Three novel mutations of microphthalmos identified in two Chinese families

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Research article

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Abstract

Background: To identify the underlying genetic defect responsible for microphthalmos eyes in two three-generation Chinese families.

Methods: In our study, we screened 425 potential eye disease-related genes of the proband of a three-generation Chinese family diagnosed with microphthalmos using next-generation sequencing-based target capture sequencing. Variants were filtered and analyzed to identify possible disease-causing variants before Sanger sequencing validation.

Results: We enrolled two families with microphthalmos (Family 1: microphthalmos with congenital ocular coloboma and Family 2: simple microphthalmos). Two novel heterozygous mutations, PXDN c.3165C>T (p.Pro1055Pro) and PXDN c.2640C>G (p.Arg880Arg), were found in Family 1, and CRYBB2 c.481G>A (p.Gly161Arg) was found in Family 2, but none of the mutations were found in the unaffected individuals, who were phenotypically normal. Multiple orthologous sequence alignment (MSA) revealed that the CRYBB2 p.Gly161Arg mutation was a deleterious effect mutation.

Conclusions: The three novel mutations found in our study extend our current understanding of the genetic basis of microphthalmos and provide early presymptomatic diagnosis and emphasize the significance of genetic diagnosis of microphthalmos.

Background

Microphthalmos is a rare, inherited or sporadic congenital eye development defection and is responsible for approximately 3–12% visual impairment in children. It is characterized by a short axial length (AL < 20.0–21.0 mm) with thickened choroid and sclera [1, 2], a small anterior chamber depth (ACD < 2.2 mm) and a small cornea (horizontal corneal diameter < 11 mm) [3]. According to the anatomical difference and American Society of Cataract and Refractive Surgery (ASCRS) cataract clinical committee, microphthalmos can be divided into four types: simple microphthalmos (or nanophthalmos), microphthalmos with other congenital ocular coloboma, relative anterior microphthalmos (RAM) and axial high hyperopia [2, 4].

Genetic alterations are now a major cause of microphthalmos. The hereditary mode of nanophthalmos is autosomal dominant in many patients and sporadic in others [5, 6]. As a developmental eye disease, most of the related genes involved in microphthalmos are also ocular development-associated genes, including SOX2, OTX2, PAX6, and GJA8. Although over 30 genes have been implicated in eye development, we are currently able to explain the genetic bases of these defects in less than half of patients [5]. Moreover, recent studies on microphthalmia mainly rely on clinical observation, and novel related genes and mutations in microphthalmos have rarely been explored.

Hence, in our study, we studied the novel mutation genes of microphthalmos in two Chinese families (Family 1: microphthalmos with congenital ocular coloboma and Family 2: simple microphthalmos) and reported three novel mutations. Hopefully, our study will be helpful for microphthalmos prediction, prevention and molecular treatment.

Methods

Subjects

To make a precise diagnosis, we performed targeted next-generation sequencing (NGS) of microphthalmos-related genes. All featured participants provided written consent to participate, and the parents/guardians of any minors that participated provided written consent to participate on their behalf. The study was approved by the ethics committee of Eye and ENT Hospital of Fudan University and was conducted according to the principles of the Declaration of Helsinki.

Our study involved two family members from three generations who underwent detailed history ophthalmic examination, including best corrected visual acuity (BCVA) testing, slit lamp biomicroscopy, IOL master 500 (Carl Zeiss Meditec, Germany), dilated fundus examination, B scan, and SD-OCT (Spectralis HRA + OCT, Heidelberg, Engineering, Inc., Heidelberg, Germany). Family and medical history, including age of onset and other related clinical manifestations, was obtained. Blood samples were collected from the peripheral blood and stored at 4 °C before further analysis.

Genetic analyses

We extracted the genomic DNA from the family from peripheral blood on Feb. 2018 according to the manufacturer's standard procedure using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) [7]. A capture panel (NimbleGen, Madison, USA) of microphthalmos genes has been previously designed and assessed by our group. The capture panel comprised all exons together with the flanking exon and intron boundaries (± 15 bp) of 425 genes that are most frequently involved in common inherited eye diseases (the capture probes were custom designed and produced by BGI).

The library was enriched by array hybridization according to a previously published procedure, followed by elution and postcapture amplification. Then, qualification and NGS targeted sequences were further analyzed on the Illumina HiSeq 2000 platform (Illumina, Inc., San Diego, CA, United States) in collaboration with BGI-Shenzhen (Shenzhen, Guangdong, China) as previously reported [8, 9]. The filtered sequencing was then aligned to the human genome reference (hg19) using the Burrows Wheeler Aligner (BWA) Multi-Vision software package [10]. After alignment, the output files were used to perform sequencing coverage and depth analysis of the target region, single-nucleotide variants (SNVs) and INDEL calling.

We used the following 4 databases to test annotation of all identified variants with minor allele frequency (MAF) > 0.1% to eliminate benign variants: dbSNP137, HapMap Project, 1000 Genomes Project and Exome Variant Server. Finally, the variant prioritizations were performed, combining total depth, quality score, MAF, potential deleterious effect and the existence of mutation reports in common databases such as the Human Gene Mutation Database (HGMD), Online Mendelian Inheritance in Man (OMIM). All mutations and potential pathogenic variants were validated using conventional Sanger sequencing or a second spectrum sequencing method. Segregation analysis was performed if DNA from family members was available.

Results

Clinical examination and pedigree analysis

A total of 17 members from two three-generation families (Fig. 1) were included in the study. The clinical information of the four affected members is listed in Table 1. The proband of Family 1 (P5), a 29-year-old woman, has suffered from poor vision in both eyes since birth. Ophthalmic examination revealed a BCVA HM in both eyes. The axial length measurement showed the extreme short axial length (OD: 18.36 mm, OS: 19.83 mm). We also

found an extremely small cornea diameter (OD: 6.5 mm, OS: 7.2 mm) after corneal diameter evaluation. Fundus examination and B scan both revealed severe choroid colomoba (Fig. 2a). After careful examination of her son, we found that P5 and her son (P9) suffered from the same eye disease: microphthalmos with congenital ocular coloboma and congenital cataract (Fig. 2b).

Table 1
Clinical characteristics of the four affected patients

Patients	Family 1(DLL)		Family 2 (FBF)	
	Patient 5	Patient 9	Patient 4	Patient 7
Age	29	2	31	7
BCVA (OD/OS)	HM/HM	/	FC/FC	0.05/FC
Axial length (mm)(OD/OS)	18.36/19.83	15.77/15.62	16.99/16.02	16.57/16.34
Anterior chamber depth (OD/OS)	1.07/1.21	/	/	/
Cornea diameter (mm) (OD/OS)	6.5/7.2	6.4//6.1	9.7/9.0	9.3/9.1
Intraocular pressure (OD/OS)	8.2/13.9	/	/	/
B scan	choroid coloboma		normal	
Diagnosis	microphthalmos with congenital ocular coloboma		simply microphthalmos	

The proband of Family 2 (P4), a 31-year-old woman, has suffered from progressive decreased vision in both eyes for over 10 years. Ophthalmic examination also revealed extremely short eyes (axial length: OD: 16.99 mm, OS: 16.02 mm) and small cornea (OD: 9.7 mm, OS: 9.0 mm) with congenital cataract. Fundus examination and a B scan showed moderate normal fundus. After careful examination of her daughter (P7), P4 and her daughter were diagnosed as the same disease: simple microphthalmos.

Genetic analyses

We performed a targeted NGS approach on the probands (P5 of Family 1 and P4 of Family 2). The targeted gene length was 1,106,466 bp of 425 genes, and the mean depth of the target region was 170.45 with 99.95% coverage. After the data acquisition and bioinformation analysis, two novel heterozygous mutations, PXDN c.3165C > T (p.Pro1055Pro) and PXDN c.2640C > G (p.Arg880Arg), were found in the proband (P5) of Family 1 (Fig. 3 and Table 2), and CRYBB2 c.481G > A (p.Gly161Arg) was found in Family 2 (Fig. 4 and Table 2). These mutations, PXDN c.3165C > T (p.Pro1055Pro), PXDN c.2640C > G (p.Arg880Arg) and CRYBB2 c.481G > A (p.Gly161Arg), were extremely rare in the control population, with frequencies of 0.0082, 0.0082 and 0 in the 1000 Genomes Project, respectively. Moreover, the three mutations in the SNV database of over 200 Chinese populations were less than 0.01.

Table 2
Mutations identified in family 1 and family 2

Family	Gene	NCBI reference sequence	Nucleotide change	Amino acid substitution	Chromosomal location	Gene subregion	Allele status
Family 1	PXDN	NM_012293	c.3165C > T	p.Pro1055Pro	chr2:1652387	EX17/CDS17	Het.
	PXDN	NM_012293	c.2640C > G	p.Arg880Arg	chr2:1652912	EX17/CDS17	Het.
Family 2	CRYBB2	NM_000496	c.481G > A	p.Gly161Arg	chr22:25627602	EX6/CDS5	Het.

Sanger sequencing was then performed to validate the variants in Family 1. The proband's son (P9) of Family 1 carried the same heterozygous mutations (c.3165C > T and c.2640C > G) of the PXDN gene (Fig. 3 and Table 2). However, the two mutations were not found in the proband's parents. However, the two mutations did not lead to amino acid changes.

We used the spectrum sequencing method to validate the variant in Family 2 after the negative result of the Sanger sequencing method. The proband's daughter (P7) in Family 2 carried the same heterozygous mutation (c.481G > A) of the CRYBB2 gene. The mutation was not found in the proband's parents. Multiple orthologous sequence alignment (MSA) using Polyphen revealed that CRYBB2 and its subsequent sequences were highly conserved amino acids across different species (Fig. 5), suggesting that the mutation may lead to a deleterious effect.

Discussion

As a high-throughput, low-cost and high-efficiency sequencing technology, NGS technology has been widely used in clinics to identify many rare diseases [7, 11]. The identified mutations and genes have become an important tool to explore potential genetic etiology and guide appropriate treatment for eye diseases [7, 12]. In our study, we comprehensively screened 425 genes involved in common, inherited eye diseases and successfully identified 3 potentially causative mutations for microphthalmos, PXDN c.3165C > T (p.Pro1055Pro), PXDN c.2640C > G (p.Arg880Arg) and CRYBB2 c.481G > A (p.Gly161Arg), in two Chinese families. Based on the mutation analysis and the clinical measurement, we concluded that these three novel mutations could provide early presymptomatic diagnoses and emphasize the significance of the genetic diagnosis of microphthalmos.

The human CRYBB2 (crystalline beta B2) gene is located on chromosome 22q and is encoded in 7 exons. The CRYBB2 gene is a dimer at low concentrations but can form oligomers under physiological conditions. The CRYBB2 gene has contributed to the identical human lens crystalline protein-protein binding [13, 14], structural constituent of human lens [15] and structural molecule activity [16]. Multiple studies have revealed that CRYBB2 gene mutations (including p.Q155X [17–19], p.E167X [20]) were associated with congenital autosomal dominant cataracts. In our study, we also found a CRYBB2 novel mutation (p.Gly161Arg) in simply microphthalmos eyes in Family 2. The proband and her daughter shared the same mutation of the CRYBB2 gene, which adhered to the dominant heredity as previous papers. Furthermore, MSA using Polyphen revealed that CRYBB2 and its subsequent sequences were highly conserved amino acids across different species, suggesting that the CRYBB2 p.Gly161Arg mutation was a deleterious mutation. To the best of our knowledge, the CRYBB2 p.Gly161Arg mutation was

originally reported in this study. However, the proband and her daughter had microphthalmos and congenital cataract, and whether the CRYBB2 p.Gly161Arg mutation is a causative mutation of microphthalmos or the congenital cataract needs further in vitro study.

The human PXDN gene is located on chromosome 2p and is encoded in 24 exons. The gene is expressed in corneal epithelium and is secreted into the extracellular matrix. Mutations in the PXDN gene were associated with congenital recessive corneal opacification and other ocular anomalies as well as microphthalmia and anterior segment dysgenesis. Choi et al [21] reported PXDN Tyr398Thrfs*40 and PXDN Gln316Pro mutations in three families with congenital cataracts, microcornea, sclerocornea and developmental glaucoma. The defective PXDN gene has been shown to impair sulfilimine bond formation in collagen IV, a constituent of the basement membrane, implying that eye defects result from the loss of basement membrane integrity in the developing eye. In our study, we also found that PXDN gene mutations c.3165C > T (p.Pro1055Pro) and c.2640C > G (p.Arg880Arg) in microphthalmos with congenital ocular coloboma eyes. The two mutations have not been previously reported. Furthermore, the heredity model was prone to be autosomal dominant in Family 1, which implied that the PXDN gene mutations c.3165C > T (p.Pro1055Pro) and c.2640C > G (p.Arg880Arg) might be different from the other PXDN gene mutations. Although the two mutations did not result in amino acid changes, we also concluded that PXDN sequencing should be considered in microphthalmos with anterior segment dysgenesis.

To the best of our knowledge, this study used the first NGS-based assay specifically designed for the confirmation and early diagnosis of microphthalmos in two Chinese families' pedigrees reported to date. In addition, we found 3 potentially causative mutations for microphthalmos, PXDN c.3165C > T (p.Pro1055Pro), PXDN c.2640C > G (p.Arg880Arg) and CRYBB2 c.481G > A (p.Gly161Arg), that are likely responsible for microphthalmos. These genetic mutation patterns are novel, and the functions and interactions of the PXDN and CRYBB2 genes should be further investigated. This study not only provides a guide to the attending clinician on the management and prognosis of the patient but also extends the phenotypic spectrum of PXDN- and CRYBB2- associated microphthalmos and enhances our current understanding of the genetic basis of microphthalmos.

Abbreviations

MSA:Multiple orthologous sequence alignment; AL:Axial length; ACD:Anterior chamber depth; RAM:Relative anterior microphthalmos; NGS:Next-generation sequencing; BCVA:Best corrected visual acuity; SNVs:Single-nucleotide variants; MAF:Minor allele frequency

Declarations

Acknowledgments

Not applicable.

Authors' contributions

Study concept and design (TZ, YL); data collection (YT, JX); analysis and interpretation of data (YT, JX); writing the manuscript (YT); critical revision of the manuscript (JX, TZ, YL); administrative, technical, or material support (TZ, YL); supervision (TZ,YL). All authors have read and approved the manuscript.

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Availability of data and materials

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

Ethics approval and consent to participate

This study was approved by the ethics committee of the Eye and Ear, Nose, and Throat (ENT) Hospital of Fudan University and was conducted according to the principles of the Declaration of Helsinki. The written informed consent was obtained from the patient.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests. Dr. Lu is a member of the editorial board of this journal.

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Figures

Figure 1

Proband of the two families.

Figure 2

a Slit lamp photograph (left) and B scan (right) of proband (P5) of Family 1. The yellow arrow shows the severe choroid colomaba. b Slit lamp photograph (left) and B scan (right) of proband (P4) of Family 2.

Figure 3

PXDN gene mutations found in Family 1.

Figure 4

CRYBB2 gene mutation found in Family 2.

Figure 5

Multiple orthologous sequence alignment (MSA) for CRYBB2 p.Gly161Arg mutation.