# Detection of A Novel PAX6 Mutation In A Chinese Family With Multiple Ocular Abnormalities

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# Abstract

**Background:**

Aniridia is a congenital, panocular disease which could affect cornea, anterior chamber angle, iris, lens, retina and optic nerve. *PAX6* loss-of-function mutations were the most common cause of aniridia. Mutations throughout the *PAX6* gene have been linked to a range of ophthalmic abnormalities. Distinct mutations at a given site in *PAX6* lead to distinctive phenotypic findings. This study aimed to characterize genetic mutations associated with congenital aniridia in a Chinese family.

**Methods**:

The proband and family underwent ophthalmologic examinations as well as exome sequencing. Results have been confirmed by Next Generation Sequencing.

**Results**:

A novel mutation(c.114_119delinsAATTTCC:p.Pro39IlefsTer17)in the *PAX6* gene was identified in subjects -1, III-1 and III-2 in these family who exhibited complete aniridia and cataract. Proband and the proband's brother also exhibited glaucoma, high myopia, and foveal hypoplasia.

**Conclusions**

We identified a novel *PAX6* frameshift heterozygous deletion mutation in a Chinese family and infered this mutation a probable cause of various eye abnormalities in carriers.

**Trial registration**We did not do any health-related interventions on the participants.


# Background

Aniridia is an eye disorder defined as partial or completet absence of iris, and it can be either congenital or caused by injury. Congenital aniridia is a sporadic[1], rare condition which affects 1:64,000–1:96,000 people. Up to two-thirds of patients exhibit an autosomal dominant form of aniridia[2].

While the absence of iris is the most salient hallmark of this condition, congenital aniridia is also associated with abnormalities in cornea, retina, lens, anterior chamber angle, and the optic nerve. Most aniridia patients exhibit macular hypoplasia, nystagmus, and significant visual impairment, besides a smaller subset of patients suffering from optic nerve hypoplasia[3]. In addition, patients with aniridia often suffer from a range of secondary ocular complications including cataracts, aniridic keratopathy, and glaucoma. The latter of these conditions affecting up to 70% of aniridic patients[4].

To date, over 500 mutations in *PAX6* gene and its regulatory regions have been characterized. Many of these mutations account for PAX6 haploinsufficiency which leads to significant ocular and systemic abnormalities[5]. However, few studies have characterized hybrid frameshift deletion insertion *PAX6*

mutations associated with ocular diseases in Chinese families. In the present study, we therefore describe a novel *PAX6* mutation that was found to be associated with congenital aniridia in a Chinese family.

# Methods

**2.1.Subjects:**The proband and the proband's family with a history of congenital aniridia were recruited at the Aier Eye Hospital of Changsha, the father of the proband had also been diagnosed with aniridia at a different hospital(Figure 1).This study was approved by the Aier Eye Hospital of Changsha ethics committee and was consistent with the Declaration of Helsinki, with all subjects providing informed consent to participate.

**2.2.Clinical Evaluation:** Thorough ophthalmologic examination were performed to the proband and her brother, including tests of visual acuity, intraocular pressure(IOP),slit-lamp analyses, anterior segment photography, visual field tests(Humphrey 750,Carl Zeiss, Germany), funduscopy, ultrasonic B analyses(Chiescan Quantel Medical, France), gonioscopic analyses, OCTA(optical coherence tomography angiography)assessments(RTVue-XR Avanti,v2017.1.0;OptoVue,Inc.,CA,USA),and ultrasound biomicroscope(UBM)assessments(SW China),the proband's other family members underwent a simple slit lamp examination.

### 2.3.Mutation screening

**2.3.1.Genome DNA extraction**:About 4 ml of venous blood was sampled from proband and proband's brother. The genomic DNA samples were stored at -20℃ before using.

### 2.3.2.Library construction

1.Genome-wide library construction: DNA enzymatic fragmentation and genome-wide library construction were carried out using the DNA library construction kit of YEASEN Biology Company（Hieff NGS® OnePot DNA Library Prep Kit for Illumina®，YEASEN).

2.Construction of clinical whole exon group capture library:

XGen Exome Research Panel V1.0 （Integrated DNA Technologies, Inc.，USA）of IDT company was used to capture the clinical all exon group library and constructed the clinical all exon group library of the proband and his brother.

**2.3.3.Clinical total exome sequencing**：Two-terminal sequencing was performed on the Illumina (san diego, ca) sequencing platform using PE 150 patterns.

### 2.3.4.Sanger sequencing

Primer3Plus（http://www.primer3plus.com/cgi-bin/dev/primer3plus.cgi）was used to design primers for PAX6 gene C. 114_119delinsAATTTCC(P :Pro39fs) site and in-silico PCR（http://genome.ucsc.edu/cgi-bin/hgPcr）was used to verify the specificity of the primer(Table 1). PCR amplification products of family

members were sequenced using ABI3730s AUTOMATIC DNA sequence Analyzer (3730 DNA Analyzer), and sequencing results were analyzed and compared using CodonCode Aligner software (CodonCode Corporation, USA).

Table 1.Sequencing primer details.

| Primer Name | Sequence | Amplified fragment length (bp) | Purpose |
|---|---|---|---|
| SG2560_F | 5'-TACAGTAAGAAATGAAGAGAGGGCGTT-3' | 499 | PCR amplification/sequencing forward primers |
| SG 2560_R | 5'-GGGCACGGTTGCTTGGACT -3' | | PCR amplification/sequencing reverse primers |

**2.3.5. Analysis:** Raw reads of low quality were removed, and the remaining reads were mapped to the UCSC(University of California Santa Cruz) hg19 reference genome(http://genome.ucsc.edu/). Single nucleotide variations(SNVs)and insertion-deletion(InDel)mutations were detected using the HaplotypeCaller function of the Genome Analysis ToolKit(GATK,http://software broadinstitute org/gatk/).These annotated variants were then filtered based on the Annovar(http://www.openbioinformatics.org/annovar/)database,The databases used in mutative pathogenicity prediction included SIFT(http://sift.jcvi.org), Polyphen2_HDIV(http://genetics.bwh.harvard.edu/pph2), Polyphen2_HVAR(http://genetics.bwh.harvard.edu/pph2), LRT(http://www.genetics.wustl.edu/jflab/lrt_query.html),MutationTaster(http://www.mutationtaster.org/), MutationAssessor(http://mutationassessor.org/r3/), FATHMM(http://fathmm.biocompute.org.uk), PROVEAN(http://provean.jcvi.org/index.php), MetaSVM(https://omictools.com/meta-svmtool), MetaLR(http://www.ensembl.info/tag/metalr/), M-CAP(http://bejerano.stanford.edu/mcap/), fathmm-MKL_coding(http://fathmm.biocompute.org.uk/fathmmMKL.htm). Quality control requirements:data volume>=6GB,average coverage>=150X,30X coverage>=98.5%,Q30 Qualification rate (%)>=89.17%).

2.3.6.Interpretation:The guidelines of the American College of Medical Genetics and Genomics(ACMG)were used to facilitate appropriate data analysis Table 3. Only those genetic variations with known, definitive genetic associations were analyzed. Genes with unknown pathogenicity or functionality were omitted from these analyses. In addition, common benign polymorphic variants, synonymous variants, and intronic variants not altering mRNA splicing were not included in these analyses unless they have previously been reported in the literature as being pathogenic or were included in the database.

Table 3.Classification basis referred to ACMG guide.

| ACMG | Description of evidence | Classification results |
|---|---|---|
| PVS1 | The pax6 gene in the clingen database(https://clinicalgenome.org/) was recorded with a single dose sensitive gene with a score of 3 . | Pathogenic variation |
| PM2_Supporting | It is a rare variant, not included in the Genome Aggregation Database (gnomAD,https://gnomad.broadinstitute.org/about) East Asian database. | |
| PP4 | The patient's clinical symptoms and family history were anastomotic with PAX6 gene abnormality. | |

# Results

### 3.1 Clinical data

### Proband

A 13-year old girl presented to our hospital complaining of bilateral blurred vision with no history of surgery or medical treatment of either eye. Her IOPs were 44 mmHg OD and 38mmHg OS detected by Goldmann tonometry, with her best-corrected visual acuity (BCVA) 20/100 OD and 20/125 OS. Refractive errors were -8.5 D OD and-6.0 D OS, with eyeball axis length (AL)values 26.3 mm OD and 26.0 mm OS. The anterior chamber in both eyes appeared normal, and peripheral angles were opened in both eyes. A slit lamp examination showed the presence of bilateral peripheral cataracts and posterior capsular opacification, while UBM examination revealed iris coloboma. A further fundus examination found large optic disc with bilateral glaucomatous cupping and peripapillary atrophy, while OCTA examination displayed diffuse superior and inferior RNFL (retinal nerve fibre layer) thinning, reduced wiVD(whole image vessel density),idVD(inside disc vessel density),and ppVD(peripapillary vessel density)vessel density, and significant foveal hypoplasia(Table 2). Visualfield tests highlighted bilateral glaucomatous visual field defects (Figure 2).The proband had no other discomfort, especially hearing loss or abnormal olfaction.

Table 2.RNFL,wiVD(whole image vessel density),idVD(inside disc vessel density),and ppVD(peripapillary vessel density)values for the proband and the proband’s brother.

| Patient | RNFL(μm) | | wiVD% | | idVD% | | PPVD% | |
|---|---|---|---|---|---|---|---|---|
| eye | OD | OS | OD | OS | OD | OS | OD | OS |
| proband | 78 | 80 | 43.6 | 39.5 | 32.8 | 40.2 | 41.7 | 41.8 |
| proband 's brother | 85 | 82 | 42.3 | 40.6 | 43.8 | 33.4 | 43.4 | 42.8 |

### Brother of the Proband

The 23-year-old brother of the proband reported a history of glaucoma which had been diagnosed one year prior in a different hospital, and was taking IOP-lowering eye drops since then.

His BCVA in both eyes was 20/80, with refractive error values of-9.5 D OD and-10.25 D OS, and with eyeball AL values of 26.7 mm OD and 26.5 mm OS. He exhibited many of the same ophthalmic abnormalities as his sister did, including complete aniridia, cataracts, glaucoma, high myopia, and foveal hypoplasia. In addition, the brother exhibited a decreased VD compared to what was normally observed in healthy eyes. The superior and inferior RNFL of the brother's eyes were thicker compared to his sister's eyes(Table 2). He exhibited less pronounced bilateral glaucomatous visual field defects(Figure 3). Proband's brother had no hearing loss or abnormal olfaction either.

### Other family members of the Proband

The proband's father had such limited vision as light perception, simple check showed severe cataracts and complete aniridia. Proband's mother and grandparents had no obvious eye problems.

### 3.2 Mutation analysis

Second-generation sequencing analyses demonstrated the presence of a heterozygous frameshift deletion mutation(c.114_119delinsAATTTCC:p.Pro39IlefsTer17)in exon 5 of the *PAX6* gene. This mutation, which consisted of a 6 bp deletion and a 7 bp insertion, resulted in a frameshift in the *PAX6* gene from the 39th proline codon resulting in the generation of a premature stop codon (Figure 4). Based on the ACMG guidelines, the mutation is a pathogenic variant (Table 3).

# Discussion

By analyzing a Chinese family with histories of congenital aniridia, we herein identified a novel hybrid mutation(c.114_119delinsAATTTCC:p.Pro39IlefsTer17)in the *PAX6* gene. This mutation consisted of a 6 bp deletion and a 7 bp insertion that resulted in the premature truncation of the PAX6 protein. The affected brother and sister patients exhibited shared ophthalmic abnormalities including cataracts, nystagmus, glaucoma, aniridia, and macular fovea hypoplasia. The *PAX6* gene was first characterized by Ton et al.in 1991[6] found on chromosome 11p13. *PAX6* encodes a transcriptional regulator that is important for the development of organs and tissues including the eyes. *PAX6* expression is detectable in the iris, lens, optic disc, corneal epithelium, ciliary body, retinal neuroepithelium, and retinal pigment epithelium. In 2005,Tzoulaki et al. characterized human *PAX6* mutations and found that mutations throughout this gene were associated with aniridia and its related phenotypes[7].In an additional study of 95 Chinese patients with aniridia, You et al.found that *PAX6* loss-of-function mutations were the most common cause of aniridia[8].The identified *PAX6* mutation in these siblings(c.114_119delinsAATTTCC:p.Pro39IlefsTer17)resulted in a frameshift from the 39th codon of this gene, leading to the premature generation of a stop codon. In light of prior studies, we hypothesized that

this mutation was likely to be the primary cause of aniridia and other observed ophthalmic abnormalities in these siblings.

In their prior study of 95 Chinese aniridia patients, You et al. identified 47 different mutations associated with the aniridia phenotype including 6 frameshift InDel mutations,12 nonsense mutations,2 missense mutations,1 run-on mutation,1 synonymous mutation, and 15 mutations that altered mRNA splicing[8].The human gene mutation database(HGMD)currently includes 479 pathogenic *PAX6* mutations gene(http://www.hgmd.cf.ac.uk/ac/gene.php?gene=PAX6). In total, 20 reports to date have described cases of patients with both insertion and deletion mutations in the *PAX6*, and such combination mutations are generally more likely to be associated with serious ophthalmic abnormalities. Our observations of abnormalities including aniridia and glaucoma in the patients in the present study are thus consistent with these prior studies.

The PAX6 protein is composed of four domains: two DNA-binding domains, including an N-terminal 128 amino acid paired box domain(PD)and a 61 amino acid homeodomain(HD),as well as a 79 amino acid glycine-rich hinge region and a C-terminal proline-rich serine transactivation domain[9, 10].Mutations throughout the *PAX6* gene have been linked to a range of ophthalmic abnormalities, with distinct mutations at a given site within this gene leading to distinct phenotypic findings. Glaucoma manifested at an earlier age and was more severe in the proband than in her brother in the present study. Two primary models have been proposed to describe the penetrance of *PAX6* mutations. Dominant-negative *PAX6* mutations are thought to enhance PAX6 binding to DNA, leading to abnormal dominant-negative effects as a result of premature PAX6 truncation[11].Other *PAX6* mutations are better described by a dose-effect model wherein premature termination codons(PTCs)within the *PAX6* open reading frame lead to premature protein truncation as a result of nonsense-mediated mRNA decay(NMD).In such a dose-effect model, a single wild-type allele of *PAX6* is insufficient to facilitate normal ocular development, leading to the observed ophthalmic abnormalities[12].Subtle phenotypic differences between patients with different *PAX6* mutations may thus be attributable to slight differences in intracellular PAX6 levels[13].In the present study, we identified a novel heterozygous frameshift mutation in *PAX6* that resulted in a frameshift from the 39th proline codon and the generation of a premature stop codon. This mutation began in exon 5 in the PD domain and led to the truncation of the LNK(Linker, glycine-rich hinge region)HD, and PST domains of the PAX6 protein, resulting in a shortened peptide that is unlikely to be functional[14].Haploinsufficiency is likely to explain the observed aniridia phenotype in the subjects of the present study ,although the mechanistic link between genotype and phenotype in these patients remains to be fully characterized in future studies.

# Conclusions

In summary, we identified the novel heterozygous c.114_119delinsAATTTCC:p.Pro39IlefsTer17 mutation the *PAX6* gene as a putative cause of aniridia in a Chinese family. These results expand the spectrum of known mutations that can cause PAX6-triggered congenital aniridia, while also enhancing current

understanding regarding the genetic etiology of this condition. Furtherly, our findings may have the potential to aid in the genetic diagnosis of aniridia.

# Abbreviations

IOP intraocular pressure

OCTA optical coherence tomography angiography

UBM ultrasound biomicroscope

SNVs single nucleotide variations

InDel insertion-deletion

GATK Genome Analysis ToolKit.

ACMG The guidelines of the American College of Medical Genetics and Genomics

BCVA best-corrected visual acuity

RNFL retinal nerve fibre layer)thinning,

wiVD whole image vessel density

idVD inside disc vessel density

ppVD peripapillary vessel density

VD vessel density

HGMD The human gene mutation database

PD paired box domain

HD homeodomain

NMD nonsense-mediated mRNA decay

PTCs premature termination codon

# Declarations

**Ethics approval and consent to participate:**Approved by Changsha Aier Eye Ethics Committee (2019)KYPJ001.Written informed consent was obtained from the proband,the proband's brother and their father.

**Consent for publication:**We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed.We further confirm that the order of authors listed in the manuscript has been approved by all of us.The brother and the father of the the proband that was a minor gave written consent for their personal or clinical details along with any identifying images to be published in this study.

**Availability of data and materials:**All data generated or analysed during this study are included in this published article[and its supplementary information files].

**Competing Interests:**The authors declare that they have no competing interests.

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**Authors'contributions:**Contributions of authors involved in conception and design of study (JY, XC); Collection, analysis and interpretation of data (YJ, ZY, F,MD); Writing the article (JY); Critical revision of the article (ZY, XC). All authors have read and approved the manuscript in its current state.

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# Figures

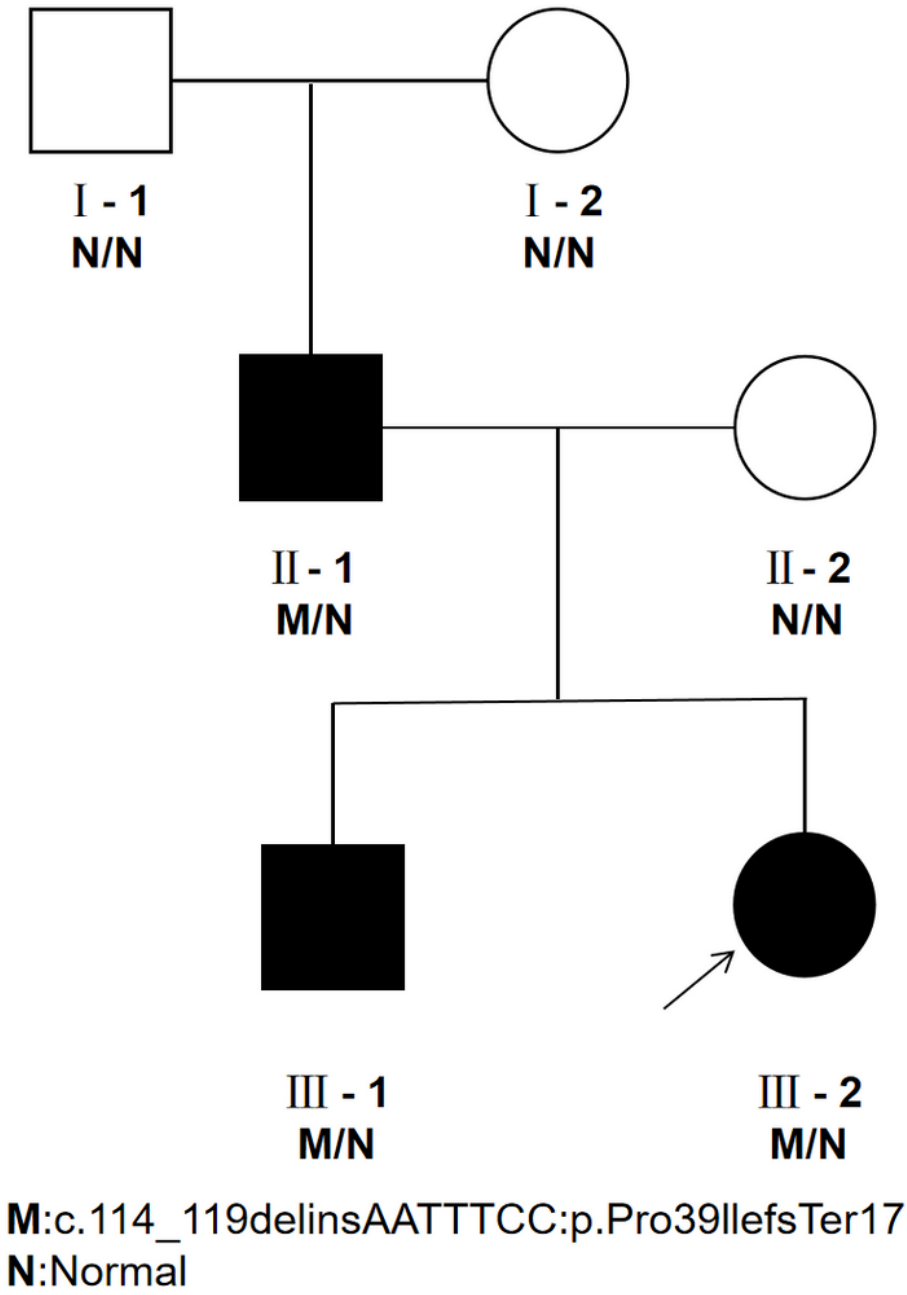
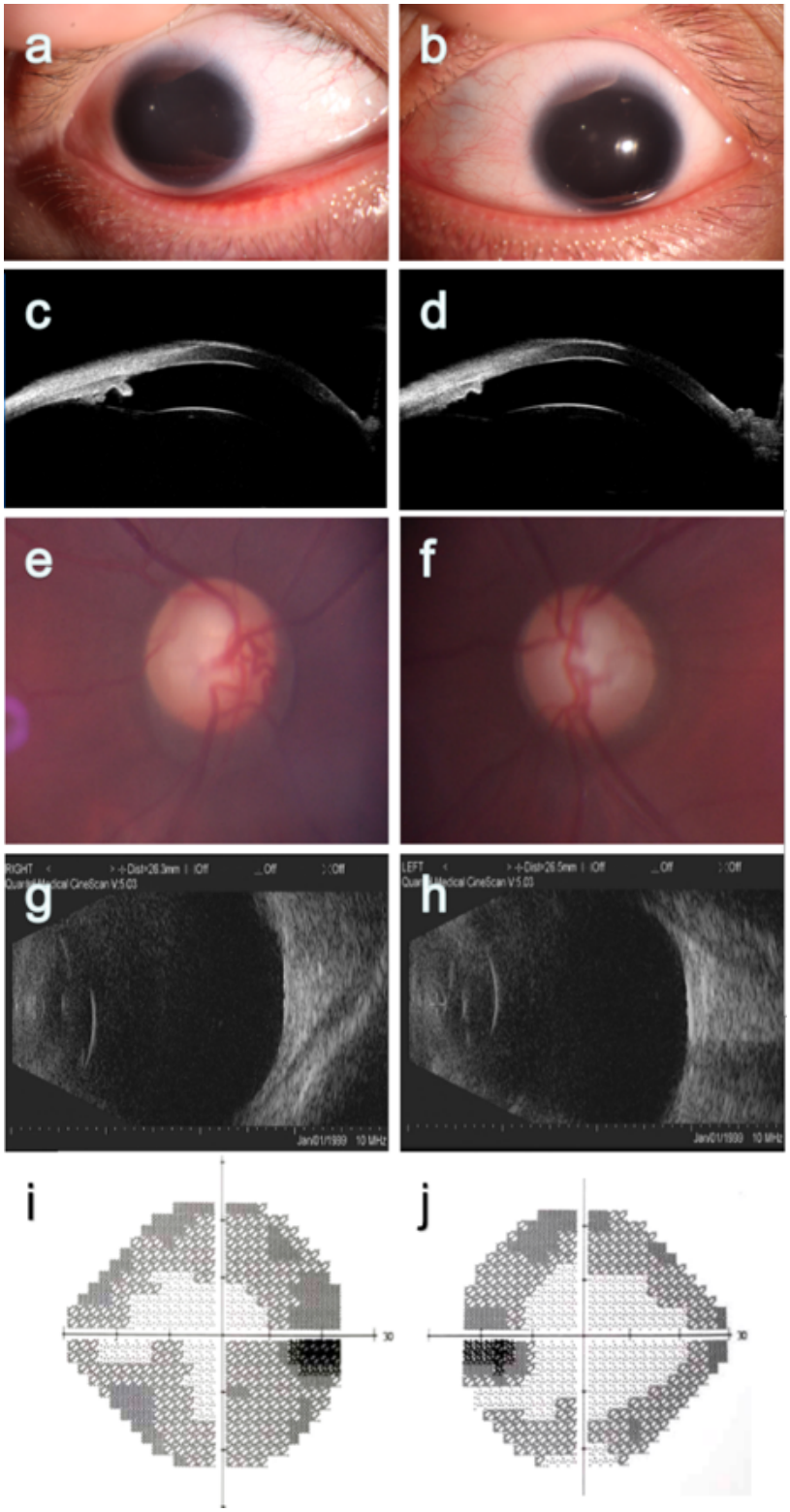


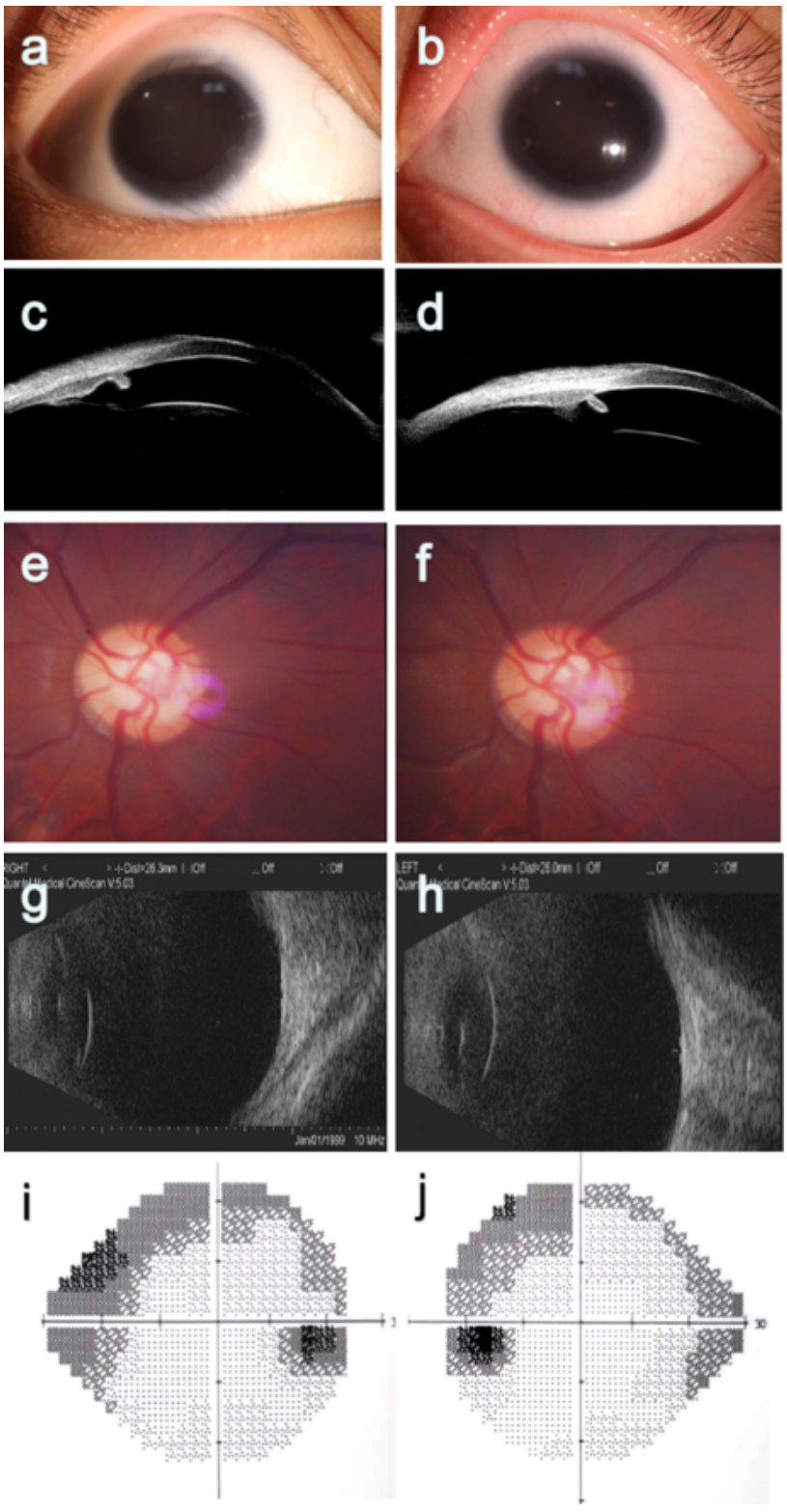

**Figure 1**

Pedigree of a Chinese family with aniridia. Squares and circles correspond to males and females, respectively. Black and white shapes correspond to affected and unaffected individuals, respectively. The proband is indicated with an arrow.




## Figure 2

Clinical findings in the proband. The right and left columns correspond to the left and right eyes, respectively. Images of the anterior segment(a,b)and horizontally scanned UBM images of the anterior chamber(c,d).Images of the optic disc(e,f),images of B-mode ultrasound(g,h)and pattern deviation plots from the Humphrey750 visual field test(i,j).Vertically scanned ultrasonic B images of the eyeball.




## Figure 3

Clinical findings in the proband's brother. The right and left columns correspond to the left and right eyes, respectively. Images of the anterior segment(a,b)and horizontally scanned UBM images of the anterior chamber(c,d).Images of the optic disc(e,f),images of B-mode ultrasound(g,h)and pattern deviation plots from the Humphrey750 visual field test(i,j).Vertically scanned ultrasonic B images of the eyeball.

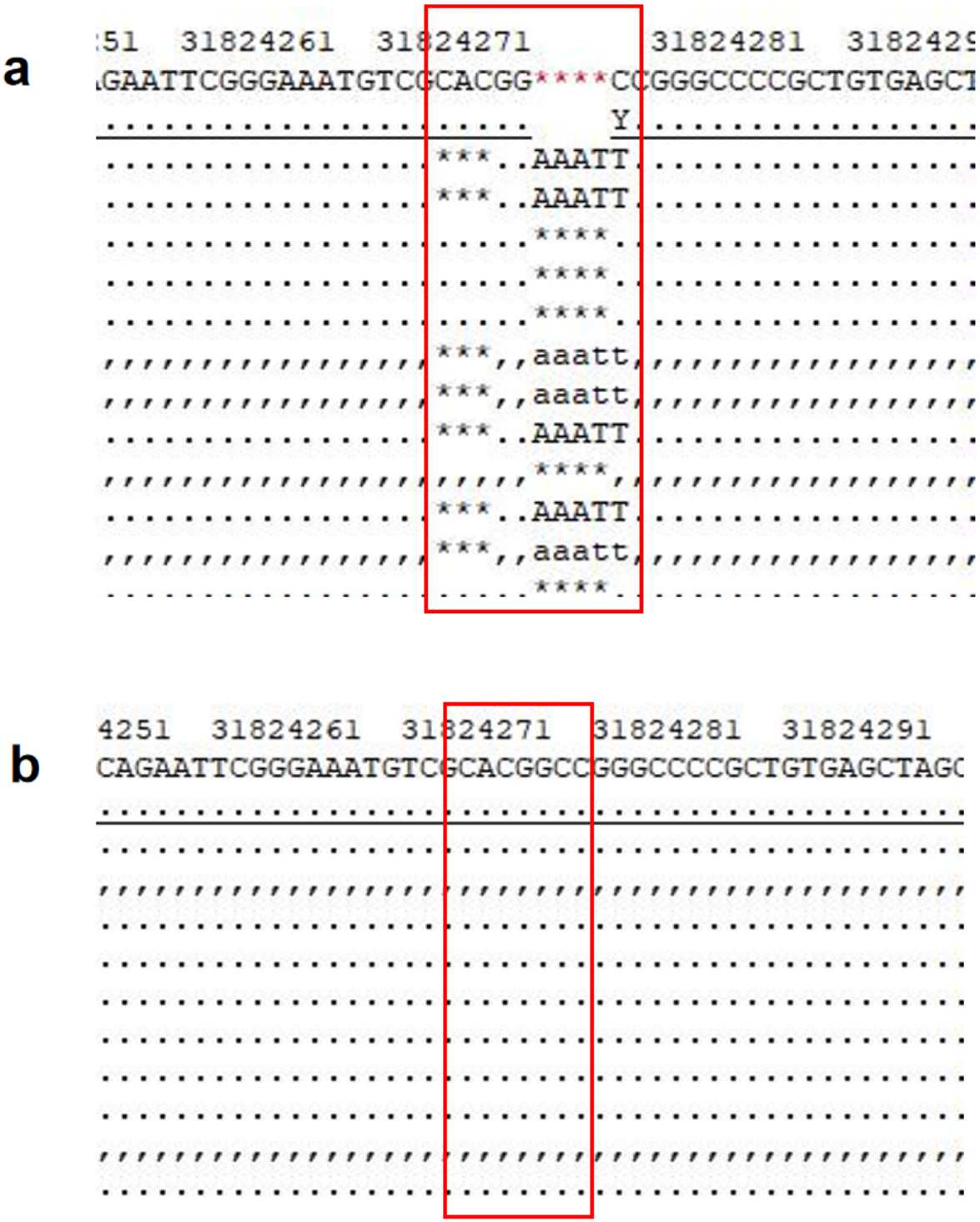



**Figure 4**

Identification of a novel mutation of .a:Next Generation Sequencing revealed the presence of a frameshift heterozygous deletion mutation(red box)in the proband, the proband's brother and proband's father(a), b:Sequencing map of normal phenotypic family members

## Supplementary Files

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