Molecular Vision **2004; 10:233-9** <http://www.molvis.org/molvis/v10/a29>
Received 17 July 2003 | Accepted 24 March 2004 | Published 29 March 2004

©2004 Molecular Vision

Special fasciculiform cataract caused by a mutation in the γD-crystallin gene

Xingchao Shentu,[1] Ke Yao,[1,2] Wen Xu,[1] Shu Zheng,[3] Songnian Hu,[4] Xiaohua Gong[5]

(The first two authors contributed equally to this publication)

[1]Eye Center, Affiliated Second Hospital, College of Medicine and [2]Institute of Ophthalmology, Zhejiang University, Hangzhou, China; [3]Cancer Institute of Zhejiang University, Hangzhou, China; [4]Huada Gene Institute, Hangzhou, China; [5]Department of Cell Biology, The Scripps Research Institute, La Jolla, CA

Purpose: To identify ultrastructure changes and genetic defects associated with a special fasciculiform autosomal dominant congenital cataract (ADCC) affecting a large Chinese family.
Methods: Members of the Chinese family affected by this special fasciculiform ADCC were studied. Clinical examinations, light and transmission electron microscopy of the removed lens tissue, linkage analyses using polymorphisms of microsatellite markers, and mutational analyses of candidate genes by direct sequencing were performed.
Results: The thirteen affected individuals in the family showed no variability between one another. The lens fiber cells showed focal degeneration, dense globular intracellular deposits, and an enlarged intercellular space. This ADCC was associated with a locus on chromosome 2q33-35 (maximum lod score [Z_{max}]=3.34; θ=0.05). Mutational analyses of the candidate genes (γ-crystallin; *CRYG*), identified a C->A heterozygous transversion at nucleotide position 70 in the γD-crystallin gene (*CRYGD*) exon2, which co-segregated with the presence of ADCCs and was not observed in 100 unrelated controls.
Conclusions: This study identified ultrastructure changes and genetic defects associated with a special fasciculiform ADCC affecting a large Chinese family. It appeared to be caused by a missense mutation in the *CRYGD* gene, further supporting the notion that alterations to *CRYG* play an important factor in human cataract formation.

Congenital cataracts may be caused by intrauterine embryopathies, single gene defects, or chromosomal rearrangements. They are a common abnormality of the eye and account for approximately one third of all pediatric visual loss [1]. Because these congenital cataracts result in cloudy imaging onto the retina, the abnormal development of visual cortical synaptic connections results in amblyopia, which causes affected children irreversible visual loss without suitable intervention. About 25% of all congenital cataracts are inherited [2,3], with the most common being nonsyndromal autosomal dominant congenital cataracts (ADCC).

In general, congenital cataracts are bilateral and characterized by the location and structure of the opacities, which include shape, size, color, and the presence or absence of refractivity. However, phenotypic variability has been documented among and within families [4-6], whereby identical mutations can result in different phenotypes [7,8]. These identical cataracts have also been mapped to different loci [9,10]. Although congenital cataracts are phenotypically and genetially heterogeneous, more than twenty loci have been shown to be linked with congenital cataracts and the disease associated mutations have been identified in 19 genes, including αA-crystallin [11], αB-crystallin [12], βA1-crystallin [13], βB1-crystallin [14], βB2-crystallin [15], γC-crystallin [16], γD-crystallin [17], γE-crystallin [18], beaded filament structural protein 2 [19], heat shock transcription factor 4 [20], gap junction protein alpha-3 [21], gap junction protein alpha-8 [22], paired-like homeodomain transcription factor-3 [23], ferritin [24], galactokinase 1 [25], glucosaminyl (N-acetyl) transferase 2 [26], major intrinsic protein of lens fiber (MIP) [27], lens intrinsic membrane protein 2 (LIM2) [28], and paired box gene 6 [29].

In this report we studied a large, four-generation Chinese family containing members affected by special fasciculiform ADCC. Using linkage analysis, we mapped an associated locus to 2q33-35, which contains the γ-crystallin genes (*CRYG*). The *CRYG* gene family has six members including γA-crystallin (*CRYGA*), γB-crystallin (*CRYGB*), γC-crystallin (*CRYGC*), γD-crystallin (*CRYGD*), γE-crystallin (*CRYGE*), and γF-crystallin (*CRYGF*). We identified a mutation within exon 2 of the γD-crystallin gene (*CRYGD*), which was present in all affected family members.

METHODS

Clinical Evaluation: The family was ascertained through the Eye Center of Affiliated Second Hospital College of Medicine, Zhejiang University, Hangzhou, China. Informed consent in accordance with the Declaration of Helsinki and the Zhejiang Institutional Review Board approval was obtained

Correspondence to: Ke Yao, Eye Center, Affiliated Second Hospital College of Medicine, Zhejiang University & Institute of Ophthalmology, Zhejiang University, 88 Jiefang Road, Hangzhou 310009, China; Phone: 0086-571-87783897; FAX: 0086-571-87783897; email: xlren@zju.edu.cn

from all participants. Informed consent was also obtained from the subjects after explanation of the nature and possible consequences of the study. Thirty-seven individuals participated in the study, 13 affected individuals and 24 unaffected individuals of whom 12 were spouses (Figure 1). Affected status was determined by a history of cataract extraction or ophthalmologic examination, which included slit lamp examination with dilated pupils, visual acuity testing, intraocular pressure measurement, and fundus examination. The cataract phenotype of the patients who previously had cataract extraction was obtained from history records.

Pathology and ultrastructure: Anterior lens capsules with attached lens epithelial cells were obtained by capslotomy and the lens material was aspirated by double cannula during cataract surgery. Part of the extracted lens material was fixed in formalin overnight and embedded in paraffin. Paraffin embedded sections (5 μm) were then subjected to HE staining. The remaining lens material was fixed in 2.5% glutaraldehyde for 30 min and postfixed in 1% osmic acid for 1 h at 4 °C. After dehydration with a graded series of ethanol concentrations, specimens were embedded in epon resin. Ultrathin sections (60-90 nm thickness) were stained with lead citrate and uranyl acetate and then observed with transmission electron microscopy (JEM-1200, Tokyo, Japan) at 60 Kv.

Genotyping and linkage analysis: Blood specimens (5 ml) were collected in EDTA and leukocyte genomic DNA was extracted. Microsatellite markers and their distances were obtained from the Cooperative Human Linkage Center (CHLC) and Genethon [30]. Microsatellites were amplified by polymerase chain reaction (PCR) with fluorescent labeled primers (Shenyou Bi-technology LTD, Shanghai, China) following standard methods. PCR products were denatured for 3 min at 90 °C and immediately placed on ice, then resolved on a 5% sequencing gel (FMC BioProducts, Copenhagen, Denmark) using a DNA sequencer (ABI Prism™ 377; Perkin-Elmer Applied Biosystems, Foster city, CA). Data were collected and analyzed using the followinmg computer programs; ABI Prism™ 377-18 Collection (Genescan) and Genescan analysis® 2.1 (Perkin-Elmer Applied Biosystems). For autosomal dominant inheritance, a disease-gene frequency of 0.0001 and full penetrance was assumed. Two point lod scores between the cataract locus and markers were calculated by the MLINK program of LINKAGE package (version 5.1) for a full range of θ values.

Mutational analysis of CRYG*:* We screened all exons of the candidate gene, *CRYG*, by direct sequencing for mutations. The genomic sequence of *CRYG* was obtained from the National Center for Biotechnology Information (*CRYGA*, OMIM 123660; *CRYGB*, OMIM 123670; *CRYGC*, OMIM 123680, *CRYGD*, OMIM 123690). Nine gene specific PCR primer pairs (Sangon Biological Engineering Technology and Service Co., LTD, Shanghai, China) were designed to amplify the 12 exons and flanking intron sequences of *CRYGA*, *CRYGB*, *CRYGC*, and *CRYGD* (Table 1). PCR products were subsequently purified using QIAquick Gel Extraction kit and were sequenced using Big Dye Primer Cycle Sequencing kit (Perkin-Elmer Applied Biosystems) and a DNA sequencer (ABI Prism™ 377, Perkin-Elmer Applied Biosystems). Data were

Table 1. Primers for amplification of Γ-crystallin (CRYG) gene coding regions

Primer	Sequence	Product size
CRYGA exon1/f	5'-GTCAGCTGGAAGGAACATCC-3'	291 bp
CRYGA exon1/r	5'-TGAGGACAACTCGAAAATGC-3'	
CRYGA exon2/f	5'-GGGTCAGGCCTTGCTATTCT-3'	450 bp
CRYGA exon2/r	5'-CCATGTCTATTGGGGGTCTG-3'	
CRYGA exon3/f	5'-GGATTGATTGAACCTGGGAG-3'	659 bp
CRYGA exon3/r	5'-GGTGAAAGTTGCAGTGAGCA-3'	
CRYGB exon1-2/f	5'-GGTGGTGCATGCCTGTAA-3'	679 bp
CRYGB exon1-2/r	5'-GCCCTTTTGTGTGATTTCCT-3'	
CRYGB exon3/f	5'-GGGAGGTGTAGGGACTGGAG-3'	515 bp
CRYGB exon3/r	5'-TGCTTCCCATCATGAAAACAT-3'	
CRYGC exon1-2/f	5'-GCAGTATGTACAGGACAGCGTTA-3'	644 bp
CRYGC exon1-2/r	5'-CCTCCCTGTAACCCACATTG-3'	
CRYGC exon3/f	5'-ATTCCATGCCACAACCTACC-3'	527 bp
CRYGC exon3/r	5'-CCCACCCCATTCACTTCTTA-3'	
CRYGD exon1-2/f	5'-GCTTATGTGGGGAGCAAACT-3'	650 bp
CRYGD exon1-2/r	5'-CAGCAGCCCTCCTGCTAT-3'	
CRYGD exon3/f	5'-GAAACAGCTATGACCATGCAC ACTTGCTTTTCTTCTCTTT-3'	435 bp
CRYGD exon3/r	5'-ATACGACTCACTATAGGGCAAG ACACAAGCAAATCAGTGCC-3'	

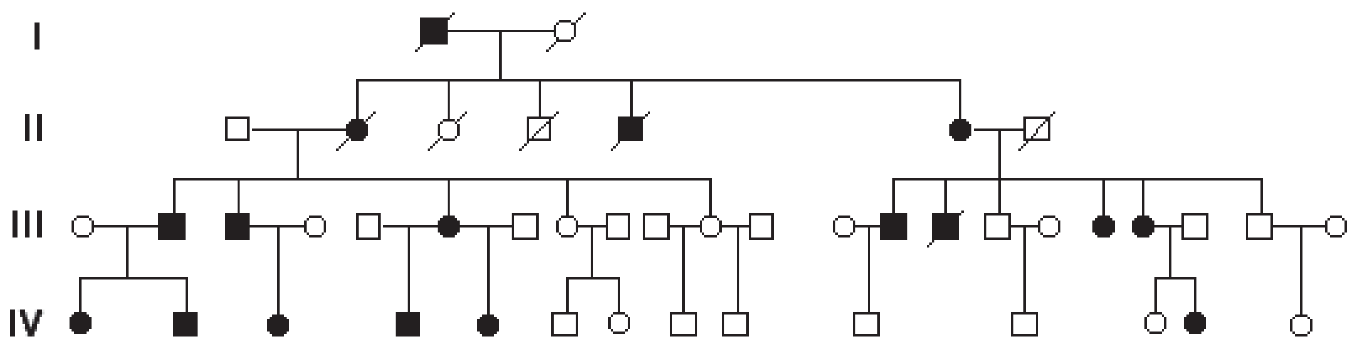

Figure 1. Pedigree of autosomal dominant congenital cataract (ADCC). Detailed pedigree of the ADCC family with the disease segregating in four generations. Squares and circles symbolized males and females respectively. Clear and blackened symbols denote unaffected and affected individuals, respectively.

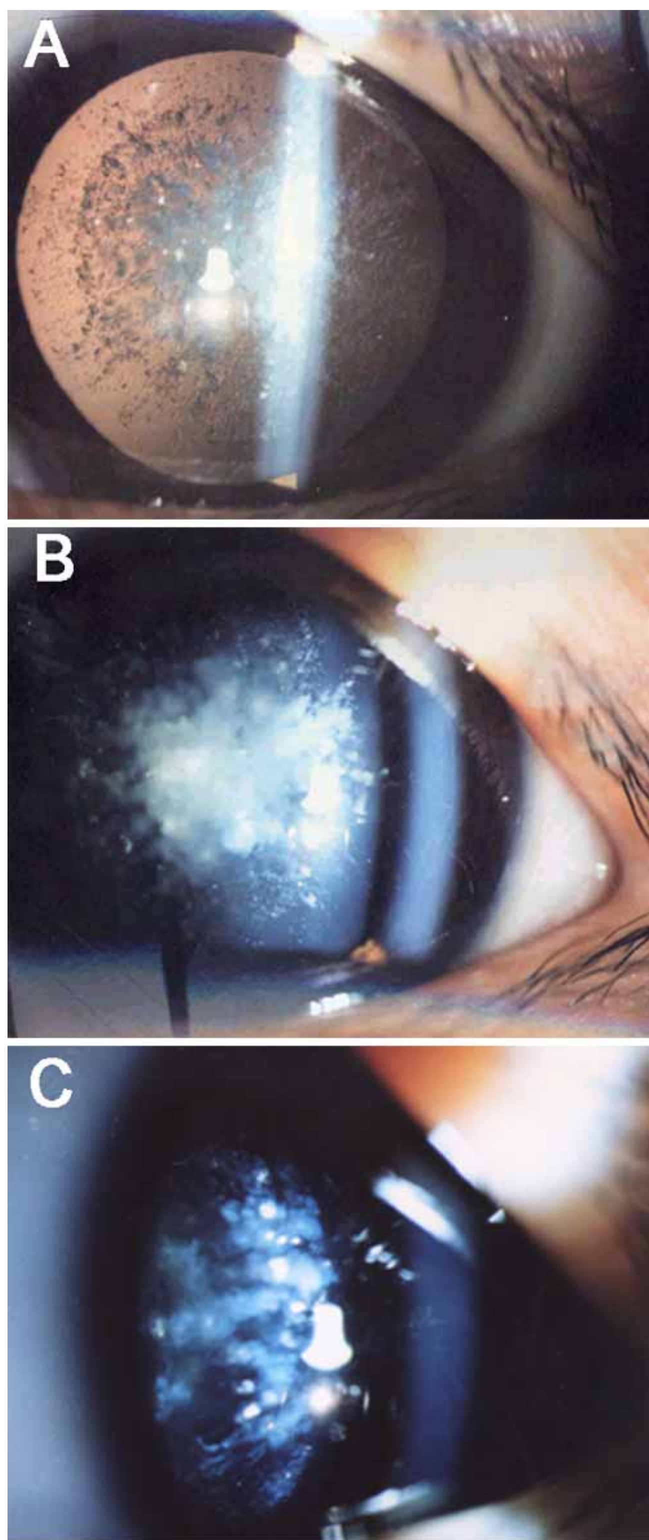

Figure 2. Slit lamp photographs of the eye of affected individuals. All affected family members showed a special fasciculiform cataract characterized by corroliform opacification, involving embryonic and fetal nuclei, and a fiberglasslike opacity projecting in different directions from the central zone with a radially oriented metal-like refractivity. **A**: Front view of the eye of an affected individual. **B**: Slit lamp view of the lens. **C**: Slit view of the eye of another affected individual.

collected and analyzed by the following computer programs; the ABI Prism™ 377-18 Collection (sequence run) and Sequencing analysis 3.3 (Perkin-Elmer Applied Biosystems). For screening, two unaffected and two affected individuals were compared. After detecting a probable mutation, all members of the family and 100 unrelated controls, who had undergone full ophthalmic examinations to be sure of free of eye diseases, were screened for the proposed disease associated mutation.

RESULTS

Clinical evaluation: All affected family members showed a special fasciculiform cataract characterized by corroliform opacification, which included embryonic and fetal nuclei, and a fiberglasslike opacity projecting in different directions from the central zone with a radially oriented metal-like refractivity (Figure 2A,B). This special fasciculiform cataract was a congenital cataract and there was no progressive development of lens opacities since birth. The degree of opacification of

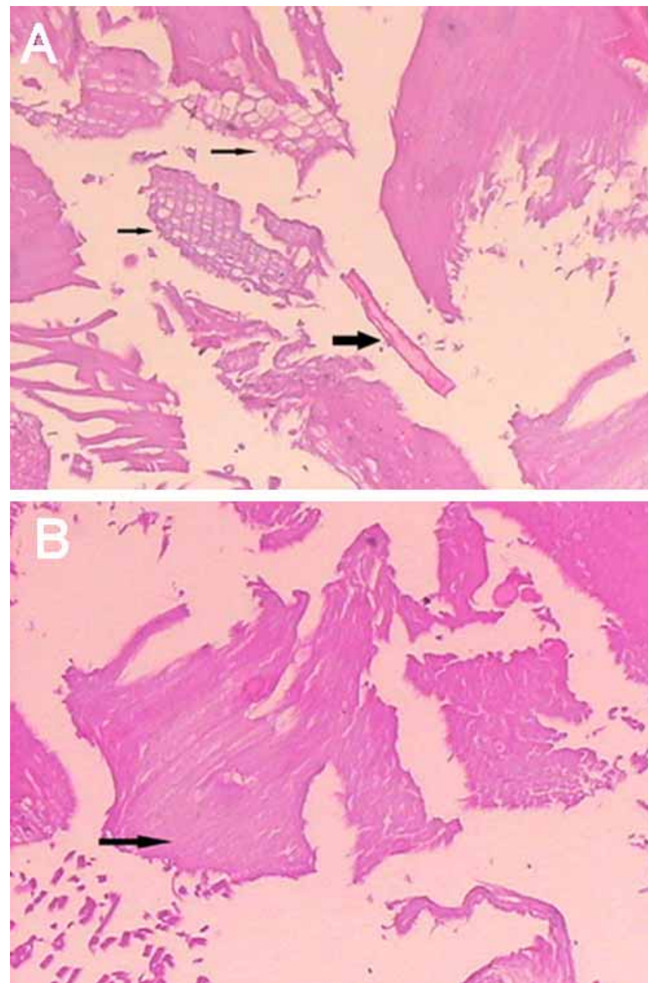

Figure 3. Pathology micrographs of removed lens tissue from affected individuals, by HE staining. There were focal degeneration alterations in the lens fiber cells. **A**: Reticular change (small arrow) and crystal precipitation (large arrow). **B**: Mucus-like degeneration (arrow).

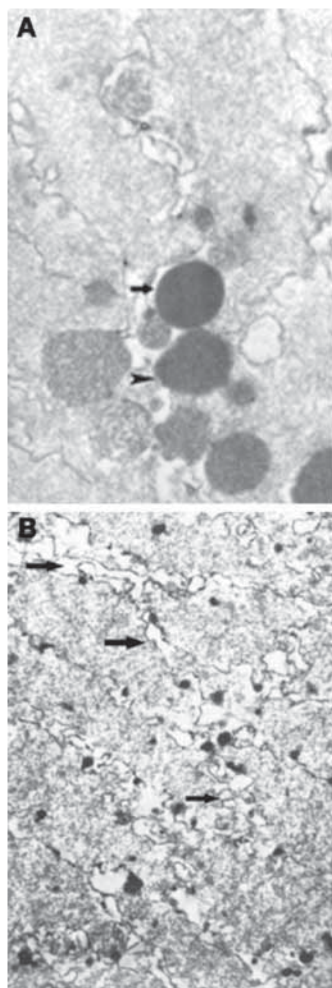

Figure 4. Transmission electron micrographs of removed lens tissue from affected individuals. **A**: There were foci of dense, globular deposits (0.05 to 0.5 μM in diameter) in the cytosol of the lens fiber cells, either surrounded by a single membrane (arrow) or deposited freely in the cytoplasm (arrowhead). **B**:The lens fiber cell borders were highly irregular and there was increased space (about 0.1 to 0.5 μM in width) between the cells (arrows).

the cataract showed no variability among the affected family members (Figure 2C). Also, there was no family history of other ocular or systemic abnormalities aside from age related disorders. Based on the presence of affected individuals in each of the four generations, equal numbers of affected males and females, and male to male transmission, autosomal dominant inheritance of the cataract was demonstrated.

Pathology: There were no detectable abnormal alterations to the lens epithelial cells by HE staining. However, the lens fiber cells showed manifestations of degeneration, including focal mucus-like degeneration, reticular change, crystal precipitation, and irregular refracility (Figure 3).

Ultrastructure: There were no characteristic or consistent alterations to the lens epithelial cells. However, lens fiber cells displayed abnormal intercellular and intracellular alterations. Normally, there are tight contacts between the lens fiber cells and a uniformly fine granular appearance within the cytoplasm. However, in affected samples there were foci of dense, globular intracellular deposits 0.05 to 0.5 μM in diameter. They were either surrounded by a single membrane or deposited freely in the cytoplasm (Figure 4A). The cell borders were highly irregular and there were enlarged spaces (0.1 to 0.5 μM in width) between the cells (Figure 4B).

Linkage and mutation analysis: Thirty members of the affected family, including 13 affected individuals, 9 unaffected individuals, and 8 spouses were genotyped and studied by microsatallite marker linkage analysis. For chromosome 2q33-35, around the γ-crystallin locus, seven locus genotypes and inferred haplotypes were showed (Figure 5) and two point lod scores were summarized (Table 2). Significant linkage was found with markers D2S2208 ($[Z_{max}]$=3.34; θ=0.05).

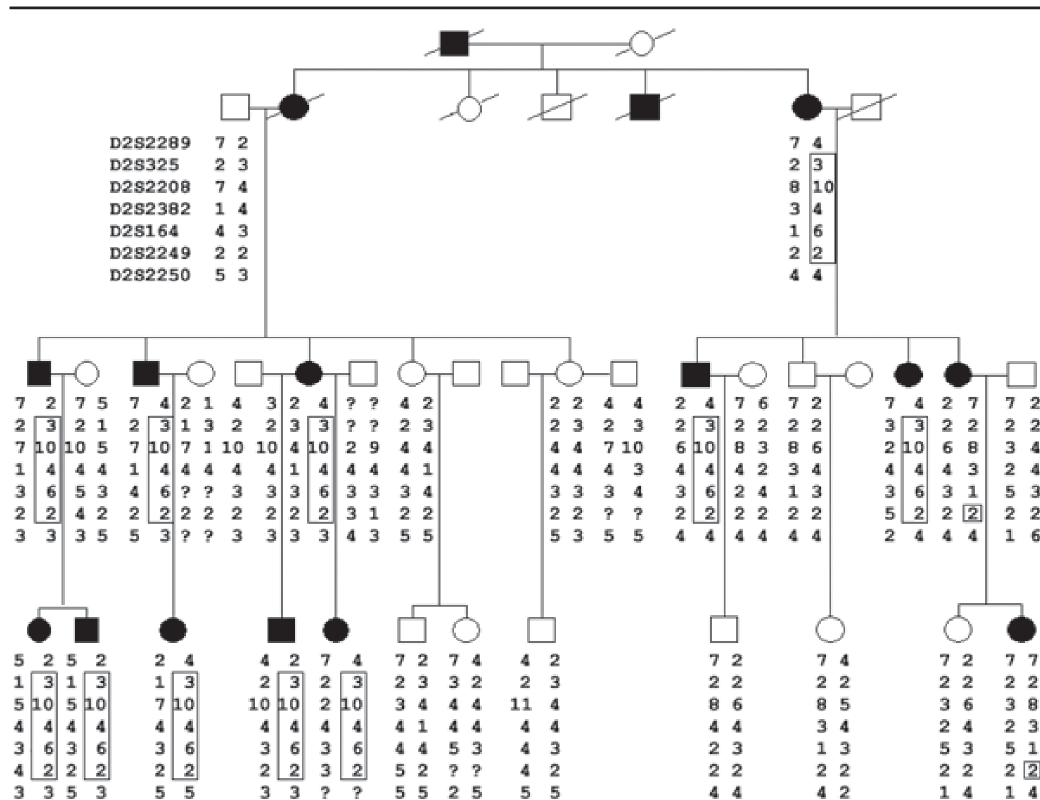

Figure 5. Pedigree of the cataractous Chinese family. Pedigree of the Chinese family with special fasciculiform cataract, showing seven locus genotypes and inferred haplotypes. Individuals designated as affected on the basis of family history or clinical records were indicated by blackened symbols. The affected haplotype was indicated by a vertical box.

Mutation analysis of the *CRYG* genes in the affected family detected two heterozygous transversions. Direct sequencing of the amplified exons of *CRYGA* and *CRYGC* failed to document any sequence change. *CRYGB* showed a A->C heterozygous transversion at nucleotide position 331 (I110L) in exon 3, which was an SNP (dbSNP:796287) loci and not disease specific. This polymorphic change did not co-segregate with cataract in the family. The change was observed in some (not all) affected family members and some unaffected family members. Sequencing of *CRYGD* revealed a C->A heterozygous transversion at nucleotide position 70 in exon 2 (Figure 6), which was predicted to substitute the conserved amino acid proline for a threonine (P23T). This sequence change was observed in all affected family members and was not observed in all unaffected family members, spouses in the family, and 100 unrelated control individuals.

DISCUSSION

Cataracts are a leading cause of blindness worldwide [31] and the number of cases are increasing due to an increased aging population. Unfortunately, the mechanism of lens opacification remains unknown. The study of congenital cataracts, which are usually the result of autosomal dominant inheritance, is useful in attempting to ascertain cataract related genetic defects. Genes that have been previously reported to be associated with ADCC are crystallins [11-18], connexins [21,22], cytoskeletal structure protein [19], and homeobox gene [23].

Among the reported genes, the *CRYG* genes were of great interest in this study. Although congenital cataracts are phenotypially and genetically heterogeneous, the position and degree of the opacity provide clues to the time and cause of the cataract. The balance of proteins and water in a regular array of cells is critical in maintaining the transparency of the lens. Modifications that reduce the effective solubility of lens proteins can lead to progressive protein aggregation and the formation of light scattering centers in the lens [32,33]. Crystallins constitute more than 90% of the soluble proteins in the human lens. They are made up of α-crystallin (40%), β-crystallin (35%), and γ-crystallin (25%) [34].The congenital cataracts in this study were characterized by opacification of the lens nucleus, which was made up of enucleated lens fiber cells. The opacification was denser in the center than in the periphery. Also, there were many structural alterations to the lens fiber cells at the microscopic level suggesting that a special protein expressed only in the lens fiber cells was the most probable cause of the pathologic events leading to the formation of high molecular weight aggregates and deposition.

The *CRYG* genes encode γ-crystallin, a major component of the lens structural protein. In addition, it is only expressed in differentiated fiber cells of the human lens [35]. Also, γ-

Table 2. Two point lod scores for linkage between cataract locus and chromosome 2 markers

Marker	LOD scores by recombination fraction (θ) 0.0	0.01	0.05	0.1	0.2	0.3	0.4	Zmax	θmax
D2S2289	-∞	-3.65	-1.08	-0.16	0.43	0.50	0.34	-	
D2S325	-∞	0.14	0.65	0.71	0.55	0.31	0.11	0.71	0.1
D2S2208	-∞	2.96	3.34	3.23	2.64	1.84	0.89	3.34	0.05
D2S2382	-∞	0.81	1.33	1.39	1.20	0.84	0.43	1.39	0.1
D2S164	-∞	0.92	2.02	2.24	2.01	1.45	0.70	2.24	0.1
D2S2249	0.20	0.20	0.17	0.14	0.08	0.04	0.01	0.20	0
D2S2250	-∞	-2.21	-0.91	-0.42	-0.06	0.03	0.02	-	

For chromosome 2q33-35, around the γ-crystallin locus, two-point lod scores were summarized. Significant linkage was found with markers D2S2208 (Z_{max}=3.34; θ_{max}=0.05).

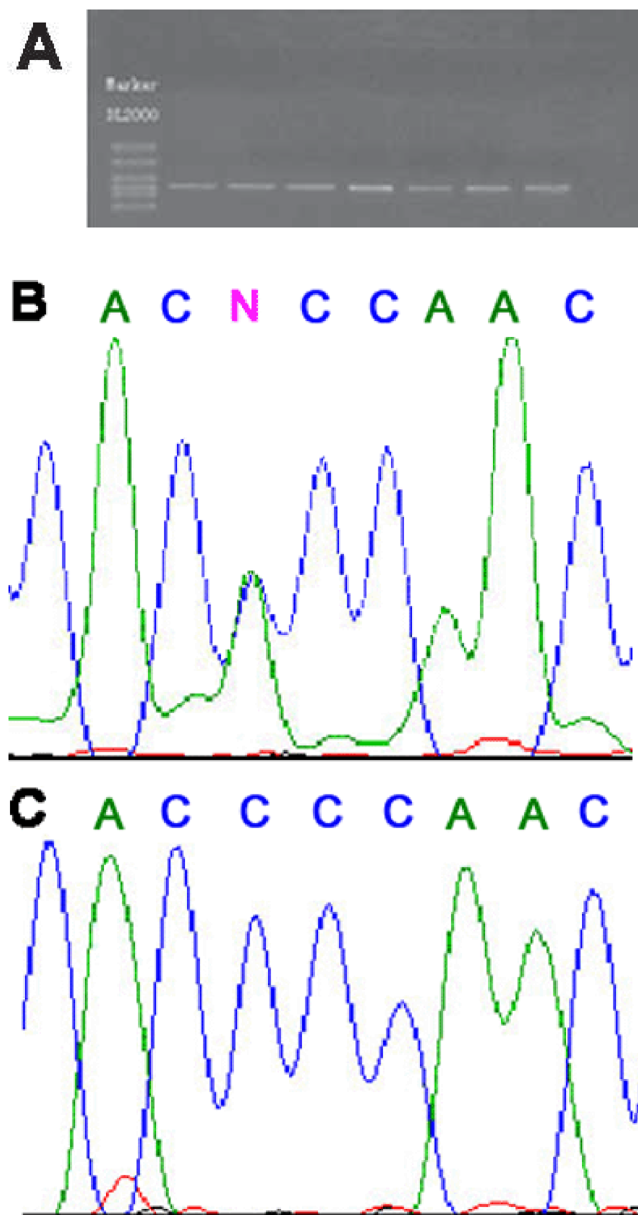

Figure 6. Mutational analysis of the γD-crystallin (*CRYGD*) gene. **A**: *CRYGD* exon 1 and 2 amplified from genomic DNA of the ADCC affected family and analyzed on a 1.5% agarose gel. **B**: Sequence chromatograms showing a C->A heterozygous transversion at nucleotide position 70 in *CRYGD* exon 2, which was predicted to substitute the amino acid proline for a threonine (P23T), found in all affected individuals. **C**: The sequence change was not observed in all unaffected family members, spouses in the family, and 100 unrelated control individuals.

crystallin is associated with high protein concentrations and high refractive indices in the human lens. Thus, it plays a key role in maintaining the development and transparency of the lens [36-38]. The improper folding of γ-crystallin would cause protein aggregation and deposition leading to cataract formation. The *CRYG* genes are associated with cataract formation in human. In fact, Coppock-like cataracts [39] and cataracts with a dust-like opacity [17], an aculeiform phenotype [16], or a deposition of crystals [40] have been determined to be due to mutations in the *CRYG* genes. Interestingly, the crystal precipitations we found in the lens cortices of our affected family members were very similar to the prismatic crystals found by Kmoch et al. [40].

The *CRYG* gene family has six members; *CRYGA*, *CRYGB*, *CRYGC*, *CRYGD*, *CRYGE*, and *CRYGF*. *CRYGE* and *CRYGF* are considered to be pseudogenes, by virtue of an in-frame stop codon in an exon [41]. In combination with linkage and gene mutational analysis, we identified a mutation in *CRYGD*, a C->A heterozygous transversion at nucleotide position 70 in exon 2, which was predicted to substitute the conserved amino acid proline for a threonine (P23T) in the γD-crystallin protein. This mutation co-segregated with the cataract in the affected family and was not observed in 100 unrelated controls, implying that it was associated with ADCC rather than a rare polymorphism. It was noteworthy that the same mutation had recently been suggested to cause the lamellar cataract in an Indian family [42] and the cerulean cataract in a large Moroccan family [43]. Both the lamellar cataract in an Indian family, which was a perinuclear shaped cataract with a transparent embryonic nucleus, and the cerulean cataract in a large Moroccan family, which was a cerulean blue dot cataract and characterized by a very early onset of lens opacities and a rapid progression towards blindness, were clinically different from our special fasciculiform cataract, which was not progressive in development of lens opacities and showed a special corroliform opacification including embryonic and fetal nuclei and a fiberglasslike opacity projecting in different directions from the central zone with the radially oriented metal-like refractivity. Interestingly, during galley processing of this article, Mackay et al. [44] reported that an autosomal dominant form of coral-shaped cataract segregating in a three generation Caucasian pedigree was also caused by the P23T mutation in *GRYGD*. As no clinical photographs of this coral-shaped cataract were presented and it was only "...described as coralliform or axial, having long trumpet-shaped opacities grouped toward the center of the lens and projecting radially forward resembling a piece of coral" [44], it is not clear that this coral-shaped cataract is the same as, or even similar to, our special fasciculiform cataract. An explanation for the observation of three different phenotypes with the same mutation may be related to the influence of an unidentified modifier gene or to sequence variations that may influence *CRYGD* expression. The phenotypic heterogeneity of congenital cataracts has been reported before [8].

In summary, we have identified a missense mutation (P23T) in exon 2 of the *CRYGD* gene which is linked and associated with a special fasciculiform ADCC affecting a large Chinese family. This is the first time to report a mutation in *CRYGD* resulting in special fasciculiform ADCC. Future studies will include crystallization of the mutant γD-crystallin and the generation of a transgenic animal model of this mutation. These studies will help us to better understand cataract formation and will provide a useful system for testing strategies for cataract prevention.

ACKNOWLEDGEMENTS

This work was financed by the key project of the Natural Science foundation of Zhejiang Province of china.

REFERENCES

1. Jensen S, Goldschmidt E. Genetic counselling in sporadic cases of congenital cataract. Acta Ophthalmol (Copenh) 1971; 49:572-6.
2. Phelps Brown N, Bron AJ. Lens disorders: a clinical manual of cataract diagnosis. Oxford: Butterworth-Heinemann; 1995.
3. Merin S. Inherited cataracts. In: Merin S, editor. Inherited eye diseases: diagnosis and clinical management. New York; Marcel Dekker; 1991. p. 86-120.
4. Marner E, Rosenberg T, Eiberg H. Autosomal dominant congenital cataract. Morphology and genetic mapping. Acta Ophthalmol (Copenh) 1989; 67:151-8.
5. Nettleship E. Seven new pedigrees of hereditary cataract. Trans Ophthalmol Soc U K 1909; 29:188-211.
6. Mackay D, Ionides A, Berry V, Moore A, Bhattacharya S, Shiels A. A new locus for dominant “zonular pulverulent” cataract, on chromosome 13. Am J Hum Genet 1997; 60:1474-8.
7. Litt M, Carrero-Valenzuela R, LaMorticella DM, Schultz DW, Mitchell TN, Kramer P, Maumenee IH. Autosomal dominant cerulean cataract is associated with a chain termination mutation in the human beta-crystallin gene CRYBB2. Hum Mol Genet 1997; 6:665-8.
8. Gill D, Klose R, Munier FL, McFadden M, Priston M, Billingsley G, Ducrey N, Schorderet DF, Heon E. Genetic heterogeneity of the Coppock-like cataract: a mutation in CRYBB2 on chromosome 22q11.2. Invest Ophthalmol Vis Sci 2000; 41:159-65.
9. Francis PJ, Berry V, Bhattacharya SS, Moore AT. The genetics of childhood cataract. J Med Genet 2000; 37:481-8.
10. He W, Li S. Congenital cataracts: gene mapping. Hum Genet 2000; 106:1-13.
11. Litt M, Kramer P, LaMorticella DM, Murphey W, Lovrien EW, Weleber RG. Autosomal dominant congenital cataract associated with a missense mutation in the human alpha crystallin gene CRYAA. Hum Mol Genet 1998; 7:471-4.
12. Berry V, Francis P, Reddy MA, Collyer D, Vithana E, MacKay I, Dawson G, Carey AH, Moore A, Bhattacharya SS, Quinlan RA. Alpha-B crystallin gene (CRYAB) mutation causes dominant congenital posterior polar cataract in humans. Am J Hum Genet 2001; 69:1141-5.
13. 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.
14. 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.
15. Vanita, Sarhadi V, Reis A, Jung M, Singh D, Sperling K, Singh JR, Burger 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.

16. Heon E, Priston M, Schorderet DF, Billingsley GD, Girard PO, Lubsen N, Munier FL. The gamma-crystallins and human cataracts: a puzzle made clearer. Am J Hum Genet 1999; 65:1261-7.
17. Stephan DA, Gillanders E, Vanderveen D, Freas-Lutz D, Wistow G, Baxevanis AD, Robbins CM, VanAuken A, Quesenberry MI, Bailey-Wilson J, Juo SH, Trent JM, Smith L, Brownstein MJ. Progressive juvenile-onset punctate cataracts caused by mutation of the gammaD-crystallin gene. Proc Natl Acad Sci U S A 1999; 96:1008-12.
18. Brakenhoff RH, Henskens HA, van Rossum MW, Lubsen NH, Schoenmakers JG. Activation of the gamma E-crystallin pseudogene in the human hereditary Coppock-like cataract. Hum Mol Genet 1994; 3:279-83.
19. Conley YP, Erturk D, Keverline A, Mah TS, Keravala A, Barnes LR, Bruchis A, Hess JF, FitzGerald PG, Weeks DE, Ferrell RE, Gorin MB. A juvenile-onset, progressive cataract locus on chromosome 3q21-q22 is associated with a missense mutation in the beaded filament structural protein-2. Am J Hum Genet 2000; 66:1426-31.
20. Bu L, Jin Y, Shi Y, Chu R, Ban A, Eiberg H, Andres L, Jiang H, Zheng G, Qian M, Cui B, Xia Y, Liu J, Hu L, Zhao G, Hayden MR, Kong X. Mutant DNA-binding domain of HSF4 is associated with autosomal dominant lamellar and Marner cataract. Nat Genet 2002; 31:276-8.
21. Mackay D, Ionides A, Kibar Z, Rouleau G, Berry V, Moore A, Shiels A, Bhattacharya S. Connexin46 mutations in autosomal dominant congenital cataract. Am J Hum Genet 1999; 64:1357-64.
22. Berry V, Mackay D, Khaliq S, Francis PJ, Hameed A, Anwar K, Mehdi SQ, Newbold RJ, Ionides A, Shiels A, Moore T, Bhattacharya SS. Connexin 50 mutation in a family with congenital “zonular nuclear” pulverulent cataract of Pakistani origin. Hum Genet 1999 Jul-Aug; 105:168-70.
23. Semina EV, Ferrell RE, Mintz-Hittner HA, Bitoun P, Alward WL, Reiter RS, Funkhauser C, Daack-Hirsch S, Murray JC. A novel homeobox gene PITX3 is mutated in families with autosomal-dominant cataracts and ASMD. Nat Genet 1998; 19:167-70.
24. Aguilar-Martinez P, Biron C, Masmejean C, Jeanjean P, Schved JF. A novel mutation in the iron responsive element of ferritin L-subunit gene as a cause for hereditary hyperferritinemia-cataract syndrome. Blood 1996; 88:1895.
25. Stambolian D, Ai Y, Sidjanin D, Nesburn K, Sathe G, Rosenberg M, Bergsma DJ. Cloning of the galactokinase cDNA and identification of mutations in two families with cataracts. Nat Genet 1995; 10:307-12.
26. Yu LC, Twu YC, Chang CY, Lin M. Molecular basis of the adult i phenotype and the gene responsible for the expression of the human blood group I antigen. Blood 2001; 98:3840-5.
27. Berry V, Francis P, Kaushal S, Moore A, Bhattacharya S. Missense mutations in MIP underlie autosomal dominant ‘polymorphic’ and lamellar cataracts linked to 12q. Nat Genet 2000; 25:15-7.
28. Pras E, Levy-Nissenbaum E, Bakhan T, Lahat H, Assia E, Geffen-Carmi N, Frydman M, Goldman B, Pras E. A missense mutation in the LIM2 gene is associated with autosomal recessive presenile cataract in an inbred Iraqi Jewish family. Am J Hum Genet 2002; 70:1363-7.
29. Azuma N, Yamaguchi Y, Handa H, Hayakawa M, Kanai A, Yamada M. Missense mutation in the alternative splice region of the PAX6 gene in eye anomalies. Am J Hum Genet 1999; 65:656-63.
30. Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J. A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 1996; 380:152-4.
31. Thylefors B, Negrel AD, Pararajasegaram R, Dadzie KY. Global data on blindness. Bull World Health Organ 1995; 73:115-21.
32. Harding JJ, Crabbe MJC. The lens: development, proteins, metabolism and cataract. In: Davson H, editor. The eye. Vol 1B. Orlando (FL): Academic Press; 1984. p. 207-492.
33. Benedek GB. Cataract as a protein condensation disease: the Proctor Lecture. Invest Ophthalmol Vis Sci 1997; 38:1911-21.
34. Hejtmancik JF. The genetics of cataract: our vision becomes clearer. Am J Hum Genet 1998; 62:520-5.
35. Ibaraki N, Lin LR, Reddy VN. Effects of growth factors on proliferation and differentiation in human lens epithelial cells in early subculture. Invest Ophthalmol Vis Sci 1995; 36:2304-12.
36. Papaconstantinou J. Molecular aspects of lens cell differentiation. Science 1967; 156:338-46.
37. Delaye M, Tardieu A. Short-range order of crystallin proteins accounts for eye lens transparency. Nature 1983 Mar 31-Apr 6; 302:415-7.
38. Wistow GJ, Piatigorsky J. Lens crystallins: the evolution and expression of proteins for a highly specialized tissue. Annu Rev Biochem 1988; 57:479-504.
39. Heon E, Priston M, Billingsley GD, et al. Clarifying the role of gamma crystalline in congenital CCL cataract. Am J Hum Genet 1999; 65(suppl):A19.
40. Kmoch S, Brynda J, Asfaw B, Bezouska K, Novak P, Rezacova P, Ondrova L, Filipec M, Sedlacek J, Elleder M. Link between a novel human gammaD-crystallin allele and a unique cataract phenotype explained by protein crystallography. Hum Mol Genet 2000; 9:1779-86.
41. Meakin SO, Breitman ML, Tsui LC. Structural and evolutionary relationships among five members of the human gamma-crystallin gene family. Mol Cell Biol 1985; 5:1408-14.
42. Santhiya ST, Shyam Manohar M, Rawlley D, Vijayalakshmi P, Namperumalsamy P, Gopinath PM, Loster J, Graw J. Novel mutations in the gamma-crystallin genes cause autosomal dominant congenital cataracts. J Med Genet 2002; 39:352-8.
43. Nandrot E, Slingsby C, Basak A, Cherif-Chefchaouni M, Benazzouz B, Hajaji Y, Boutayeb S, Gribouval O, Arbogast L, Berraho A, Abitbol M, Hilal L. Gamma-D crystallin gene (CRYGD) mutation causes autosomal dominant congenital cerulean cataracts. J Med Genet 2003; 40:262-7.
44. Mackay DS, Andley UP, Shiels A. A missense mutation in the gammaD crystallin gene (CRYGD) associated with autosomal dominant "coral-like" cataract linked to chromosome 2q. Mol Vis 2004; 10:155-62.

The print version of this article was created on 29 Mar 2004. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.