

# Cerulean cataract mapped to 12q13 and associated with a novel initiation codon mutation in MIP

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**Purpose:** To identify the genetic defect in a large Chinese family with autosomal dominant cerulean cataract.

**Methods:** Genomic DNA and clinical data were collected from the family. Candidate gene sequencing and genome-wide linkage analysis were used to disclose the molecular basis responsible for cerulean cataract in the family.

**Results:** Initially, sequencing analysis of the three genes (beta-B2-crystallin [*CRYBB2*], gamma-D-crystallin [*CRYGD*], and V-MAF avian musculoaponeurotic fibrosarcoma oncogene homolog [*MAF*]) known to cause cerulean cataract failed to find any mutation. Then, genome-wide linkage analysis mapped the disease to chromosome 12q13-q22 between D12S85 and D12S351, with a maximum lod score of 4.10 at  $\theta=0$ . Sequence analysis of the major intrinsic protein of lens fiber gene (*MIP*), a gene known to cause other types of cataract in the linkage interval, detected a novel heterozygous initiation codon mutation, c.2T>C (p.Met1?). This mutation was present in all patients with cerulean cataract but was not present in any of the 13 unaffected family members nor in 96 control individuals.

**Conclusions:** Cerulean cataract was found in a large family and is caused by a novel initiation codon mutation in *MIP*. This study adds a new member in the existing list of genes causing cerulean cataract and expands the mutation spectrum and phenotypic association of *MIP* mutations.

About one third of infant blindness is due to congenital cataracts [1,2]. Congenital cataracts can result in significant vision loss by impairing visual development. Genetic factors played an important role in the development of congenital cataracts, with up to 25% of them hereditary [3-5]. Hereditary congenital cataracts can be inherited as an autosomal dominant, autosomal recessive, or X-linked trait, where the autosomal dominant trait is most commonly described. So far, mutations in at least 21 genes have been identified to be responsible for a subset of nonsyndromic congenital cataracts while a substantial number of causative genes remain to be determined [6,7].

Clinical phenotypes of hereditary congenital cataract are highly heterogeneous. Specific clinical signs may be more frequently related to one or a few causative genes but establishment of genotype-phenotype correlation is usually difficult in most cases. Cerulean cataract (OMIM 115660) is a specific type of cataract characterized by predominantly bluish opacifications in the superficial layers of the fetal nucleus as well as the adult nucleus of the lens. At least four loci for cerulean cataract have been identified, including

cerulean type congenital cataract-1 (CCA1; OMIM 115660, 17q24) [8], CCA2 (OMIM 601547, 22q11.2-q12.2) [9], CCA3 (OMIM 608983, 2q33-q35) [10], and CCA4 (OMIM 610202, 16q22-q23) [11]. Mutations in 3 genes have been identified to be responsible for cerulean cataract, i.e., the beta-B2-crystallin gene (*CRYBB2*, OMIM 123620) [12], the gamma-D-crystallin gene (*CRYGD*, OMIM 123690) [12], and the V-MAF avian musculoaponeurotic fibrosarcoma oncogene homolog gene (*MAF*, OMIM 177075) [11].

In this study, cerulean cataract was found in a five-generation Chinese family. An initial scan of the three genes known to cause cerulean cataract did not detect any mutation. A subsequent genome-wide linkage study mapped the cerulean cataract locus to chromosome 12q13-q22. Sequencing the candidate gene in the linkage interval identified a novel c.2T>C (p.Met1?) mutation in the major intrinsic protein of lens fiber gene (*MIP*, OMIM 154050).

## METHODS

**Family with cerulean cataract:** A five generation family with congenital cerulean cataract was identified from the Eye Hospital of Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China. The family was originated from Central China and moved to Shenzhen, China in recent years. Written informed consent was obtained from the participating individuals or their guardians before the

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TABLE 1. PRIMERS USED TO AMPLIFY GENOMIC FRAGMENTS OF CANDIDATE GENES.

Gene name	Primer ID	Primer sequence (5'-3')	Product length (bp)	Annealing temperature (°C)
<i>CRYBB2</i>	1F	TTGGGGCCAGAGGGGAGTG		
	1R	TGGGCTGGGGAGGGACTTTC	353	66
	2F	AGGTCCCACGGCTGCTTAT		
	2R	GGCTGCCAGACCCCAAACT	421	64
	3F	GTGGGTAAAGGCAGCATAGC		
	3R	GGCAGAGAGAGGGAGTAGGG	378	68
	4F	GGTGCACTGGGAAGAGAGTG		
	4R	GAAGCCAGAGGTCAGCAGAG	397	60
	5F	GAGGCTTCACCCTTCCTAGTG		
<i>CRYGD</i>	5R	GCAGACAAGTTGCAAGTCAC	389	69
	1-2F	GGGCCCTTTTGTGCGGTTCT		
	1-2R	GTGGGGAGCAAACCTCTATTGA	643	65
	3F	TGCTCGGTAATGAGGAGTTT		
<i>MAF</i>	3R	AAATCAGTGCCAGGAACACA	506	63
	1aF	GAGCGAGGGAGCACATTG		
	1aR	CCGGTTCCTTTTCACTTCA	352	60
	1bF	AACTGGCAATGAGCAACTCC		
	1bR	GTGGTGGTGGTGGTGGTAGT	548	60
	1cF	CCGCACTACCACCACCAC		
	1cR	CTGGTTCTTCTCCGACTCCA	432	60
	1dF	AGCTGGTGACCATGTCTGTG		
	1dR	AGAAGTAGCAAGCCACACC	407	53.5
	2F	AAATCCTGAGTAAGTGCCATTCA		
	2R	GTTGCATTCCGGGAAACTT	575	60
<i>MIP</i>	1F	GACTGTCCACCCAGACAAGG		
	1R	TCAGGGAGTCAGGGCAATAG	492	64~57
	2F	TGAAGGAGCACTGTTAGGAGATG		
	2R	AGAGGGATAGGGCAGAGTTGATT	500	64~57
	3F	CCAGACAGGGCATCAGT		
	3R	TGGTACAGCAGCCAACAC	373	64~57
	4F	AAGGTGTGGGATAAAGGAGT		
	4R	TTCTTCATCTAGGGGCTGGC	429	64~57
	SeqE1R	AAGGCACGGAGCAGGGACATC		

collection of clinical data and genomic samples. This study was approved by the Internal Review Board of the Zhongshan Ophthalmic Center and followed the tenets of the Declaration of Helsinki and the Guidance of Sample Collection of Human Genetic Diseases (863-Plan) by the Ministry of Public Health of China.

Genomic DNA was prepared from venous leukocytes [13].

**Mutational screening:** Bioinformation of *CRYBB2*, *CRYGD*, and *MAF* was obtained from the National Center for Biotechnology Information (NCBI). Polymerase chain reaction (PCR) was used to amplify the coding exons and adjacent intronic sequences of the 3 genes using the primers referred to the previous publication [14] with modification (Table 1). PCR amplifications were carried out in 20- $\mu$ l reactions containing 80 ng genomic DNA. PCR cycles consisted of a denaturizing step at 95 °C for 5 min, followed by 35 cycles of amplification (at 95 °C for 30 s, at 53.5~69 °C for 30~60 s for 35~40 cycles, and at 72 °C for 30 s), and a final extension at 72 °C for 5 min. The nucleotide sequences

of PCR products were determined with the ABI BigDye Terminator cycle sequencing kit v3.1 on an ABI 3100 genetic analyzer (ABI Applied Biosystems, Foster City, CA). Variations were identified by importing the sequencing results from patients and consensus sequences from the NCBI human genome database into the SeqManII program of the Lasergene package (DNASTar Inc., Madison, WI).

**Genotyping and linkage analysis:** Genotyping for all participating family members was performed using 5'-fluorescently labeled microsatellite markers as previous described [15]. Briefly, a genome-wide scan was carried out using panels 1 to 27 of the ABI PRISM linkage Mapping Set Version 2 (Applied Biosystems). PCR was conducted at 94 °C for 8 min, followed by 10 cycles of amplification at 94 °C 15 s, 55 °C 15 s, and 72 °C 30 s; then 20 cycles at 89 °C 15 s, 55 °C 15 s, 72 °C 30 s; finally at 72 °C for 10 min. After mixing with GENESCAN™ 400HD (ROX™) standard (Applied Biosystems) and deionized formamide, the amplicons were denatured at 95 °C for 5 min and then immediately placed on ice for 5 min. The amplicons were separated on an ABI 3100

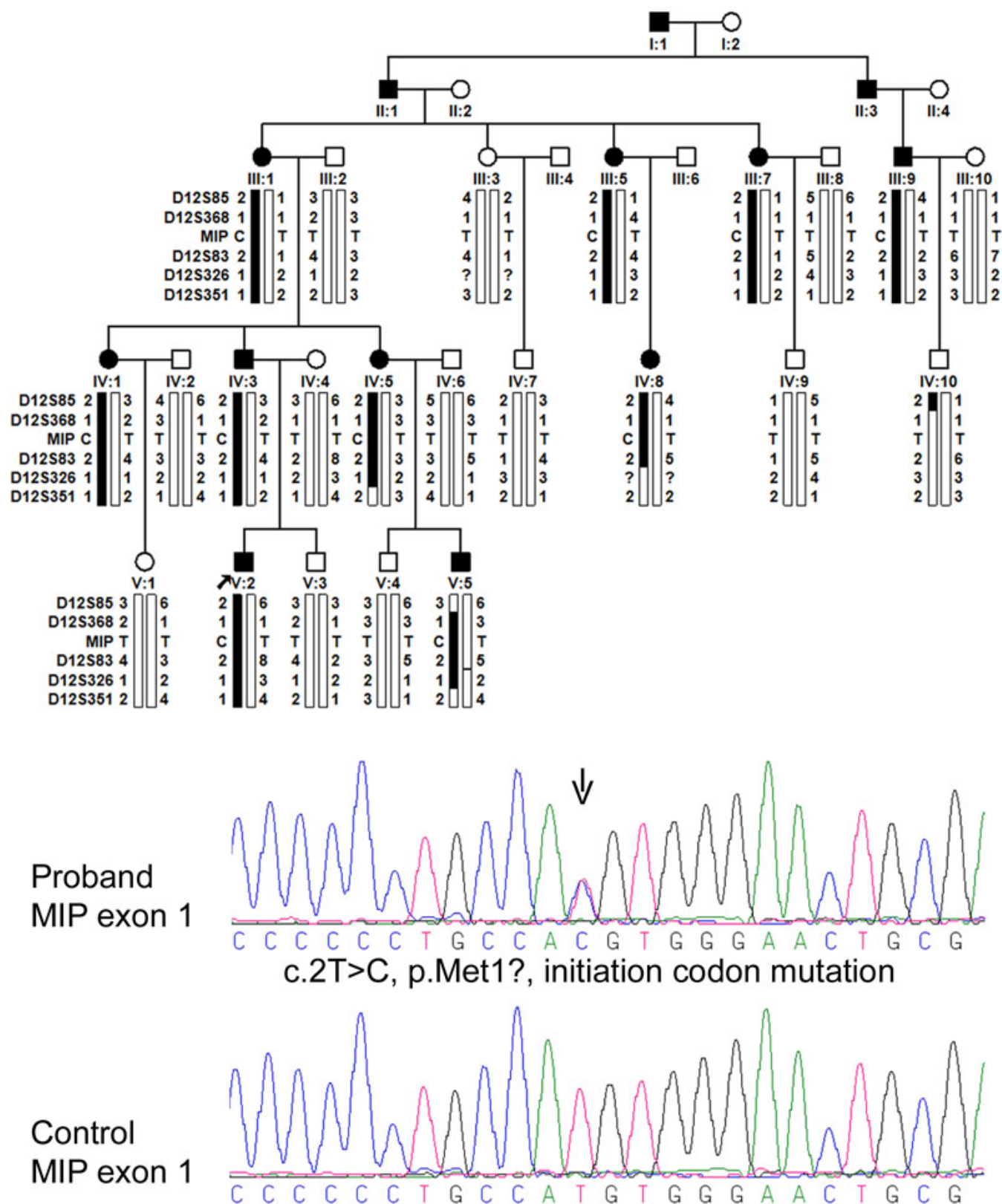


Figure 1. Pedigree, haplotypes on chromosome 12q and *MIP* mutation. Pedigree and haplotypes are shown on top. Filled squares (male) or circles (female) represent individuals affected with cerulean cataract. Bars filled with black indicate the chromosomal regions that are derived from the ancestral disease-associated haplotype. Sequence tracing of the *MIP* mutation is shown at bottom. Arrow indicates the site with double peaks, where a heterozygous T to C variant affects the second nucleotide of the ATG initiation codon for *MIP*.

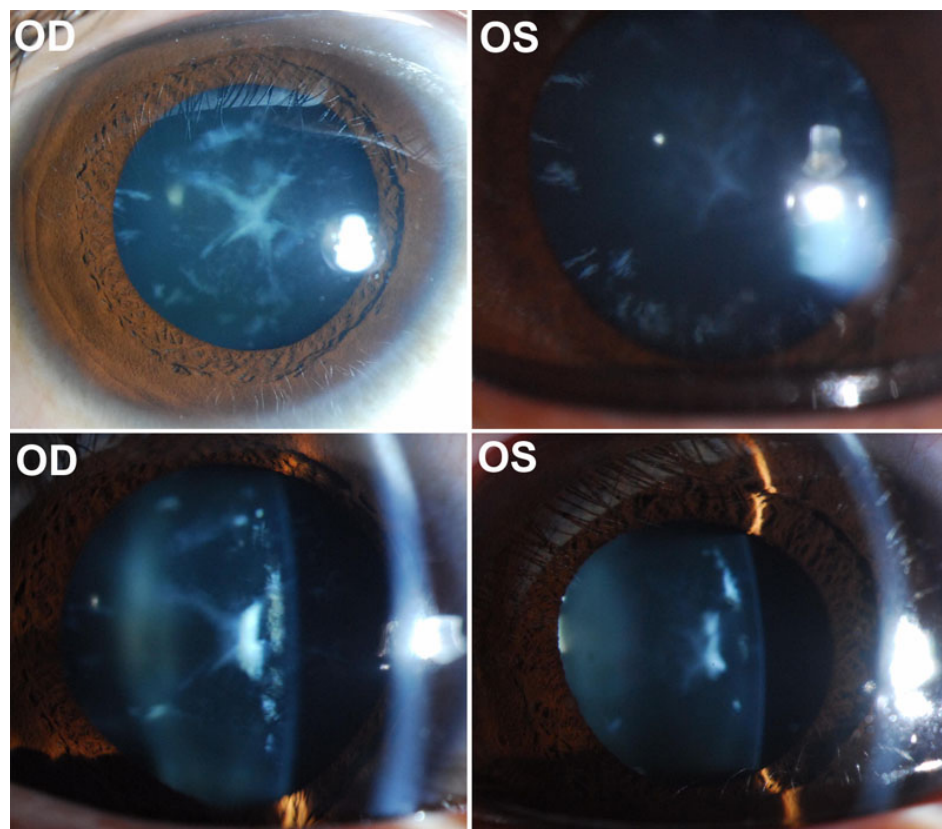


Figure 2. Lens photograph of the proband (V2). The photos were taken when the proband (V:2) was 19 years old. Cerulean cataract was observed in the right (OD) and left (OS) eyes, showing lamellar, Y-suture, and punctate lens opacities.

Genetic Analyzer (Applied Biosystems). Genotyping data were analyzed using the Gene Mapper version 3.5 software package (Applied Biosystems). Two-point linkage analysis was performed by using the [MLINK](#) program of the FASTLINK implementation of the LINKAGE program package [16,17]. The cerulean cataract in the family was analyzed as an autosomal dominant trait with full penetrance and with a disease-gene allele frequency of 0.0001. Haplotypes were generated using the Cyrillic 2.1 program (Cyrillic Software, Wallingford, Oxfordshire, UK) and confirmed by inspection. The criteria for establishing linkage have been previously described. Briefly, a lod score of 3 is accepted as significant evidence for linkage for autosomal diseases while a lod score of 2 is considered to be significant linkage for X-linked diseases [18,19].

**Mutation identification in MIP:** Primers used to amplify the 4 coding exons and their adjacent intronic region of MIP were the same as those in the previous report [20], except that a new primer was synthesized for additional reverse sequencing of exon 1 (Table 1). PCR amplifications were performed in 20- $\mu$ l reactions containing 80 ng genomic DNA. Touchdown PCR amplification consisted of a denaturing step at 95 °C for 5 min, followed by 35 cycles of amplification (at 95 °C for 30 s, at 64–57 °C for 30 s starting from 64 °C with decreasing by 0.5 °C every cycle for 14 cycles until remaining at 57 °C for 21 cycles, and at 72 °C for 40 s), and a final extension at

72 °C for 10 min. The nucleotide sequences of PCR products were determined with an ABI BigDye Terminator cycle sequencing kit v3.1 as described in the section for mutational screening. Any variant detected was initially confirmed by bidirectional sequencing and then evaluated in 192 chromosomes of 96 normal controls. Mutation description followed the recommendation of the Human Genomic Variation Society ([HGVS](#)).

## RESULTS

The disease in the family passed at least five generations. Twenty three individuals, including 10 affected and 13 unaffected, participated in this study (Figure 1). The cataract in all subjects were cerulean (Figure 2), but with different morphology (lamellar, punctuate, and/or Y-sutural) in different patients (Table 2). Although affected subjects complained of visual blur, their visual acuity is within the normal range or only mildly reduced. The fundus was normal in 17 eyes of the 10 affected subjects. Macular degeneration was observed in two eyes of one affected subject and traumatic retinal detachment was present in one eye of one affected subject. Nine affected subjects tested had normal color vision. Systemic examination did not find any significant abnormality.

Initially, three genes known to cause cerulean cataract including *CRYBB2*, *CRYGD*, and *MAF* were analyzed by



TABLE 2. CLINICAL INFORMATION OF THE AFFECTED FAMILY MEMBERS IN THE FAMILY.

ID #	Gender	Age	Visual acuity		Cataract phenotype*	Fundus OU (OD;OS)	Color vision
			OD	OS			
III:1	F	66	0.04	0.1	C, L, Y	MD	N/A
III:5	F	52	1.0	1.0	C, L, P	normal	normal
III:7	F	45	0.8	0.5	C, L, P	normal	normal
III:9	M	44	1.0	1.0	C, L, P, Y	normal	normal
IV:1	F	45	1.0	1.0	C, L, P	normal	normal
IV:3	M	42	HM	1.2	C, L, P, Y	TRD;normal	normal
IV:5	F	39	1.0	1.0	C, L, P, Y	normal	normal
IV:8	F	24	1.2	1.2	C, L, P	normal	normal
V:2	M	19	0.8	1.2	C, L, P, Y	normal	normal
V:5	M	8	1.0	1.0	C, P	normal	normal

\*Note: C=cerulean; L=lamellar; p=punctate; Y=Y suture. MD=macular degeneration; TRD=traumatic retinal detachment.

TABLE 3. TWO-POINT LOD SCORES OF FAMILY FOR MARKERS AROUND *MIP*.

Markers	Position		Lod score at $\theta=$						
	cM*	Mb#	0.00	0.01	0.05	0.10	0.20	0.30	0.40
D12S85	62.70	47.34	-inf	-0.53	0.69	1.04	1.07	0.79	0.39
D12S368	67.30	52.63	1.62	1.62	1.58	1.47	1.16	0.79	0.41
<i>MIP</i>		56.85	3.80	3.73	3.43	3.05	2.24	1.40	0.59
D12S83	76.50	60.89	4.10	4.03	3.73	3.34	2.52	1.63	0.73
D12S326	87.60	77.97	1.80	1.76	1.59	1.38	0.94	0.51	0.16
D12S351	97.10	91.91	-inf	-0.07	1.07	1.33	1.24	0.88	0.43

\*Genethon. #Homo genome (Build 37.2) Chr1 Primary\_Assembly.

Sanger dideoxy sequencing. After complete analysis of the coding and adjacent intronic regions of the three genes, no mutation was identified. Then, a genome-wide linkage analysis was performed. Genome wide linkage scan mapped the cerulean cataract locus to chromosome 12q13-q22 between D12S85 and D12S351, with a maximum lod score of 4.10 at  $\theta=0$  (Figure 1, Table 3). One gene known to cause other types of cataract is present in the linkage interval, i.e., *MIP*. Subsequently, sequencing the coding regions of *MIP* identified a novel heterozygous c.2T>C (p.Met1?) mutation in exon 1 (Figure 1). The heterozygous c.2T>C mutation was present in all subjects with cerulean cataract but neither in any of the 13 unaffected family members nor in 96 control individuals. The mutation itself could establish linkage with a maximum lod score of 3.8 at  $\theta=0$  (Table 3). The c.2T>C mutation affect the initiation codon, which may result in no production of protein or activation of a new translation initiation site.

### DISCUSSION

Previously, mutations in *CRYBB2*, *CRYGD*, and *MAF* have been identified to be responsible for three types of cerulean

cataract [9-12], i.e., CCA2, CCA3, and CCA4. The causative gene for CCA1 at 17q24 is still unknown [8]. Here in this study, we identified a large family with autosomal dominant cerulean cataract. Initial mutational screening excluded *CRYBB2*, *CRYGD*, and *MAF* as the causative genes. A genome wide linkage analysis mapped the disease to chromosome 12q13-q22. Sequence analysis of *MIP* in the linkage interval identified a novel heterozygous c.2T>C mutation that cosegregated with the cataract and was not present in controls. All lines of evidence suggest that the *MIP* mutation is the cause of cerulean cataract in this family. This might add a new member in the existing list of genes causing cerulean cataract when mutated.

Nine different mutations in *MIP* have been identified in 9 families with different types of autosomal dominant cataract, including seven missense mutations (c.97C>T [p.R33C] [21], c.319G>A [p.V107I] [22], c.401A>G [p.E134G] [23], c.413C>G [p.T138R] [23], c.530A>G [p.Y177C] [24], c.559C>T [p.R187C] [25], and c.698G>A [p.R233K] [26]), one splicing site mutation that activates a cryptic splicing acceptor in the 3'UTR region (c.607-1G>A

[p.V203fs] [20]), and one deletion resulted in frameshift (c. 638delG [p.G213VfsX46] [27]). These mutations are located in exon 1 (2 families), exon 2 (2 families), exon 3 (2 families), and exon 4 (3 families, including 1 families in 3' end of intron 3). Initiation codon mutation identified in this study represents a different type of mutation, which has been rarely reported in cataract. Of the great number of mutations identified in nearly 200 genes and loci [7], only two mutations involving the initiation codon have been reported in beta B1-crystallin (*CRYBB1*) and galactokinase 1 (*GALK1*), respectively [28, 29].

Previously, phenotypes of the cataract in the 9 families with *MIP* mutation included nuclear polymorphic and lamellar [23]; punctate and lamellar [30]; nuclear punctate, suture, and cortical [27]; total [21]; punctate and polymorphic [26]; snail-like [20]; Y-sutural, nuclear pulverulent, and nuclear [22]; Nuclear [25]; congenital nuclear [24]. Cerulean cataract as a major finding has not been described in the previous studies.

In summary, cerulean cataract was found in a large family and is caused by a novel initiation codon mutation in *MIP*. This study expands the mutation spectrum and phenotypic association of *MIP* mutations.

# ACKNOWLEDGMENTS

The authors thank all patients and family members for their participation. This study was supported by the National Natural Science Foundation of China (30800615 to X.X.), the National Science Fund for Distinguished Young Scholars (30725044 to Q.Z.) and the Fundamental Research Funds of State Key Lab of Ophthalmology, Sun Yat-sen University.

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