

# Identification of a missense mutation in MIP gene via mutation analysis of a Guangxi Zhuang ethnic pedigree with congenital nuclear cataracts

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**Abstract.** At present, congenital cataract is the world's leading cause of blindness among children. The aim of the present study was to determine and analyze the genetic disorder associated with a congenital nuclear cataract in a three-generation family of Guangxi Zhuang ethnicity. A total of 3 affected individuals and 5 unaffected family members underwent appropriate comprehensive medical examinations, mainly of the eyes. The white blood cells of the family members were collected and genomic DNA was extracted from 100 healthy individuals, as the control group. The sequences of candidate genes were determined by polymerase chain reaction amplification followed by direct sequencing. The functional consequences of the mutation were analysed with biology software. A missense mutation (c.97C>T) was found in exon 1 of major intrinsic protein of lens fiber (MIP) gene. Therefore, the arginine of the highly conserved codon 33 was changed to cysteine. This mutation was identified in the affected family members, but not identified in unaffected family members or the 100 normal controls. The mutation in the MIP gene is the genetic cause of the congenital cataract in the ethnic Guangxi Zhuang family.

## Introduction

Congenital cataract is an important cause of blindness in children globally (1). A total of 10.7-14.0% of the affected children are blind (1). This lens disease exhibits clinical and genetic heterogeneity; autosomal dominant inheritance is the most common. Currently, an increasing number of genes have been identified as associated with various forms of congenital cataracts. These genes include crystallin genes

[crystallin  $\alpha$  A (CRYAA) (2), crystallin  $\alpha$  B (CRYAB) (3), crystallin A1/A3 (CRYBA1/A3) (4), crystallin A4 (CRYBA4) (5), crystallin B1 (CRYBB1) (6), crystallin B2 (CRYBB2) (7), crystallin B2 (CRYBB3) (8), crystallin  $\gamma$  C (CRYGC) (9), crystallin  $\gamma$  D (CRYGD) (10) and crystallin  $\gamma$  S (CRYGS) (11)], transcription factors [heat shock transcription factor 4 (12), paired-like homeodomain transcription factor 3 (13) and MAF bZIP transcription factor (14)], skeleton protein genes [beaded filament structural proteins 1 (15) and 2 (16)], membrane transporter genes [major intrinsic protein of lens fiber (MIP) (17), gap junction protein  $\alpha$  8 (GJA8) (18), gap junction protein  $\alpha$  3 (GJA3) (19) and lens intrinsic membrane protein 2 (20)], glucosaminyl (N-acetyl) transferase 2 (21), charged multivesicular body protein 4B (22), and transmembrane protein 114 (23). Elucidating the structure and functional characteristics of these candidate genes and their protein products may aid in understanding the occurrence of cataracts, and the functional and structural implications of their mutations may provide important clues for understanding the disease etiology. In the present study, a heterozygous c.97C>T transition mutation of the MIP gene was identified in a family from the Chinese Guangxi Zhuang Autonomous Region with congenital nuclear cataract. The mutation completely cosegregated with the disease. This is the first cataract-associated mutation identified among patients of Guangxi Zhuang ethnicity.

## Materials and methods

**Clinical data and sample collection.** A three-generation Chinese Zhuang family (Fig. 1) with congenital nuclear cataract was recruited from the People's Hospital of Guangxi Zhuang Autonomous Region (Nanning, China). The study included eight family members, including three affected individuals (II:2, III:2 and IV:1) and five unaffected individuals (II:1, II:3, II:4, III:1 and III:3). All participants underwent physical and ophthalmic examinations. An image of the lens opacity of the proband was captured (Fig. 2). A total of 100 Guangxi Zhuang ethnicity subjects without congenital cataract were recruited as normal controls. All patients included in the present study provided written informed consent for participation and publication. A total of 5 ml of venous blood was collected from family

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members and controls using BD Vacutainer® Blood Collection tubes (BD Biosciences, San Jose, CA, USA) containing EDTA. Genomic DNA was extracted by QIAamp DNA Blood kits (Qiagen Sciences, Inc., Gaithersburg, MD, USA). The present study was approved by the Institutional Review Committee of the People's Hospital of Guangxi Zhuang Autonomous Region and followed the provisions of the Declaration of Helsinki.

**Mutation detection.** Known protein coding regions of candidate genes associated with autosomal dominant congenital cataract, including CRYAA, CRYAB, CRYBA1, CRYBB2, CRYGC, CRYGD, CRYGS, GJA3, GJA8 and MIP, were amplified using polymerase chain reaction (PCR). The primer sequences were listed in Table I. The PCR mixtures was as follows: 12.5  $\mu$ l 2 xTaq PCR Mastermix (Tiangen Biotech Co., Ltd., Beijing, China), 1  $\mu$ l forward primer, 1  $\mu$ l reverse primer, 1  $\mu$ l genomic DNA and ddH<sub>2</sub>O up to a volume of 25  $\mu$ l. PCR cycling conditions consisted of the following: An initial denaturation at 94°C for 7 min, 40 cycles of denaturation at 94°C for 30 sec, annealing at 62°C for 30 sec and extension at 72°C for 45 sec, then a final extension at 72°C for 8 min and a last hold at 4°C.

**Bioinformatics analysis.** PCR products were sequenced by ABI 3730 automated sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) from both directions. The results of sequencing were analyzed with Chromas (2.3 edition; Technelysium Pty Ltd, South Brisbane, Australia) and compared with reference sequences from the NCBI database (<https://www.ncbi.nlm.nih.gov/>). Bioinformatics analysis of wild-type and mutant MIP protein sequences was conducted using a polymorphism phenotyping v2 (PolyPhen-2) software (version 2.0; <http://genetics.bwh.harvard.edu/pph2/>), and the effects of mutations on biochemical properties were predicted. PolyPhen-2 was based on position-specific independent counting from multiple sequence alignments (24), was used to predict whether the amino acid substitutions affected the protein function. The hydrophilicity of wild-type and mutant protein products was analyzed using online biological software program Misc Protein Analysis ([https://fasta.bioch.virginia.edu/fasta\\_www2/fasta\\_www.cgi?rm=misc1](https://fasta.bioch.virginia.edu/fasta_www2/fasta_www.cgi?rm=misc1)).

## Results

**Clinical data.** There were 4 affected individuals among the 10 family members (Fig. 1). The proband (IV:1) was a one-year old male whose great-grandmother (I:2), grandmother (II:2) and father (III:2) had poor eyesight in their childhood. Among them, one (I:2) succumbed to mortality and two (II:2 and III:2) were examined prior to cataract removal. The proband exhibited a bilateral cataract characterized as a central nuclear opacity involving the embryonic and fetal nuclei with posterior polar opacities (Fig. 2). There was no family history of other eye conditions or systemic diseases.

**Mutation analysis.** Direct sequencing of the candidate genes indicated that in the MIP gene position 97, as a result of the C-T transition, the highly conserved arginine was substituted by cysteine in the codon 33 (Fig. 3). This mutation was detected in all affected members, however, it was not observed in the unaffected family members or normal

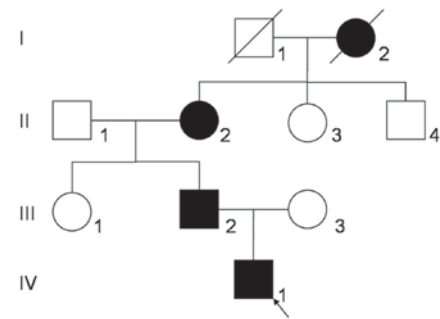


Figure 1. Pedigree chart of the family. A total of eight members of a three-generation Chinese Zhuang family were involved in the present study, including three affected individuals (II:2, III:2, and IV:1) and five unaffected individuals (II:1, II:3, II:4, III:1 and III:3). Circles represent females and squares indicate males. Shaded shapes indicate affected individuals. A diagonal line through a symbol indicates a deceased person. The arrow indicates the proband.

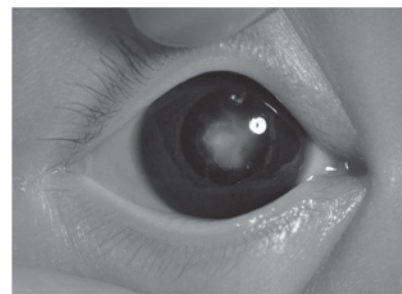


Figure 2. Phenotype of the proband. The proband exhibited a bilateral cataract characterized by central nuclear opacity involving embryonic and fetal lens nuclei with posterior polar opacities.

controls. No significant nucleotide polymorphisms were identified in other candidate genes.

**Bioinformatics analysis.** Bioinformatics analysis with PolyPhen-2 revealed that the replacement in the MIP gene at position 33 from R to C scored 0.999 (sensitivity, 0.14; specificity, 0.99) and was predicted as possibly damaging. The hydrophobicity of this variant was markedly elevated (Fig. 4).

## Discussion

MIP is a member of the water-channel family of proteins. It is the most abundant type of membrane protein in the mature lens, expressed only in end-stage differentiated fibrous cells (25). The function of MIP, as a water channel and an adhesion molecule in the lens fiber, contributes to the formation of the small intercellular space of the lens fiber, which is necessary for lens transparency and adaptation (26).

MIP gene is located on chromosome 12q13 and several mutations in MIP are associated with human genetic cataracts (27). At present, 20 different mutations have been identified to cause human congenital cataracts, including p.M1T, p.R33C, p.V107I, p.R113X, p.E134G, p.T138R, p.D150H, p.G165D, p.A169PfsX15, p.L170PfsX31, p.Y177C, p.R187C, p.N200GfsX12, p.W202X, c.606+1G>A splicing, p.V203fs, p.G213VfsX46, p.G215D, p.Y219X and p.R233K, and 3 variations have been found to be associated with age-related cataracts (rs2269348, rs117788190 and rs74641138) (27). According to the amino acid sequences,

Table I. The primers used for PCR.

Name	Primer sequence (5'→3')		Product length (base pair)
	Forward	Reverse	
CRYAA-1	AGCAGCCTTCTTCATGAGC	CAAGACCAGAGTCCATCG	584
CRYAA-2	GGCAGGTGACCGAAGCATC	GAAGGCATGGTGCAGGTG	550
CRYAA-3	GCAGCTTCTCTGGCATGG	GGGAAGCAAAGGAAGACAGA	511
CRYAB-1	AACCCCTGACATCACCATT	AAGGACTCTCCCGTCCTAGC	250
CRYAB-2	CCATCCCATTCCTTACCTT	GCCTCCAAAGCTGATAGCAC	350
CRYAB-3	TCTCTCTGCCTCTTTCCTCA	CCTTGGAGCCCTCTAAATCA	400
CRYBA1-1	GGCAGAGGGAGAGCAGAGTG	CACTAGGCAGGAGAAGTGGG	550
CRYBA1-2	AGTGAGCAGCAGAGCCAGAA	GGTCAGTCACTGCCTTATGG	508
CRYBA1-3	AAGCACAGAGTCAGACTGAAGT	CCCCTGTCTGAAGGGACCTG	463
CRYBA1-4	GTACAGCTCTACTGGGATTG	ACTGATGATAAATAGCATGAACG	355
CRYBA1-5	GAATGATAGCCATAGCACTAG	TACCGATACGTATGAAATCTGA	597
CRYBA1-6	CATCTCATACCATTTGTGTTGAG	CATCTCATACCATTTGTGTTGAG	528
CRYBB2-1	GTTTGGGGCCAGAGGGGAGTGGT	TGGGCTGGGGAGGGACTTTCAGTA	350
CRYBB2-2	CCTTCAGCATCCTTTGGGTTCTCT	GCAGTTCTAAAAGCTTCATCAGTC	330
CRYBB2-3	GTAGCCAGGATTCTGCCATAGGAA	GTGCCCTCTGGAGCATTTTCATAGT	360
CRYBB2-4	GGCCCCCTCACCCATACTCA	CTTCCCTCTGCCTCAACCTAATC	230
CRYBB2-5	CTTACCCTTGGGAAGTGGCAATGG	TCAAAGACCCACAGCAGACAAGTT	600
CRYGC-1	TGCATAAAATCCCCTTACCG	CCTCCCTGTAACCCACATTG	514
CRYGC-2	TGGTTGGACAAATTCTGGAAG	CCCACCCCATTCACCTTCTTA	430
CRYGD-1	CAGCAGCCCTCCTGCTAT	GGGTCCTGACTTGAGGATGT	550
CRYGD-2	GCTTTTCTTCTCTTTTATTTCTGG	AAGAAAGACACAAGCAAATCAGT	308
CRYGS-2	GAAACCATCAATAGCGTCTAAATG	TGAAAAGCGGGTAGGCTAAA	575
CRYGS-3	AATTAAGCCACCCAGCTCCT	GGGAGTACACAGTCCCCAGA	479
CRYGS-4	GACCTGCTGGTGATTTCCAT	CCTGTGGCGAGCACTGTAT	974
GJA3-1	CGGTGTTTCATGAGCATTTTC	CTCTTCAGCTGCTCCTCCTC	450
GJA3-2	GAGGAGGAGCAGCTGAAGAG	AGCGGTGTGCGCATAGTAG	450
GJA3-3	TCGGGTTCCCACCCTACTAT	TATCTGCTGGTGGGAAGTGC	300
GJA8-1	CCGCGTTAGCAAAAACAGAT	CCTCCATGCGGACGTAGT	420
GJA8-2	GCAGATCATCTTCGTCTCCA	GGCCACAGACAACATGAACA	330
GJA8-3	CCACGGAGAAAACCATCTTC	GAGCGTAGGAAGGCAGTGTC	350
GJA8-4	TCGAGGAGAAGATCAGCACA	GGCTGCTGGCTTTGCTTAG	500
MIP-1	TCTCGGCTCATCTCCCAGTT	GGCAATAGAGAGACAGGACAC	635
MIP-2	TGAAGGAGCACTGTTAGGAGATG	AGAGGGATAGGGCAGAGTTGATT	500
MIP-3	CCAGACAGGGCATCAGT	TGGTACAGCAGCCAACAC	677
MIP-4	AAGGTGTGGGATAAAGGAGT	TTCTTCATCTAGGGGCTGGC	389

Summary of the product lengths and primers used for the amplification of all exons of candidate genes associated with autosomal dominant congenital nuclear cataracts. CRYAA, crystallin  $\alpha$  A; CRYAB, crystallin  $\alpha$  B; CRYBA1, crystallin A1; CRYBB2, crystallin B2; CRYGC, crystallin C; CRYGD, crystallin D; CRYGS, crystallin S; GJA8, gap junction protein  $\alpha$  8; GJA3, gap junction protein  $\alpha$  3; MIP, major intrinsic protein of lens fiber.

the members of the water-channel family are predicted to share a common protein topology consisting of six transmembrane domains and five extracellular loops (28). Out of them, the first extracellular loop contains the following residues: 33R, 34W, 35A, 36P, 37G, 38P, 39L and 40H (28). The mutation investigated in the current study was in the first residue of the first extracellular loop of the MIP protein.

In the present study, a missense mutation c.97C>T in MIP gene leading to substitution of arginine with cysteine (p.R33C) was found in a Chinese family with congenital central nucleus

and posterior polar cataract. This mutation co-segregated with the disease phenotype and was not found in the 100 unrelated control individuals. The p.R33C substitution was reported in a Chinese family (29) and an Australian sporadic case (30). In 2007, Gu *et al* (29), was the first to report the c.97C>T mutation in a Chinese family with an autosomal dominant total cataract. This was the first reported case of cataracts caused by a mutation located outside the transmembrane portion of MIP. The authors hypothesized that the p.R33C substitution may allow for the formation of intermolecular disulfide bonds and thereby

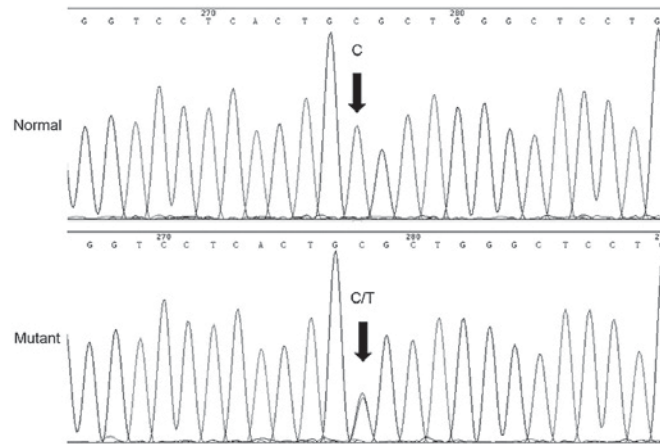


Figure 3. Heterozygous C>T transition in major intrinsic protein of lens fiber. The sequence chromatogram (forward strand) shows a heterozygous C>T transition resulting in a change of arginine to cysteine at codon 33. The black arrows show the normal wild-type and mutant loci, respectively.

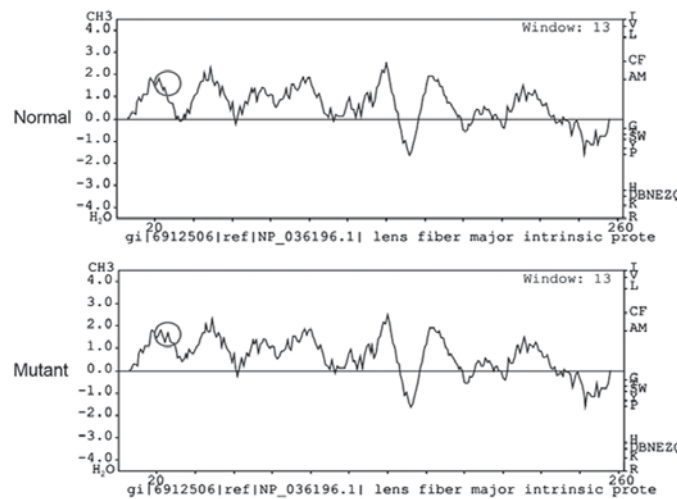


Figure 4. Decrease in hydrophilicity in the mutant form. The x-axis represents the position of amino acids. The numerical y-axis represents the hydrophilicity value and the alphabetical y-axis represents the one letter code of the amino acids. The regions of interest are marked with circles. The increase in hydrophobicity in the mutant form is evident.

destabilize the wild-type structure of MIP. The abnormal formation of the disulfide bonds may affect the position of MIP in the plasma membranes (29). Ma *et al* (30) identified p.R33C in an Australia sporadic congenital cataract case by using the next generation sequencing technique, which further confirmed the R33C mutation in MIP is associated with congenital cataract.

In the Chinese and Australian families reported in the previous studies, the phenotype was described as a full cataract (29,30). In the present study, the phenotype was described as a bilateral central nuclear opacity involving embryonic and fetal lens nuclei and posterior polar opacity. The family analyzed in the present study was of Guangxi Zhuang ethnicity, which is the most populous minority in the Guangxi Zhuang Autonomous Region (31). The present study provided additional information for the understanding of the genetic diversity of the Chinese nation. It was demonstrated that the phenotypic heterogeneity of the p.R33C mutation in MIP may be associated with the occurrence of congenital cataracts.

The molecular consequence of the p.R33C mutation in MIP should be further examined to provide an in-depth

understanding of the pathogenesis of congenital cataracts. Additional studies may examine MIP mutations, which cause cataracts, to gain a greater understanding of the basis of MIP-mediated cataractogenesis.

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#### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.



## Authors' contributions

ZZ analyzed and interpreted the data, and was a major contributor in writing the manuscript. LLi made substantial contributions to conception of the study. LLu performed the Genomic DNA extraction, gel electrophoresis, polymerase chain reaction analysis, polymerase chain reaction product sequencing and drafted the manuscript. LM made substantial contributions to design of the study and acquisition of data. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

The present study was approved by the Institutional Review Committee of the People's Hospital of Guangxi Zhuang Autonomous Region and followed the provisions of the Declaration of Helsinki. Written informed consent to participate was obtained from all patients included in the present study.

## Patient consent for publication

Written informed consent for publication was obtained from all patients included in the present study.

## Competing interests

The authors declare that they have no competing interests.

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