

A novel mutation in *CRYBB2* responsible for inherited coronary cataract

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LABORATORY STUDY

Abstract

Purpose To study the molecular pathogenesis of a Chinese family with coronary form of cataract.

Methods One Chinese three-generation family with inherited coronary cataract phenotype was recruited. Five affected and seven unaffected family members attended our study. Genome-wide linkage analysis was applied to map the disease loci, and two candidate genes from a locus on chromosome 1 and a locus on chromosome 22 were sequenced for mutation identification. Software at the ExPasy proteomics server was utilized to predict the mutation effect on proteins.

Results Whole genome linkage analysis indicated some regions on chromosome 1, 10, and 22, with LOD score values greater than 1. Within these loci, the *GJA8* and *CRYBB2* genes, located in the two loci with the highest LOD score of 1.51 on chromosomes 1 and 22, respectively, were sequenced. A novel mutation c.92C>G in exon 2 of *CRYBB2* causing S31W was identified in all five patients. It was not found in 95 unrelated controls. This missense sequence alteration likely enhanced the local solubility. Around the mutation site, a lipocalin signature motif was predicted by ScanProsite.

Conclusions A novel disease-causing mutation S31W in *CRYBB2* was identified in a Chinese cataract family. It is the first reported mutation for coronary cataract. Functional characterization should be carried out to evaluate the biological effects of this mutant. *Eye* (2009) 23, 1213–1220; doi:10.1038/eye.2008.222; published online 11 July 2008

Keywords: *CRYBB2* mutation; inherited cataract; crystallin

Introduction

Congenital cataract refers to lens opacity presented at birth or shortly thereafter. It is

responsible for approximately one-tenth of childhood blindness worldwide, with prevalence estimated to be about 1–6 per 10 000 live births in most populations.^{1,2} Autosomal dominant congenital cataract (ADCC) is the major inherited mode of congenital cataract. So far, at least 18 genes with more than 60 mutations are known to be responsible for isolated ADCC that is not associated with systemic diseases.³ Functions and properties of these genes are widely diversified. They include nine crystallin genes: *CRYAA*, *CRYAB*, *CRYBB1*, *CRYBB2*, *CRYBA1*, *CRYBA4*, *CRYGC*, *CRYGD*, *CRYGS*; four membrane transport protein genes: *MIP*, *TMEM114*,⁴ *GJA8*, *GJA3*; one cytoskeletal protein gene: *BFSP2*; three transcription factor genes: *PITX3*, *MAF*, and *HSF4*; and one chromatin modifying protein gene: *CHMP4B*.⁵

Crystallins account for 90% of water-soluble proteins in the human lens. After synthesis, they exist throughout the lifespan of the host without turnover in mature lens fiber cells. The correct expression of native crystallins and maintenance of their native properties against metabolic and environmental insult are crucial for lens transparency. There are three crystallin families: α -, β -, and γ -crystallins. α -Crystallins (*CRYAA*) that account for more than half of the total crystallins in human lens.⁶ Apart from being structural proteins that help to maintain light refraction through the lens, they also function as chaperones during lens development.⁷ Human β - and γ -crystallins belong to a super family and they share highly homologous sequences.

β -crystallins can be further classified into the acidic and basic subtypes. Acidic β -crystallins include $\beta A1$ -, $\beta A2$ -, $\beta A3$ -, $\beta A4$ -crystallins (*CRYBA1*, *CRYBA2*, *CRYBA3*, and *CRYBA4*, respectively), and basic β -crystallins include $\beta B1$ -, $\beta B2$ -, $\beta B3$ -crystallins (*CRYBB1*, *CRYBB2*, and *CRYBB3*, respectively). *CRYBB2* is the most abundant β -crystallin.⁶ It is also the most soluble and least modified crystalline protein during

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aging.^{8,9} During the first year of life, the relative amounts of *CRYBB2* and *CRYAA* in lens change drastically. *CRYBB2* increases from 12 to 24% and α -crystallin decreases from 30 to 18%, with a total percentage maintained at 42%. It seems that *CRYBB2* is replacing *CRYAA*.⁸ *CRYBB2* likely plays a contributive role in lens development, but the functional significance is not yet known.

In this study, we investigated the gene lesion that is associated with inherited coronary cataract in a Chinese family.

Materials and methods

Sample collection and DNA extraction

A Chinese family with autosomal dominant inherited cataract was recruited at the First Affiliated Hospital, Medical College, Zhejiang University, People's Republic of China. This study followed the tenets of Declaration of Helsinki. Twelve family members attended our study and signed consent forms. All of them were given detailed ophthalmic examinations, including visual acuity, intraocular pressure, slit lamp assessment, and indirect ophthalmoscopy under dilated pupils. Peripheral venous blood samples were collected, and genomic DNA was extracted using QIAamp DNA kit (Qiagen, Valencia, CA, USA). A total of 95 unrelated control subjects who were aged more than 50 years with no family history of congenital cataracts were also recruited. They did not have eye diseases except mild myopia and senile cataracts. They were given complete ophthalmologic examinations as the study subjects of the inherited cataract family.

Genome-wide linkage analysis

Whole genome scanning with 541 microsatellite markers having an average spacing of around 8 cM was carried out at deCODE (Sturlugata, Reykjavik, Iceland). Briefly,

PCR samples were set up in multiplex with fluorescently labeled primers. When the PCR was completed, 12 markers were pooled for each individual sample. The amplified DNA fragments were analysed by capillary electrophoresis on an ABI 3730 DNA analyzer. The proprietary deCODE Allele Caller software was used for automated allele calling. The relationship between family members and genotyping error was examined by PedCheck, which identifies genotype incompatibilities in linkage analysis.¹⁰ Two-point linkage analysis was conducted by MLINK between disease and markers on chromosomes 1–22. MLINK subprogram was from FASTLINKAGE version 4.1P package. A gene frequency of 0.0001 and a penetrance of 100% were assumed for ADCC.

Gene sequencing

GJA8 and *CRYBB2* were selected from the regions showing likely association with cataract as indicated by the whole genome scanning. All exons and adjacent splicing regions were sequenced with specific primers (Table 1) and BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) on an ABI PRISM™ 3130xl genetic analyzer (Applied Biosystems). The sequences were compared with references obtained from NCBI GeneBank (<http://www.ncbi.nlm.nih.gov/Genebank/>; NCBI accession numbers NM005267.3 and NM000496.2).

Computational methods

The effects of S31W on proteins were assessed using softwares at the ExPASy proteomics server (<http://ca.expasy.org/>). Protein isoelectric point (pI) and molecular weight (MW) were calculated by the program of Compute pI/MW. The protein hydrophobicity was

Table 1 Primers and conditions for *GJA8* and *CRYBB2* gene sequencing

Gene	Exon	Primer sequence	MgCl ₂ (mM)	Temp (°C)
<i>GJA8</i>	2-1F	CGGGCCCTCTTTGTCTCTAGTCC	1	57
	2-1R	AGGCCCAGGTGGCCCAACTCC		
	2-2F	CAGCCGGTGGCCCTGCC	1	TD68-60
	2-2R	GTTGCCTGGAGTGCCTGCCC		
<i>CRYBB2</i>	1F	TGCTCTCTTTCTTTGAGTAGACCTC	1.5	57
	1R	CCCATTTTACAGAAGGGCAAC		
	2F	ACCCTTCAGCATCCTTTGG	1.5	57
	2R	GCAGACAGGAGCAAGGGTAG		
	3F	GCTTGGAGTGGAACTGACCTG	1	57
	3R	GGCAGAGAGAGAAAGTAGGATGATG		
	4F	GCCCCCTCACCCATACTC	1.5	57
	4R	CCCCAGAGTCTCAGTTCCTG		
	5F	CCTAGTGGCTTATGGATGCTC	1.5	57
	5R	TCTTCACTTGGAGGTCTGGAG		

predicted by ProtScale. The polypeptide sequences were scanned for occurrence of specific motifs by ScanProsite at Prosite. The Protein 3D structure was obtained from RCSB protein data bank (<http://www.rcsb.org/pdb/home/home.do>) (PDB ID: 1ytq).

Results

Clinical phenotypes

The inherited pattern of this family was consistent with autosomal dominant mode (Figure 1a). The age of diagnosis of disease ranged from 8 to 35 years. Five family members had bilateral inherited cataract and three of them had undergone lens extraction surgeries. The remaining two patients who did not undergo cataract surgery showed round opacity distributed in the deep cortex, which surrounded the nucleus like a crown (Figure 1b). The severity of cataract in both patients was

similar. Their uncorrected visual acuities were 0.12–0.8 (Table 2). The diagnosis of bilateral coronary cataract was given by a senior ophthalmologist. None of the five affected family members have other ocular or systemic abnormalities. Other family members did not have lens opacity except for I2, who had senile cataract.

Linkage analysis

Genomewide scanning mapped some promising regions on chromosome 1, 10, and 22 with a series of logarithm of the odds (LOD) scores bigger than 1.0. (Table 3). As the family size was not big, the highest LOD scores obtained on chromosomes 1 and 22 were only 1.51. Two possible loci were noted, one on chromosome 1 flanked by markers D1S484 and D1S452, the other on chromosome 22 flanked by D22S427 and D22S539. Two candidate genes reported to be responsible for ADCC, *GJA8* and

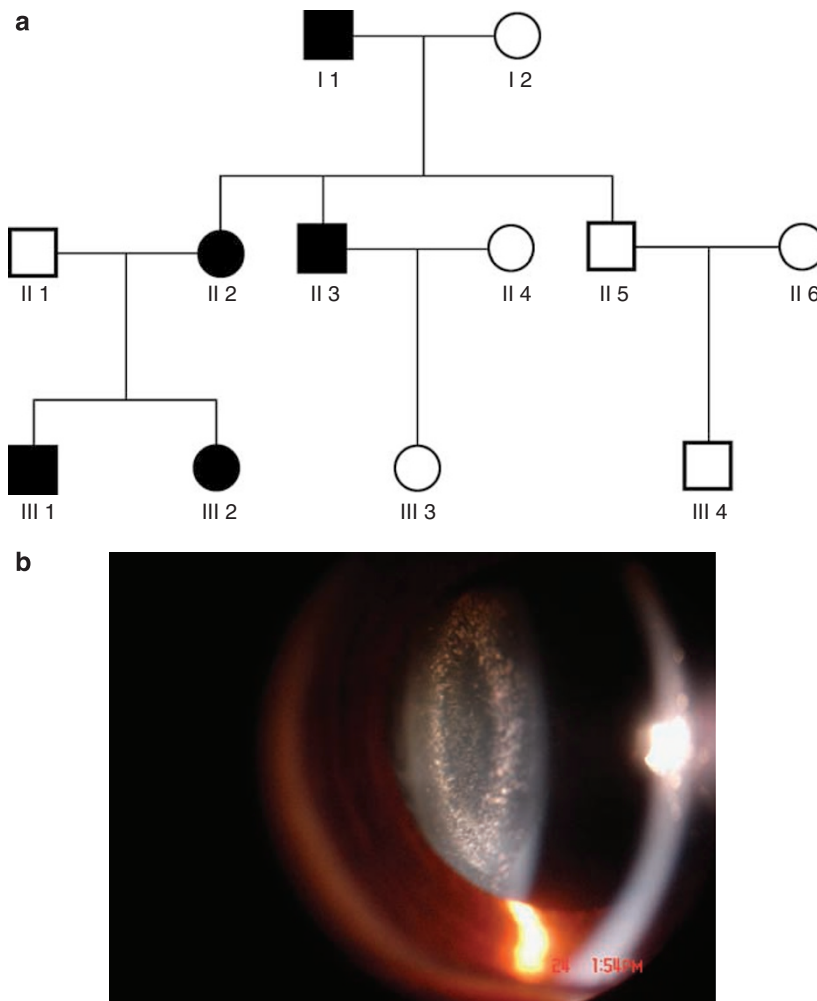


Figure 1 An inherited coronary cataract family. (a) The pedigree; (b) the lens picture taken from the patient of $\beta 1$, a 24-year-old man, the age of diagnosis was 14 years. He showed round opacity distributed in the deep cortex, which surrounded the nucleus like a crown. The severity of cataract in both two lenses was similar, and the visual acuity of both eyes was 0.8.

Table 2 Clinical information of the family members

ID	Age (years)	Sex	Type of cataract	Age of diagnosis (years)	BCVA before surgery	BCVA after surgery	Age of surgery (years), lens
I1	72	M	Bilateral, coronary cataract	8	R: 0.12, L: 0.15	R:0.3, L: 0.4	50, aphakia
I2	65	F	Bilateral, Senile cataract	55	R: 0.2, L: 0.4		No surgery, phakia
II1	47	M	Normal,		R: 1.2, L: 1.5		No surgery, phakia
II2	43	F	Bilateral, coronary cataract	35	R: 0.15, L: 0.12	R:0.6, L: 0.6	37, pseudophakia
II3	34	M	Bilateral, coronary cataract	26	R: 0.3, L: 0.2		No surgery, phakia
II4	30	F	Normal		R: 0.25, L: 0.25		No surgery, phakia
II5	40	M	Normal		R: 0.8, L: 1.0		No surgery, phakia
II6	39	F	Normal		R: 0.8, L: 0.6		No surgery, phakia
III1	24	M	Bilateral, coronary cataract	14	R: 0.8, L: 0.8		No surgery, phakia
III2	26	F	Bilateral, coronary cataract	16	R: 0.4, L: 0.4	R:0.8, L: 0.8	26, pseudophakia,
III3	3	F	Normal				No surgery, phakia
III4	12	M	Normal		R: 2.0, L: 2.0		No surgery, phakia

BCVA, best-corrected visual acuity; F, female; L, left; M, male; R, right.

Table 3 Markers with two-point LOD scores >1.0 obtained in this family by genomewide scan

Marker	Chromosome	Genetic location (cM)	Z _{max}	θ _{max}
D1S197	1p33	76.27	1.20	0.0
D1S2652	1p32.3	80.77	1.20	0.0
D1S498	1q21.3	155.89	Infinitive	0.0
D1S1653	1q23.1	164.09	0.9	0.0
D1S484	1q23.2	169.68	1.51	0.0
D1S2628	1q24.1	177.86	0.9	0.0
D1S452	1q24.3	188.85	1.51	0.0
D1S2818	1q25.3	198.30	0.60	0.0
D1S413	1q31.3	212.44	Infinitive	0.0
D10S189	10p14	19.00	1.20	0.0
D22S420	22q11.1	4.06	Infinitive	0.0
D22S427	22q11.21	8.32	1.51	0.0
D22S539	22q11.22	14.44	1.20	0.0
D22S1174	22q11.23	19.32	Infinitive	0.0

CRYBB2, were selected from these two regions for sequence analysis.

Mutation identification

Comparing with the CRYBB2 reference sequence (NCBI accession number NM000496.2), a new transversion from C to G was identified at the 92nd position (c.92C>G) in exon 2. It changed the 31st residue from serine to tryptophan (S31W) (Figure 2). The heterozygous substitution of S31W was observed in all patients, but not in 95 unrelated controls. Except for a 3-year-old unaffected girl (III3), other unaffected family members did not carry this mutation. No sequence variation was identified in the GJA8 gene.

Prediction of mutation effect on protein properties

The isoelectric point (pI) of both wild-type and mutant S31W was 6.5. The molecular weight of the mutant protein is about 23 479 Da, which was slightly higher than that of wild-type 23 380 Da. The hydrophobicity at the mutant site and the neighbouring regions obviously increased (Figure 3). Through ScanProsite, a new lipocalin signature motif was predicted in S31W mutant protein but not in wild type (Table 4). The serine at the position of 31 is located on the surface of N-terminal domain.

Discussion

We identified a novel disease causing mutation S31W in CRYBB2 in a Chinese cataract family. This missense variation was found in all the affected family members. Neither unrelated controls nor phenotypically normal family members carried this sequence change, except for a young girl who was 3 years old. Although this may suggest incomplete penetrance of disease, we noticed that the diagnosis age of patients in this family ranged from 8 to 35 years. It is still possible that the girl carrying the S31W mutation may later develop cataract. Thus, her lens transparency will be carefully followed in the future. Some ADCC genes, such as CRYAA, CRYGD, BFSP2, and MAF, have been reported to cause juvenile onset cataract. G98R in CRYAA was associated with juvenile onset cataract advancing from a peripheral ring-like opacity to a total cataract.¹¹ R14C in CRYGD was responsible for a progressive juvenile onset punctuate cataract.¹² E233del in BFSP2 was related to early childhood cataract and myopia.¹³ Another mutation in this gene R287W caused juvenile onset lamellar cataract.¹⁴ Sequence variations in

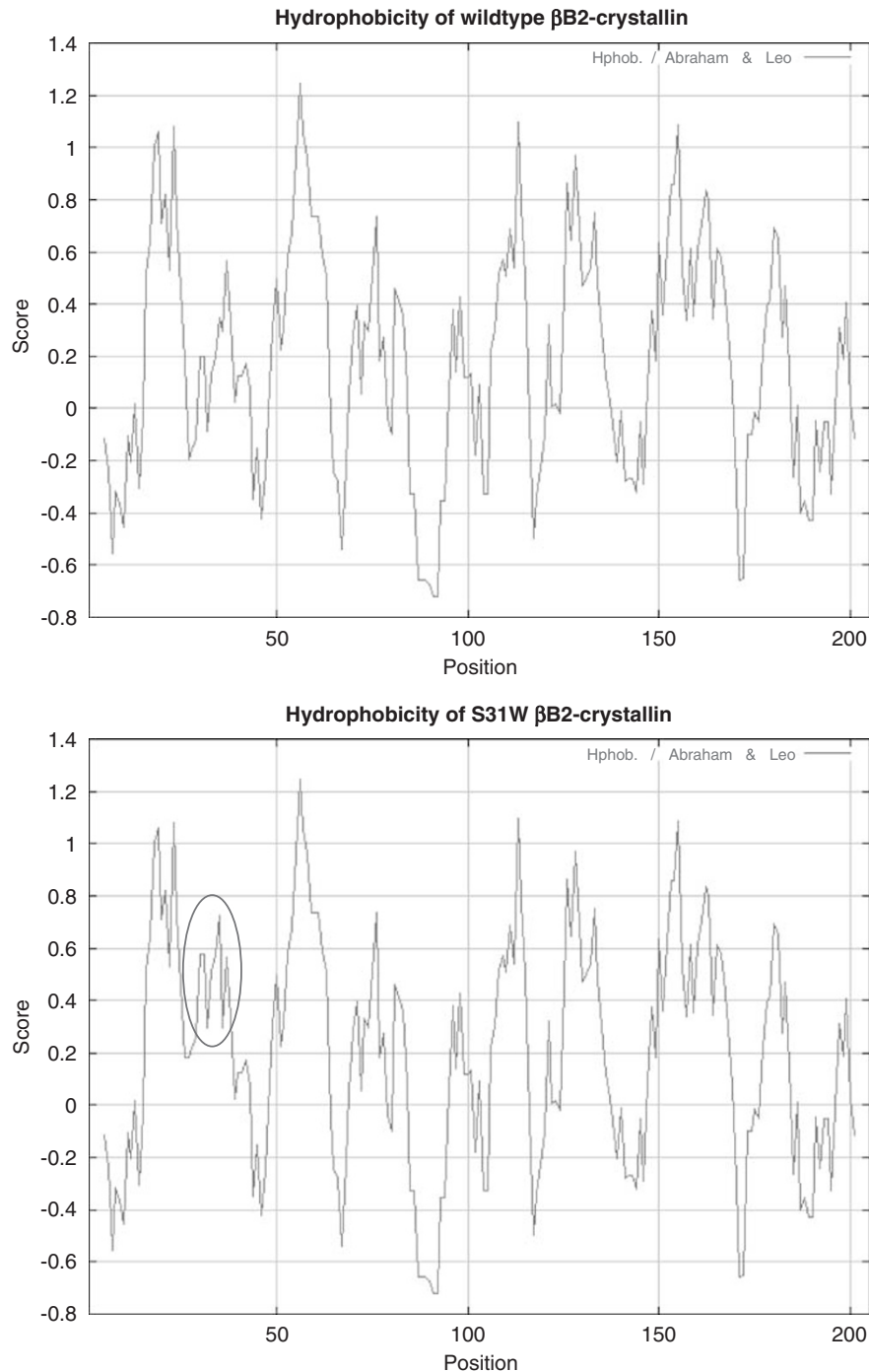


Figure 3 Hydrophobicity of wild-type and S31W mutant *CRYBB2*. The prediction was done by ProtScale program at Expasy server.

intermolecular action will lead to the change of solubility and stability of *CRYBB2*. W151C has been predicted to destroy the fourth Greek key motif and increase the protein hydrophobicity, which might affect the solubility of the mutant *CRYBB2*.¹⁷ Another mutation, Q155X, showed partial unfolded structure and decreased structure order, with reduced interactions with other proteins.²⁶ D128V mutant was supposed to cause the

random coil region between amino acids 126–139 of the mutant protein to become hydrophobic and electropositive.²³

S31W did not affect the protein pI but greatly increased the local hydrophobicity around the mutant site. Three-dimensional analysis based on the 1ytq model showed that serine at position 31 was on the surface of N-terminal domain. It is possible that the substitution at

Table 4 ScanProsite results for wild-type and S31W CRYBB2

	Wild type	S31W
LIPOCALIN	*	23–34 EQE..NFQGHWHEL
CK2_PHOSPHO_SITE	44–47: TgvE	44–47: TgvE
MYRISTYL	45–50: GVekAG	45–50: GVekAG
	149–154: GTwvGY	149–154: GTwvGY
PKC_PHOSPHO_SITE	87–89: SsR	87–89: SsR
	88–90: SrR	88–90: SrR
	96–98: SIR	96–98: SIR
	118–120: TgK	118–120: TgK
	143–145: SvR	143–145: SvR
	186–188: SvR	186–188: SvR
ASN_GLYCOSYLATION	116–119: NFTG	116–119: NFTG
AMIDATION	118–121: tGKK	118–121: tGKK

*, wild-type protein does not have this motif.

this location alters the hydrophobic interactions among CRYBB2 molecules. Moreover, it creates a lipocalin signature motif in the mutant protein. The lipocalins share characteristic conserved sequence motifs and bind small hydrophobic molecules, such as steroids, bilins, retinoids, and lipids.²⁷ They are also capable to bind cell-surface receptors and form complexes with soluble macromolecules.²⁸ Lens clarity is a result of regular packing of water soluble proteins. CRYBB2 as a structural protein plays a key role in maintaining lens transparency. We thus hypothesize that the novel mutation S31W enhanced hydrophobicity and created a calipolin signature motif that could alter the local binding ability, which would disrupt dimerization of the CRYBB2 protein or impair binding with other lens-soluble proteins. This alteration may destroy the microstructure of lens and increase light scattering, leading ultimately to lens opacity.

In summary, a novel cataract-causing mutation, c.92C>G in exon 2 of the CRYBB2 gene, which causes the 31st residue serine substituted by tryptophan (S31W), was identified in an autosomal dominant coronary cataract family. This variant likely causes cataract through obviously enhanced local hydrophobicity and formed a new lipocalin signature motif. This is the first reported mutation for coronary cataract phenotype.

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