-causing frameshift mutation in the CRYGC gene is not currently present in the ClinVar database.

Conclusions: Our findings expand the repertoire of known mutations in the CRYGC gene that cause CCs and provide new insights into the etiology and molecular diagnosis of CCs; however, the molecular mechanism of this mutation warrants further investigation.

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Congenital cataracts (CCs) are characterized by opacification of the ocular lens, which presents at birth or shortly thereafter. Congenital cataracts are the leading clinical cause of impaired vision in children, especially infants, and can result in permanent blindness.1 However, CCs can be successfully treated surgically.2 The prevalence of this condition is thought to be 1 to 6 per 10 000 live births in developed countries; however, it is reported to be 5 to 15 per 10 000 live births in developing countries.1,3

It has been suggested that 8.3% to 25% of CC cases are hereditary, most of which are autosomal dominant (AD), autosomal recessive, or X-linked.4,5 Genetic mutations in at least 42 loci have been found to be associated with inherited forms of either primary or isolated cataracts with other nominal ocular signs; thus, it is believed that these mutations are related to CC.6

The crystallin proteins alpha, beta, and gamma are the major protein components of the vertebrate eye lens, accounting for more than 90% of the total lens proteins.7,9 Several crystallin protein mutations, including αA-crystallin (CRYAA), βA1-crystallin (CRYBA1), βB1-crystallin (CRYBB1), βB2-crystallin (CRYBB2), γC-crystallin (CRYGC), γD-crystallin (CRYGD), connexin 46 (CX46), connexin 50 (CX50), and major intrinsic protein (MIP), are associated with CC.10,11

This study investigated a disease-causing heterozygous frameshift mutation, c.389_390insGCTG (p.C130fs), in the CRYGC gene. The mutation was identified in members of a Chinese family who presented with CC and microcornea. None of the previously reported mutations associated with CC were detected in any member of the family who had the condition. Our study contributes to the known mutations in CRYGC associated with CC.

Methods

Patient Data

We enrolled members of a family who presented to our hospital with AD congenital nuclear cataracts, microcornea, and nystagmus. The family originated from Quanzhou (Fujian, China) and included...
6 people: 3 affected and 3 unaffected. No other comorbidities were present.

Research was conducted in accordance with the Declaration of Helsinki. The study and all its protocols were approved by the Ethics Committee of the HongQi Hospital, MuDanJiang Medical University (approval number:201703). Informed consent was obtained from all participants and their parents/guardians.

All participants underwent the following examinations to confirm the diagnosis and to collect clinical data: ophthalmological examinations, including visual acuity, Hirschberg test, cornea diameter measurement, oculomotor examination, slit-lamp examination, retinoscopy with dilated pupil, ultrasound A/B-mode imaging, and fundoscopy. The phenotype was determined using slit-lamp photography. Unfortunately, because of the patients’ nystagmus, we failed to capture a clear photograph of the anterior segment (Table 1).

**Whole-Exome Sequencing Analysis**

A sample of venous blood was extracted from each patient (blood collection date: May 10, 2017). Whole-exome sequencing was then performed by Genokon Medical Technology Co., Ltd. The QIAamp DNA Blood Mini Kit (Qiagen) was used for genomic DNA extraction. Agarose gel electrophoresis and NanoDrop (Thermo Fisher) spectrophotometric analysis, corroborated with Qubit 3.0 (Thermo Fisher), were used to assess the concentration and quality of the extracted DNA. Genomic DNA (1.5 μg) was fragmented to a mean size of 300 base pairs, with which sequencing libraries were subsequently prepared. Afterward, the DNA fragments were ligated with sequencing adaptors (8 base pair barcoded) before hybridization with xGen Exome Research Panel v1.0 focused exome probes (IDT). Experiments were validated by assessing the capture efficiency, coverage depth, sequencing sensitivity, and reproducibility. Gauging of DNA quality and quantity was achieved by both quantitative polymerase chain reaction and the AATI Fragment Analyzer. The HiSeq X-10 platform (Illumina) was used to pool and parallel-sequence purified sequencing libraries. A sequencing yield of 10.0 Gb was produced. We achieved a sample coverage of 91%, to a 150× depth or greater.

**Reads Mapping and Variants Analysis**

Sequences were located on the reference human genome with the aid of NextGene software (SoftGenetics LLC). Databases, such as the 1000 Genomes Project (http://browser.1000genomes.org), Exome Aggregation Consortium (https://gnomad.broadinstitute.org), and dbSNP (http://www.ncbi.nlm.nih.gov/snp) were used to compare the variants. Those with a minor allele frequency greater than 0.01 in the control databases were excluded. The Sorting Intolerant from Tolerant, Polyphen-2, and Mutation Taster platforms were used for pathogenicity prediction analysis. The locations of all variants were corroborated to be in the conserved region of the gene, and the variants’ effects on the folding and function of proteins were evaluated. The guidelines of the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology for variant interpretation were used to classify variants as pathogenic (PVS1+PM2+PP4).

**Sanger Sequencing for Verification**

Sanger sequencing was used to verify whether the variant identified through exome sequencing matched the samples from the patient,
as well as to confirm the presence of the proband’s 2 children. We amplified the target sites and the flanking sequences of the genomic DNA template from each family member individually with specific primers designed using Online Design Software Primer 3.0 (http://primer3.ut.ee/).

Results

Clinical Findings

We identified a Chinese family, spanning 3 generations, with AD congenital nuclear cataracts (Fig 1). The DNA sequences of the CRYGC gene of the affected and unaffected individuals from the study population are shown in Figure 2.

The results of whole-exome sequencing were as follows:

1. One disease-associated variant was identified using Exomiser analysis by matching the proband’s phenotype and the inheritance pattern. The variant was determined to be pathogenic according to ACMG guidelines (Table 2).

2. There was no information associated with this variant in the ClinVar database.

3. There were no matched variants in 59 genes according to the ACMG SF (secondary findings) v2.0 mutation analysis.

4. The Online Mendelian Inheritance in Man database (available at https://www.omim.org/entry/123680) described known mutations in the CRYGC gene that have been shown to cause cataracts (Table 3).

5. DNA analysis of the proband’s son revealed a heterozygous frameshift mutation in CRYGC (NM_020989: exon 3: c.389_390insGCTG: p.C130fs). DNA analysis of the proband’s daughter also showed a heterozygous frameshift mutation in CRYGC (NM_020989: exon 3: c.389_390insGCTG: p.C130fs).

6. We identified the heterozygous CRYGC p.C130fs variant, including a frameshift mutation not currently reported in the ClinVar database. Sanger sequencing revealed that not only the proband but also her son and daughter carried this specific frameshift mutation.

7. The multiple sequence alignments generated using CLUSTAL X software showed that the p.c130fs of human of CRYGC is highly conserved in Homo sapiens, Mus musculus, Rattus norvegicus, Canis lupus familiaris, Pan troglodytes, and Halichoerus grypus (Fig 3).

Discussion

Whole-exome gene analysis involves all regions of the exome; in humans, this covers more than 20 000 genes, enabling the analysis of more than 85% of all human genetic diseases. Single-nucleotide variants can be detected in
Figure 2. DNA sequences of the GRYGC gene of affected and unaffected individuals in the study population. The DNA sequence chromatograms of (A) the proband, (B) individual III:1, and (C) individual III:2 (affected individuals) are shown. A heterozygous 4 base pair insertion in exon 3 results in a frameshift mutation (p.C130fs). The DNA sequence chromatograms of unaffected individuals (D) I:1, (E) I:2, (F) II:1, and (G) II:3 are also shown.

| Gene   | Chromosome | Nucleic Acid Altering | Amino Acid Altering | Mutation Type   | Protein Prediction | Genotype                  |
|--------|------------|-----------------------|---------------------|-----------------|--------------------|---------------------------|
| Proband| CRYGC      | 2q33.3 | NM_020989:exon3:c.389_390insGCTG | p.C130fs       | Frameshift mutation | MutationTaster pred (D) | Heterozygous              |
| Proband's son | CRYGC | 2q33.3 | NM_020989:exon3:c.389_390insGCTG | p.C130fs       | Frameshift mutation | MutationTaster pred (D) | Heterozygous              |
| Proband's daughter | CRYGC | 2q33.3 | NM_020989:exon3:c.389_390insGCTG | p.C130fs       | Frameshift mutation | MutationTaster pred (D) | Heterozygous              |

D = damaged.
Table 3. Gene Description of Mutations That Have Been Shown to Cause Cataracts

| Cytogenetic Locus | Physical Locus | Gene | Exon/Intron | DNA Change | Protein Change | Inheritance | Origin | Cataract Phenotype | Other Phenotype | Reference | Comment |
|-------------------|---------------|------|-------------|------------|----------------|-------------|--------|-------------------|----------------|-----------|---------|
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex2 | c.13A>C | p.T5P | AD | UK | Central zonular pulverulent (Coppock-like) | | Heon et al 1999 | 25 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex2 | c.119-123dup5bp | p.C42AfsX63 | AD | USA | Variable zonular pulverulent | | Ren et al 2000 | 26 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.502C>T | p.R168W | AD | India | Lamellar | | Santhiya et al 2002 | 27 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.502C>T | p.R168W | AD | Mexico | Nuclear | Peripupillary iris atrophy, nystagmus, myopia | | Gonzalez-Huerta et al 2007 | 28 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.502C>T | p.R168W | AD | India | Lamellar | | Devi et al 2008 | 33 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.327C>A | p.C109X | AD | China | Nuclear | Nystagmus | Yao et al 2008 | 29 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.470G>A | p.W157X | AD | China | Nuclear | Microcornea | Zhang et al 2009 | 32 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex2 | c.143G>A | p.R48H | AD | India | Nuclear pulverulent | | Kumar et al 2011 | 34 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.471G>A | p.W157X | AD | China | Nuclear | Microcornea | Guo et al 2012 | 31 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.385G>T | p.G129C | AD | China | Nuclear | Microcornea | Li et al 2012 | 30 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex2 | c.124delT | p.C42AfsX60 | AD | Korea | Congenital | Microphthalmia/ microcornea, glaucoma, corneal opacity | | Kondo et al 2013 | 35 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex2 | c.157_161dupGCGGC | c.417C>G | p.Y139X | AD | USA | congenital | Reis et al 2013 | 12 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.134T>C | p.L45P | UK | | | | Gillespie et al 2014 | 36 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.402C>G | p.Y134X | UK | | | | Gillespie et al 2014 | 37 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.497C>T | p.S166F | AD | Australia | Nuclear | Microphthalmia | Prokudin et al 2014 | 38 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.497C>T | p.S166F | AD | Australia | | | Ma et al 2015 | 39 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.280G>A | p.E94K | Sporadic | China | Total (Unilateral) | | Li et al 2016 | 39 |
| 2q33-q35          | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.337C>T | p.Q113X | Sporadic | China | Nuclear | Microcornea | Patel et al 2016 | 40 |

(Continued)
### Cytogenetic Locus | Physical Locus | Gene | Exon/Intron | DNA Change | Protein Change | Inheritance | Origin | Cataract Phenotype | Other Phenotype | Reference | Comment
--- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex2 | c.130delA | p.M44ChX59 | AD | China | Pseudophakia | | Sun et al 2017 | 17
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex2 | c.143G>A | p.R48H | AD | China | Unilateral | Optic disc coloboma | Microcornea, glaucoma | Sun et al 2017
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.432C>G | p.Y144X | AD | China | Aphakia | | Sun et al 2017
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex2 | c.136T>G | p.Y46D | AD | China | Nuclear | | Zhong et al 2017 | 41
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex2 | c.193delG | p.D65TfsX38 | AD | China | Nuclear | | Zhong et al 2017
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.417C>G | p.Y139X | AD | China | Nuclear | Microcornea | Zhong et al 2017
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.423delG | p.R142GfsX5 | AD | China | Nuclear | | Zhong et al 2017
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.423dupG | p.R142AfsX22 | AD | China | Nuclear | | Zhong et al 2017
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.432C>G | p.Y144X | AD | China | Nuclear | | Zhong et al 2017
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.497C>T | p.S166F | AD | China | Nuclear | Microcornea | Zhong et al 2017
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.505A>T | p.R169X | AD | China | Nuclear | | Zhong et al 2017
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex2 | c.17T>C | p.F6S | AD | Mexico | Nuclear | | Astiazaran et al 2018 | 42
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex2 | c.17T>C | p.F6S | AD | Mexico | Lamellar | | Astiazaran et al 2018
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex2 | c.233C>T | p.S78F | AD | China | Nuclear | | Li et al 2018 | 43
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | IVS1 | c.10-1G>A | AD | China | | | | Zhuang et al 2019 | 44
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex2 | c.192delC | p.D65TfsX38 | AD | China | Total | | Fan et al 2020 | 45
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.497C>T | p.S166F | AD | China | Total | | Fan et al 2020
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.382G>T | p.E128X | AD | India | Nuclear | | Kandaswamy et al 2020 | 46
2q33-q35 | 2:208,992,861-208,994,554 | CRYGC | Ex3 | c.432C>G | p.Y144X | AD | Turkey | | | Sekeroglu et al 2020 | 47

**AD** = autosomal dominant.

This gene encodes a member of the beta/gamma-crystallin family of proteins. Crystallins constitute major proteins of the vertebrate eye lens and are responsible for maintaining the transparency and refractive index of the lens. The Online Mendelian Inheritance in Man represents mutations in this gene that have been shown to cause cataracts. Online Mendelian Inheritance in Man No. 604307.

Source: [https://www.omim.org/entry/123680](https://www.omim.org/entry/123680)
gene-coding regions, as well as small insertion/deletion mutations.12

The main findings of our study were that the next-generation and Sanger sequencing identified the CRYGC p.C130fs variant in the proband and her 2 affected children. The proband and her 2 affected children all displayed the phenotypes associated with microcornea and cataracts, while her husband exhibited no phenotypical abnormalities. The CRYGC p.C130fs variant exhibited co-segregation in the family, matching the inheritance pattern and clinical information of the affected individuals. No mutations were identified as being related to the pathogenic genes in the ClinVar database, and there were no matched variants in 59 genes, according to ACMG SF (secondary findings) v2.0 mutation analysis.

The CRYGC gene encodes a member of the gamma-crystallin family, of which 6 genes (from γA to γF-crystallin; gene symbols: CRYGA to CRYGF) are found on human chromosome 2 q33-36.11,14,15 Crystallin proteins are crucial elements of the vertebrate eye lens and promote the preservation of the refractive index and transparency of the lens. Only CRYGC and CRYGD genes have been identified as having cataract-causing mutations in humans.16 Mutations in CRYGC are associated with various types of cataracts across genetic studies.17

Congenital cataracts may be caused by crystallin gene mutations, which change the protein—protein interactions and decrease the solubility of crystallin proteins.18,19 Stable crystallin proteins are relevant for maintaining crystal transparency and a high refractive index.2,20

Studies on the genetic etiology of CC have all used data from large families; therefore, they cannot be applied to larger population analyses. Thus, genetic analysis of CC still lags behind compared with research on other eye diseases.2,16 Identifying mutations in families with a history of CC will allow researchers to identify similar phenotypic pathogenesis and link their research with that of other studies, especially because cataracts within a single family can show significant phenotypical variation.2,21,22

Understanding the molecular basis of cataract formation may lead to the future development of nonsurgical interventions.23 Moreover, a study24 has shown that crystallin proteins are important in aging research.

Previous studies12,17,35-47 have identified several mutations in the CRYGC gene. Moreover, there have been previous reports48,49 regarding frameshift mutations in CRYGC. However, we report the novel CRYGC frameshift mutation c.389_390insGCTG (p.C130fs) in exon 3, with 4 missing bases, causing the protein sequence after the 130th amino acid codon to differ from the reference sequence, which is not currently reported in the ClinVar database.

In conclusion, we identified a pathogenic mutation (c.389_390insGCTG) in CRYGC (p.C130fs), a
heterozygous variant, and a frameshift mutation. This mutation was identified in a Chinese family whose members presented with CC and microcornea. Our findings expand the repertoire of known CRYGC gene mutations causing CC. Our findings provide valuable information for researchers and new insights into the etiology and molecular diagnosis of CC; however, the molecular mechanism of this mutation warrants further investigation.

Footnotes and Disclosures

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Abbreviations and Acronyms:
ACMG = American College of Medical Genetics and Genomics;
AD = autosomal dominant; CC = congenital cataract.
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