Molecular Vision **2008; 14:1272-1276** <http://www.molvis.org/molvis/v14/a150>
Received 16 April 2008 | Accepted 27 June 2008 | Published 9 July 2008

© 2008 Molecular Vision

A nonsense mutation in *CRYGC* associated with autosomal dominant congenital nuclear cataract in a Chinese family

Ke Yao, Chongfei Jin, Ning Zhu, Wei Wang, Renyi Wu, Jin Jiang, Xingchao Shentu

Eye Center of the 2nd Affiliated Hospital, Medical College of Zhejiang University, Hangzhou, China

Purpose: To identify the genetic defect associated with autosomal dominant congenital nuclear cataract in a Chinese family.

Methods: Family history and phenotypic data were recorded, and the phenotypes were documented by slit lamp photography. The genomic DNA was extracted from peripheral blood leukocytes. All the exons and flanking intronic sequences of *CRYGC* and *CRYGD* were amplified by polymerase chain reaction (PCR) and screened for mutation by direct DNA sequencing. Structural models of the wild type and mutant γC-crystallin were generated and analyzed by SWISS-MODEL.

Results: Sequencing of the coding regions of *CRYGC* and *CRYGD* showed the presence of a heterozygous C>A transversion at c.327 of the coding sequence in exon 3 of *CRYGC* (c.327C>A), which results in the substitution of a wild type cysteine to a nonsense codon (C109X). One and a half Greek key motifs at the COOH-terminus were found to be absent in the structural model of the mutant truncated γC-crystallin.

Conclusions: A novel nonsense mutation in *CRYGC* was detected in a Chinese family with consistent autosomal dominant congenital nuclear cataract, providing clear evidence of a relationship between the genotype and the corresponding cataract phenotype.

Hereditary congenital cataract (OMIM 604307) is an opacification of the eye lens that frequently results in visual impairment or even blindness during infancy or early childhood. Despite the great advances in the clinical management of cataracts as well as a better understanding of lens structure and function, congenital cataract remains a leading cause of blindness in children worldwide [1,2]. Irreversible visual loss can result if prompt treatment is not performed on these patients. Congenital cataracts are considered to be both phenotypically and genetically heterogeneous [3-5]. The water-soluble lens crystallins account for nearly 90% of the total lens proteins and play essential roles in maintaining the lens transparency [6]. Therefore, crystallins are good candidate genes for congenital cataract.

Crystallins are subdivided into α-, β-, and γ-crystallins with the γ-crystallin gene cluster subdivided into six genes, *CRYGA*-*CRYGF*. Only *CRYGC* (OMIM 123680) and *CRYGD* (OMIM 123690) are known to encode abundant lens γ-crystallins in humans [7,8]. The γ-crystallins have two domains with each domain composed of two exceptionally stable protein structures called “Greek key” motifs [9]. The γ-crystallins are monomeric with a molecular mass of 21 kDa and comprise about 40% of the total proteins in the mouse lens and 25% in the human lens [6,10]. As reported, mutations in *CRYGC* and *CRYGD* have been identified to cause isolated autosomal dominant congenital cataracts [11,12] as a result of altered stability, association, and/or solubility of γ-crystallins [13-16]. Indeed, in our previous study, we reported heterozygous mutations in *CRYGD* in a four-generation Chinese family with distinct fasciculiform cataract [17].

In the present study, we investigated a large Chinese family with autosomal dominant congenital nuclear cataract and detected a novel chain-termination mutation in *CRYGC* that cosegregated with the disease in the family.

METHODS

Patients and clinical data: A family of three generations was ascertained through the Eye Center of the 2nd Affiliated Hospital (Medical College of Zhejiang University, Hangzhou, China). Appropriate informed consent from each participant was obtained in accordance with the Zhejiang Institutional Review Board, and the study protocol adhered to the guidelines of the Declaration of Helsinki. Thirteen individuals (seven affected and six unaffected) from the family were enrolled in the study (Figure 1). Affected status was determined by a history of cataract extraction or ophthalmologic examination on presentation including visual function, slit lamp examination, and fundus examination with the dilated pupil. The phenotype was documented by slit lamp photography. Fifty subjects without diagnostic features of congenital cataract were recruited from the Chinese Han population in our medical examination center to serve as normal controls.

Correspondence to: Ke Yao, Ph.D., MD, Eye Center of the 2nd Affiliated Hospital, Medical College of Zhejiang University, Hangzhou, China, 310009; Phone: +86-571-87783897; Fax: +86-571-87783908; email: xlren@zju.edu.cn

TABLE 1. POLYMERASE CHAIN REACTION PRIMERS AND PRODUCT SIZES.

Name	Primer sequence (5′-3′)	Product size (bp)
GC1,2F	5′ TGCATAAAATCCCCTTACCGCTGA 3′	522
GC1,2R	5′ ACTCTGGCGGCATGATGGAAATC 3′	
GC3F	5′ AGACTCATTTGCTTTTTTCCATCCTTCTTTC 3′	407
GC3R	5′ GAAAGAATGACAGAAGTCAGCAATTGCC 3′	
GD1,2F	5′ CTTATGTGGGGAGCAAACT 3′	619
GD1,2R	5′ CAGCAGCCCTCCTGCTAT 3′	
GD3F	5′ TGCTTTTCTTCTCTTTTTATTTCTGGGTCC 3′	400
GD3R	5′ AGTAAAGAAAGACACAAGCAAATCAGTGCC 3′	

Genomic DNA preparation and molecular analysis: Blood specimens (5 ml) from all the patients and available family members were collected in EDTA. Genomic DNA was isolated as previously described [18]. Since the number of mutations leading to dominant cataracts was fairly high in the human *CRYG* gene cluster, *CRYGC* and *CRYGD* were taken as a priority to be screened as the candidate genes. The exons and flanking regions of *CRYGC* and *CRYGD* in patients II:6 and III:4 were amplified and sequenced using the primers listed in (Table 1). The cycling conditions for PCR were 38 cycles of 95 °C for 25 s, 55 °C for 25 s and 72 °C for 35 s, preceded by 5 min at 95 °C and followed by a final elongation step at 72 °C for 10 min. Any interesting sequence variation of a mutation suspect was later confirmed in the rest of the patients and unaffected family members by bidirectional sequencing of the particular exon.

Comparative modeling of γC-crystallins: Three-dimensional structures of the wild type and the mutant γC-crystallin were modeled on the basis of the crystal structure of the mouse γC-crystallin chain A [19]. The homology models were generated by SWISS-MODEL and analyzed in the Swiss-PdbViewer, version 3.7 (GlaxoSmithKline R&D, UK) [20-22].

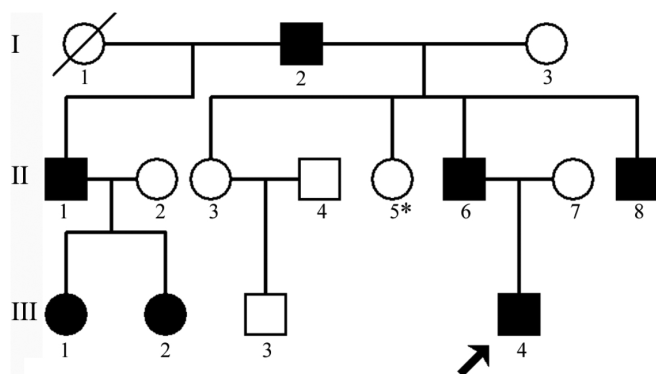

Figure 1. Pedigree of the autosomal dominant congenital cataract. The proband is marked with an arrow. Squares and circles indicate males and females, respectively. Black and white symbols denote affected and unaffected individuals, respectively. A slash through the symbol signifies that the family member is deceased. Thirteen individuals (seven affected and six unaffected) from the family were enrolled and underwent ophthalmologic examinations and genotyping in the study (II:5, marked by an asterisk, did not participate in the study).

RESULTS

Clinical evaluation: We identified isolated autosomal dominant congenital nuclear cataract in a three-generation Chinese family. Opacification of the lens was bilateral and consistent in all of the affected individuals. All embryonal, fetal, and infantile nuclei of the lens were opacified while the cortex remained transparent (Figure 2). Visual acuity ranged from light perception to 0.15 in the unoperated eyes and from 0.20 to 0.02 in the eyes that had undergone iridectomy during childhood. Obvious nystagmus was observed in all the patients except the 10-month-old proband who received phacoemulsification surgery in both eyes on presentation. There was no history of other ocular or related systemic abnormalities in the family aside from age-related changes.

Mutation analysis: Direct sequencing was performed to cover exons and flanking intron-exon boundary sequences. A heterozygous C>A transversion was identified at c.327 in exon 3 of *CRYGC* in all the affected members but not in any of the unaffected family members (Figure 3). This mutation resulted in the substitution of a wild type cysteine to a nonsense codon (C109X). The variant was completely absent in 100 chromosomes of 50 unrelated controls.

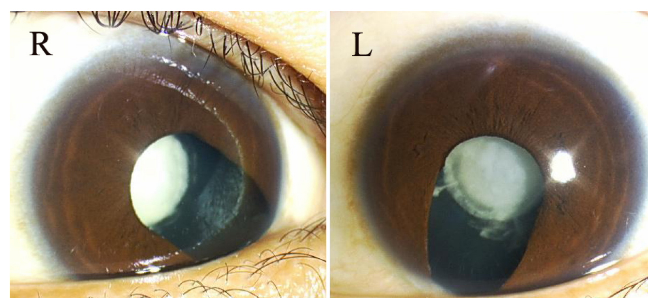

Figure 2. Slit lamp photographs of affected individual II:6. Lens opacities were located in the embryonal, fetal, and infantile nuclei of the lens while the cortex remained transparent. The patient underwent iridectomy on both eyes in his early childhood.

Comparison of wild type and mutant γC-crystallin structures: The C>A transversion at position c.327 in exon 3 led to a premature stop codon at codon 109. A truncated protein with 108 amino acids was putatively generated, 66 amino acids less than the wild type γC-crystallin, which possesses 174 amino acids (Figure 4). When modeled by SWISS-MODEL, one and a half Greek key motifs at the COOH-terminus were found to be absent in the three-dimensional structural model of the mutant γC-crystallin (Figure 5).

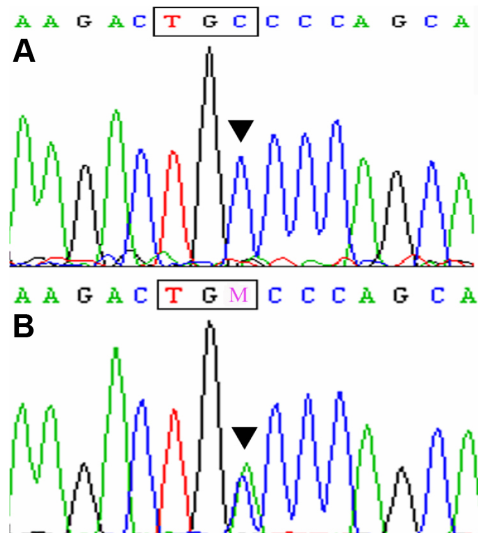

Figure 3. Forward sequence analysis of *CRYGC*. **A**: The sequence of an unaffected member (individual II:7) is shown. **B**: The sequence of an affected member (individual II:6) is shown. A heterozygous mutation was detected in the exon 3 of *CRYGC* (c.327C>A).

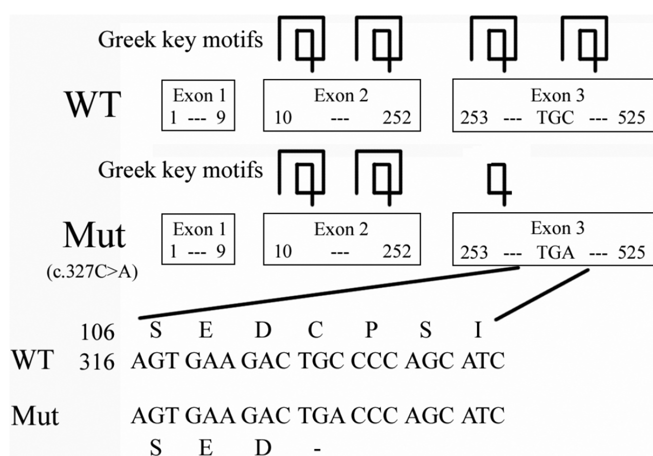

Figure 4. Influence of the mutation (c.327C>A) on γC-crystallin translation. The C>A substitution at c.327 in exon 3 leads to a premature stop codon at codon 109. A truncated protein (108 amino acids) is putatively generated in addition to a wild type γC-crystallin (174 amino acids).

DISCUSSION

In the present study, we detected a novel mutation (c.327C>A) in exon 3 of *CRYGC* in a Chinese family with autosomal dominant congenital nuclear cataract. The cataract phenotype was consistent among all the affected family members, providing a clear relationship between the genotype and the corresponding cataract phenotype. The opacification in the nuclei but not in the cortex could be explained by the fact that monomeric γC-crystallin, the major type of γ-crystallin expressed in the young human lens, is synthesized in the early life span and localized only in the central regions of the mature/aging eye lens [23,24].

To our knowledge, four mutations in *CRYGC* have been reported in the literature (listed in Table 2) [11,25-27]. The mutation detected in our present study, c.327C>A, creates a premature stop codon (C109X) and results in an in-frame stop codon at nucleotide 75 of exon 3 that may cause a truncation of 66 amino acids from the COOH-terminus of γC-crystallin. The secondary structure predicted by the Protein Prediction program (PHD) [28] shows that there are 16 β-strands (β1-β16) in γC-crystallin. The Cys109 residue located between the β10-strand and β11-strand is replaced by a nonsense codon, resulting in the loss of six β-strands after the β10-strand (Figure 4). Consequently the highly symmetric structure of γC-crystallin is lost (Figure 6).

Thus far, wild type human γC-crystallin has not been crystallized. Therefore, homology models for wild type and mutant human γC-crystallin are usually built based on the X-ray determined coordinates of mouse γC-crystallin chain A. The C109X mutation interferes with the formation of two

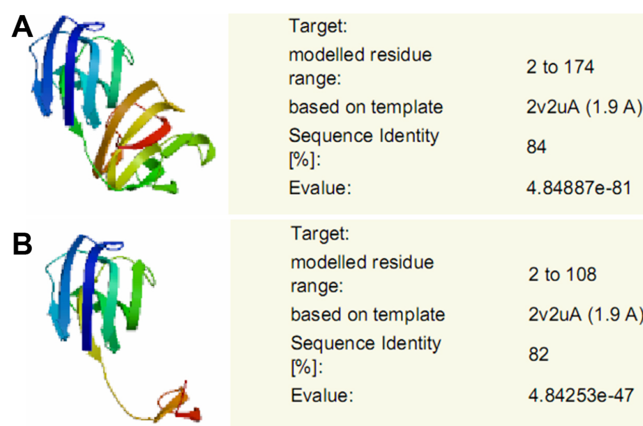

Figure 5. Structural modeling of the wild type and mutant γC-crystallins. The structure modeling is based on the X-ray determined coordinates of mouse γC-crystallin chain A using SWISS-MODEL. **A**: A structural model of the wild type γC-crystallin with 84% sequence identity is demonstrated. **B**: A structural alteration of the mutant γC-crystallin with 82% sequence identity is shown. Highly symmetric structure of γC-crystallin is disrupted when 66 amino acids are truncated from the COOH-terminus of γC-crystallin as result of c.327C>A mutation.

 © 2008 Molecular Vision

TABLE 2. HUMAN *CRYGC* MUTATIONS ASSOCIATED WITH CONGENITAL CATARACT.

Cataract Phenotypes	Mutations	Exons	Effects	Protein domains	References
Coppock-like	c.13A>C	1	p.Thr5Pro	GKM 1	[11]
Zonular pulverulent	c.123insGCGGC	2	p.Gly41delinsGlyfsX62	C-td 3 GKM loss	[25]
Lamellar/nuclear	c.502C>T	3	p.Arg168Trp	GKM 4	[26,27]
Nuclear	c.327C>A	3	p.Cys109X	C-td 1.5 GKM loss	this study

GKM=Greek key motif; C-td=COOH terminal domain.

COOH-terminal Greek key motifs. Although the function of the Greek key motifs has not been elaborated in detail, computer-based analysis suggests that it may be responsible for particular protein–protein interactions in the lens, and it is postulated to be critical in the maintenance of lens transparency [29].

It is reported that self-aggregation or quaternary structural alteration of γ-crystallin is responsible for the phenotypic association with lens opacification as well as cataractogenesis [30,31]. The truncated γC-crystallin may change the folding properties of γC-crystallin as it has been shown in a previous investigation that the COOH-terminal domain folds before and nucleates the folding of the NH_2-terminal domain in human γD-crystallin refolding [32]. The relatively loose or partially unfolded structure of mutant γC-crystallin may be susceptible to aggregation and insolubilization, which leads to cataract formation [13]. Another possible consequence of the C109X mutation may be related to the disturbances of the interactions between γC-crystallin and other crystallins [16,33]. The truncated γC-crystallin in the present study may cause a decrease or even complete loss of the ability to interact with other crystallins and may result in congenital cataract.

In conclusion, the novel nonsense mutation (c.327C>A) in *CRYGC* in this Chinese family is associated with isolated autosomal dominant congenital nuclear cataract, giving evidence of a clear relationship between the genotype and the corresponding cataract phenotype. The possible influence of the mutation on the structure as well as the function of γC-crystallin will require further investigation.

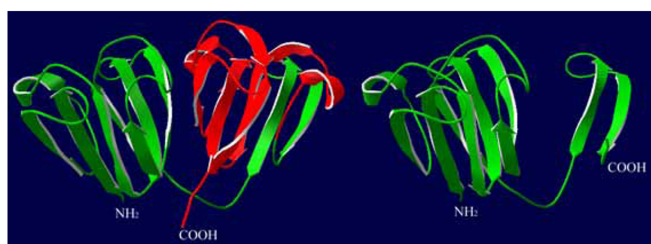

Figure 6. Comparative modeling of the full length and truncated γC-crystallins. The structural modeling was analyzed in Swiss-PdbViewer (version 3.7). When comparing the full length (left) and truncated γC-crystallins (right), the six COOH-terminal β-strands are truncated in the mutated γC-crystallin (the strands are shown in red).

ACKNOWLEDGMENTS

We are grateful to the members of the family for their participation in the study. This work was supported by Key Projects in the National Science & Technology Pillar Program in the Eleventh Five-year Plan Period (2006BAI02B04) and National Natural Science Foundation of China (30700925).

REFERENCES

1. Haddad MA, Sei M, Sampaio MW, Kara-Jose N. Causes of visual impairment in children: a study of 3,210 cases. J Pediatr Ophthalmol Strabismus 2007; 44:232-40. [PMID: 17694828]
2. Thylefors B, Negrel AD, Pararajasegaram R, Dadzie KY. Global data on blindness. Bull World Health Organ 1995; 73:115-21. [PMID: 7704921]
3. Reddy MA, Francis PJ, Berry V, Bhattacharya SS, Moore AT. Molecular genetic basis of inherited cataract and associated phenotypes. Surv Ophthalmol 2004; 49:300-15. [PMID: 15110667]
4. Hejtmancik JF. The genetics of cataract: our vision becomes clearer. Am J Hum Genet 1998; 62:520-5. [PMID: 9497271]
5. Ionides A, Francis P, Berry V, Mackay D, Bhattacharya S, Shiels A, Moore A. Clinical and genetic heterogeneity in autosomal dominant cataract. Br J Ophthalmol 1999; 83:802-8. [PMID: 10381667]
6. Wistow GJ, Piatigorsky J. Lens crystallins: the evolution and expression of proteins for a highly specialized tissue. Annu Rev Biochem 1988; 57:479-504. [PMID: 3052280]
7. Russell P, Meakin SO, Hohman TC, Tsui LC, Breitman ML. Relationship between proteins encoded by three human gamma-crystallin genes and distinct polypeptides in the eye lens. Mol Cell Biol 1987; 7:3320-3. [PMID: 3313014]
8. Brakenhoff RH, Aarts HJ, Reek FH, Lubsen NH, Schoenmakers JG. Human gamma-crystallin genes. A gene family on its way to extinction. J Mol Biol 1990; 216:519-32. [PMID: 2258929]
9. Blundell T, Lindley P, Miller L, Moss D, Slingsby C, Tickle I, Turnell B, Wistow G. The molecular structure and stability of the eye lens: x-ray analysis of gamma-crystallin II. Nature 1981; 289:771-7. [PMID: 7464942]
10. Graw J. The crystallins: genes, proteins and diseases. Biol Chem 1997; 378:1331-48. [PMID: 9426193]
11. 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. [PMID: 10521291]
12. 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 USA 1999; 96:1008-12. [PMID: 9927684]
13. Talla V, Narayanan C, Srinivasan N, Balasubramanian D. Mutation causing self-aggregation in human gammaC-crystallin leading to congenital cataract. Invest Ophthalmol Vis Sci 2006; 47:5212-7. [PMID: 17122105]
14. Fu L, Liang JJ. Alteration of protein-protein interactions of congenital cataract crystallin mutants. Invest Ophthalmol Vis Sci 2003; 44:1155-9. [PMID: 12601044]
15. Sandilands A, Hutcheson AM, Long HA, Prescott AR, Vrensen G, Loster J, Klopp N, Lutz RB, Graw J, Masaki S, Dobson CM, MacPhee CE, Quinlan RA. Altered aggregation properties of mutant gamma-crystallins cause inherited cataract. EMBO J 2002; 21:6005-14. [PMID: 12426373]
16. Liang JJ. Interactions and chaperone function of alphaA-crystallin with T5P gammaC-crystallin mutant. Protein Sci 2004; 13:2476-82. [PMID: 15322286]
17. Shentu X, Yao K, Xu W, Zheng S, Hu S, Gong X. Special fasciculiform cataract caused by a mutation in the gammaD-crystallin gene. Mol Vis 2004; 10:233-9. [PMID: 15064679]
18. Jin C, Yao K, Jiang J, Tang X, Shentu X, Wu R. Novel FBN1 mutations associated with predominant ectopia lentis and marfanoid habitus in Chinese patients. Mol Vis 2007; 13:1280-4. [PMID: 17679947]
19. Purkiss AG, Bateman OA, Wyatt K, Wilmarth PA, David LL, Wistow GJ, Slingsby C. Biophysical properties of gammaC-crystallin in human and mouse eye lens: the role of molecular dipoles. J Mol Biol 2007; 372:205-22. [PMID: 17659303]
20. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis 1997; 18:2714-23. [PMID: 9504803]
21. Schwede T, Kopp J, Guex N, Peitsch MC. SWISS-MODEL: An automated protein homology-modeling server. Nucleic Acids Res 2003; 31:3381-5. [PMID: 12824332]
22. Arnold K, Bordoli L, Kopp J, Schwede T. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. Bioinformatics 2006; 22:195-201. [PMID: 16301204]
23. Robinson NE, Lampi KJ, Speir JP, Kruppa G, Easterling M, Robinson AB. Quantitative measurement of young human eye lens crystallins by direct injection Fourier transform ion cyclotron resonance mass spectrometry. Mol Vis 2006; 12:704-11. [PMID: 16807530]
24. Lampi KJ, Ma Z, Shih M, Shearer TR, Smith JB, Smith DL, David LL. Sequence analysis of betaA3, betaB3, and betaA4 crystallins completes the identification of the major proteins in young human lens. J Biol Chem 1997; 272:2268-75. [PMID: 8999933]
25. Ren Z, Li A, Shastry BS, Padma T, Ayyagari R, Scott MH, Parks MM, Kaiser-Kupfer MI, Hejtmancik JF. A 5-base insertion in the gammaC-crystallin gene is associated with autosomal dominant variable zonular pulverulent cataract. Hum Genet 2000; 106:531-7. [PMID: 10914683]
26. 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. [PMID: 12011157]
27. Gonzalez-Huerta LM, Messina-Baas OM, Cuevas-Covarrubias SA. A family with autosomal dominant primary congenital cataract associated with a CRYGC mutation: evidence of clinical heterogeneity. Mol Vis 2007; 13:1333-8. [PMID: 17679936]
28. Rost B. PHD: predicting one-dimensional protein structure by profile-based neural networks. Methods Enzymol 1996; 266:525-39. [PMID: 8743704]
29. Crabbe MJ, Goode D. Protein folds and functional similarity; the Greek key/immunoglobulin fold. Comput Chem 1995; 19:343-9. [PMID: 8528592]
30. Pande A, Pande J, Asherie N, Lomakin A, Ogun O, King JA, Lubsen NH, Walton D, Benedek GB. Molecular basis of a progressive juvenile-onset hereditary cataract. Proc Natl Acad Sci USA 2000; 97:1993-8. [PMID: 10688888]
31. Pande A, Pande J, Asherie N, Lomakin A, Ogun O, King J, Benedek GB. Crystal cataracts: human genetic cataract caused by protein crystallization. Proc Natl Acad Sci USA 2001; 98:6116-20. [PMID: 11371638]
32. Flaugh SL, Kosinski-Collins MS, King J. Interdomain side-chain interactions in human gammaD crystallin influencing folding and stability. Protein Sci 2005; 14:2030-43. [PMID: 16046626]
33. Fu L, Liang JJ. Detection of protein-protein interactions among lens crystallins in a mammalian two-hybrid system assay. J Biol Chem 2002; 277:4255-60. [PMID: 11700327]

The print version of this article was created on 8 July 2008. 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.