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 3q26.3-3pter, 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 (AC006831) heterozygous missense mutation in exon 2 was identified, co-segregating with the disease phenotype in this family and resulting in a glycine (G) to valine residue (V) substitution in codon 18 (NP_060011).

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

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, 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]).

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 0.00001, 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).

RESULTS

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 3q26.3-3pter. 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 characteristics of the cataract in this family was the progressive appearance of opacities in secondary fibre cells, coinciding with the CRYGS expression pattern. This gene therefore became an excellent candidate gene. The CRYGS gene spans...
Table 1 Two point LOD scores for linkage between the cataract locus and 3 q markers

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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.

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 genomes8,9 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.10 It has been shown that the γS-crystallins are 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.5 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 coding for proteins made up of a single Greek key motif, which changed to two motif/one domain proteins, 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.

Figure 1 Eye photograph of individual IV:20, aged 35, from a six generation Chinese family, showing opacities located in the peripheral cortex of the lens.
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.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 y-S-crystallin gene (CRYGS). A (sense chain) and B (antisense chain): sequence chromatograms showing a 1619G→A (ACD68631) 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.
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.

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