SHORT REPORT

Gamma-S crystallin gene (CRYGS) mutation causes dominant progressive cortical cataract in humans

H Sun*, Z Ma*, Y Li, B Liu, Z Li, X Ding, Y Gao, W Ma, X Tang, X Li, Y Shen

Background: Congenital or childhood cataract is clinically and genetically a highly heterogeneous lens disorder in children. Autosomal dominant inheritance is most common. Objective: To report the identification of a mutation in the human CRYGS gene.

Subjects and methods: A large six generation family affected by progressive polymorphic cortical cataract was investigated. After excluding loci for known cataract candidate genes using 39 fluorescent microsatellite markers, a whole genome scan was carried out.

Results: The disease was associated with inheritance of a 20.7 cM locus on chromosome 3q26.3-qter, with a maximum LOD score of 6.34 (θ = 0) at marker D3S1602. Haplotype analysis indicated that the disease gene lay at approximately 2.8 Mb physical intervals between D3S1571 and D3S3570 and contained CRYGS on 3q27.3. By sequencing the CRYGS gene, a distinct 1619G→T (AC068631) heterozygous missense mutation in exon 2 was identified, co-segregating with the disease phenotype in this family and resulting in a glycine (GGG) to valine residue (GTG) substitution in codon 18 (NP_060011).

Conclusions: This report is the first description of a mutation in CRYGS with autosomal dominant cataract in humans.

RESULTS

Cataract characterised by opacities of the lens remains the leading cause of human blindness worldwide. Non-syndromic congenital cataracts have an estimated frequency of 1–6 per 10 000 live births,1 and one third of cases are familial. A strong genetic predisposition to the development of congenital cataract and age related cataract has been well documented. Recent work in molecular genetics has identified 14 genes involved in the pathogenesis of isolated inherited cataract, including seven coding for crystallins (CRYAA[MIM 123580], CRYAB[MIM123590], CRYBA1/A3[MIM123610], CRYBB1[MIM600929], CRYBB2-[MIM123620], CRYGC[MIM123680], and CRYGD-[MIM123690]), two for gap junctional channel protein (GJA3[MIM121015] and GJA8[MIM600897]), two for lens membrane protein (LIM2[MIM154045] and MIP-[MIM154050]), one for beaded filament structural protein 2 (BFSP2[MIM603212]), one for glucosaminyl (N-acetyl)transferase 2 gene (GCNT2[MIM600429]), and one for heat shock transcription factor 4 (HSF4[MIM602438]). Twin studies on age related cataract in the United Kingdom estimate that two thirds of cortical cataracts and at least half the nuclear cataracts can be explained by genetic factors.2–4 To investigate the molecular cause of cataract we studied a large Chinese family suffering from an autosomal dominant, progressive and polymorphic cortical cataract, and added another gene to growing list of genes involved in this heterogeneous monogenic disorder.

METHODS

We studied a Chinese six generation cataract family composed of 119 individuals with a dominant pattern of inheritance. Clinical information and blood specimens were obtained from 64 family members, including 14 patients. All participants had a full ocular assessment to document the phenotype. There was no evidence of other systemic and ocular defects.

After obtaining informed consent, we studied 11 loci for known candidate genes using three or four fluorescent microsatellite markers per locus, and no evidence of linkage was detected (data not shown). Subsequently a genome-wide scan consisting of 382 microsatellite markers spaced at ~10 cM intervals was carried out using ABI PRISM linkage mapping sets. This suggested a putative linkage on chromosome 3 (D3S1262 and D3S1601). We then undertook fine mapping (table 1). The order and position of the markers were obtained from the Marshfield Genetic Database (www.marshfield.org/genetics/maps). Genotyping and data collection were conducted by ABI Prism GeneMapper v.3.0 software. In the linkage analysis, we modelled the disease as an autosomal dominant inheritance with 90% penetrance, set the affected allele frequency at 0.0001, and assumed the marker allele frequency to be uniformly distributed. We carried out two point linkage analysis using the MLINK program from the LINKAGE v.5.10 software package. Pedigree and haplotype construction were undertaken using Cyrillic v.2.1 software. We screened the mutation of candidate genes by bidirectional sequencing polymerase chain reaction products (300–600 bp).

The phenotype in this family was characterised by progressive opacities in the cortex with a ground glass appearance at an early age. The cataract is progressive and cataractous changes were prominent in affected older individuals, whose vision began to deteriorate between the ages of 8 and 15 years. Phenotypic variation in the size and density of the opacities and in their position was observed among the 14 affected members, who had no other inherited ocular or systemic abnormalities. The opacities could be located in the anterior, posterior, or peripheral cortex but no opacity was observed in the fetal nuclear region (fig 1). We identified a new autosomal dominant congenital cataract locus on chromosome 3q26.3-qter. Linkage analysis gave a maximum two point LOD score of 6.34 (θ = 0.00) for marker D3S1602 (table 1).

Haplotype analysis (fig 2) indicated that the disease gene lay at approximately 2.8 Mb physical intervals between D3S1571 and D3S3570. There are around 60 genes in this region including one crystallin gene CRYGS. The characteristic of the cataract in this family was the progressive appearance of opacities in secondary fibre cells, coinciding with the CRYGS expression pattern.2 This gene therefore became an excellent candidate gene. The CRYGS gene spans
6 kb and includes three exons. An alternative short transcript of the human CRYGS gene has been reported, with a disrupted open reading frame owing to the inclusion of part of intron 1 in the mature mRNA, producing a defective protein product with potentially deleterious results for the lens transparency. Formerly known as γS-crystallin, the γS-crystallin gene is also stress inducible in the retina11 and that the carotenoid-γS-crystallin complex can protect the retina against photodamage.12 γS-Crystallin, as a dominant structural component of the adult human lens, may play an important role in maintaining lens transparency. Formerly known as βS-crystallin, γS-crystallin was renamed when the structure of the bovine gene was determined and proved to be characteristic of the γ rather than the β family.3 In β-crystallins, each of four repeated structural motifs is encoded in a separate exon, while in γ-crystallins, the four motifs are coded as fused pairs in only two exons. γS-Crystallin has an additional α helix, which is located between the third and fourth β sheets,13 and this is not found in the other γ-crystallins. γS-Crystallin resembles other γ-crystallins in gene structure and sequence, but is the most divergent member of the family. Findings based on structural and protein engineering studies or on molecular genetic analyses indicated that a series of gene alterations and fusions proceeded from crystallin ancestors based on structural and protein engineering studies or on molecular genetic analyses indicated that a series of gene alterations and fusions proceeded from crystallin ancestors to two domain/two monomer motifs, which changed to two motif/domain pairs, and then to two domain γ-type crystalline monomers, or to two domain/two monomer β type dimmers.14 In many ways, γS is a good candidate to represent the precursor of the γ-crystallins and possibly a link between the β and γ families. The γS-crystallin gene shows the expected high conservation, with 89% murine identity and 91% bovine identity (fig 4). Both the particularity of crystallins and the high degree of conservation across species suggest that CRYGS is essential for lens development.

### DISCUSSION

Three major classes of crystallins—α, β, and γ—are common to the eye lens throughout all vertebrates. The β and γ classes are made up of homologous proteins, and constitute the βγ-crystallin superfamily. CRYGS encodes γS-crystallin, a member of the βγ-crystallin superfamily. There are two groups of γ-crystallins. The best studied group consists of six genes (γA to γF), which form a single cluster in primate and rodent genomes5–7 at human chromosome 2 or mouse chromosome 1 and affect early eye and lens development. Distinct from these is the γS-crystallin gene on human chromosome 3 or mouse chromosome 16, which is expressed late but abundantly in the ocular lens. The γS gene is also expressed outside the lens, particularly in the mature retina and cornea.15 It has been shown that the γS-crystallins are stress inducible in the retina6 and that the carotenoid-γS-crystallin complex can protect the retina against photodamage.12

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*Microsatellites are from ABI PRISM linkage mapping sets in the original genome scan and the others were added in the fine mapping stage.
Classification of human inherited cataract is difficult because of the wide variation in morphologies observed. The phenotype in this family is rare, as opacities are restricted to a sector of cortical lens and the nucleus is unaffected, in contrast to lamellar cataract. This phenotype was similar to a Swiss family suffering from autosomal recessive cortical pulverulent cataract, which has been mapped to chromosome 9q13–22, but the disease gene has not yet been cloned.\textsuperscript{15}

**Figure 2** Pedigree and haplotype analysis of the six generation Chinese family. Haplotype analysis of 56 members in this Chinese family shows five microsatellite markers on chromosome 3 co-segregation in 14 patients. Squares and circles symbolise male and female individuals, respectively. Black symbols denote affected individuals. V:1 is the proband.

**Figure 3** Mutational analysis of the \( \gamma \)-crystallin gene (CRYGS). A (sense chain) and B (antisense chain): sequence chromatograms showing a 1619G→A (AC068631) heterozygous transversion, which substitutes the amino acid glycine for valine (G18V) and was found in all affected individuals. C (sense chain) and D (antisense chain): the sequence change was not observed in any unaffected family members or in 171 unrelated control individuals.
Additionally, distributions of the opacities vary not only between family members but also between eyes in the same patient. This suggests that γS-crystallin may be involved in epithelial cell growth and migration, which in turn may contribute to lens fibre organisation. Our study indicates that a single base mutation, which results in a conservative sequence change of Gly→Val at residue 18 in γS-crystallin, is associated with progressive cortical cataract. The mutant amino acid residue locates at the end of the first β strand in the first “Greek key” motif. In vitro, γS-crystallin plays an important role in suppressing the aggregation of the γ-crystallins. There is evidence that early unfolding of β- and γ-crystallins results in a substantial degree of secondary structural binding to α-crystallin. In this way the unfolding protein can lead to opacification once the endogenous α-crystallin is saturated. The G18V mutant protein may interfere with one or more of these functions, which disrupt the morphology and organisation of cortical fibre cells.

Additionally, it has been shown that the four stranded Greek key β sheet peptide, corresponding to the crystallin fold, forms an individual calcium binding site and gives rise to the first calcium ligate at the residue next to the conserved aromatic amino acid in the sequence Y/E/WXXXXXXG, located at the end of the first β strand. Glycine is necessary to form a dihedral angle, so it is irreplaceable. In the present family only this glycine mutated, probably leading to structural alterations in Ca"β" binding and storage ability. As calcium homeostasis in the lens may be disturbed and in turn trigger lens opacification, this could be a novel mechanism of cataract formation. Understanding the non-structural properties of the crystallins may be critical for an understanding of malfunction in the molecular cascades that lead to cataractogenesis, and its eventual therapeutic amelioration.

Finally, it is noteworthy that mutation of CRYGS has been found responsible for the murine Opj cataract, which is caused by a mutant protein that precipitates and aggregates in a temperature sensitive manner. The racat cataract, which is characterised by a spontaneous mutant transmitted as a recessive trait in the mouse, shares similar characteristics with the Opj cataract. These data strongly suggest a role for CRYGS in cataractogenesis.

Conclusions

Human γS-crystallin has to last a lifetime and thus requires both structural and kinetic stabilisation. In this study we have identified the first known mutation (G18V) of the CRYGS gene to be associated with autosomal dominant cataract in humans. This may help to further our understanding of the aetiology of cataract formation and of the function and properties of this gene. This study of a progressive phenotype and a CRYGS mutation may also provide insight into the cause of the more common sporadic form of age related cataract. The phenotype and location of the mutation suggest the need for further functional experiments to explore the critical nature of this residue in lens fibre organisation and long term stability.

**ELECTRONIC DATABASE INFORMATION**

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/ Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (CRYAA[MIM123580], CRYAB-[MIM123590], CRYBA1/A3[MIM123610], CRYBB1[MIM-600929], CRYBB2[MIM123620], CRYGC[MIM123680], CRYGD-[MIM123690], GJA3[MIM121015], GJA8[MIM600897], LIM2-[MIM154045], MIP[MIM154050], BFSP2[MIM603212], GCNT2-[MIM600429], HSF4[MIM602438]).


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Figure 4 CRYGS mapping and multiple protein sequence alignment in different species. Reference sequence numbers of protein are human [NP_060011], cow [NP_776717], and mouse [NP_034097]. The arrow directed the mutant amino acid residue.

Additional information and resources

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/ Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (CRYAA[MIM123580], CRYAB-[MIM123590], CRYBA1/A3[MIM123610], CRYBB1[MIM-600929], CRYBB2[MIM123620], CRYGC[MIM123680], CRYGD-[MIM123690], GJA3[MIM121015], GJA8[MIM600897], LIM2-[MIM154045], MIP[MIM154050], BFSP2[MIM603212], GCNT2-[MIM600429], HSF4[MIM602438]).

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