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 affecting the 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, with distinct mutations at a given site within this gene leading to distinct phenotypic findings. This study aimed to characterize genetic mutations associated with congenital aniridia in a Chinese family.

Methods: The proband and the proband's brother of this family underwent comprehensive ophthalmologic examinations as well as exome sequencing, with Next Generation Sequencing being used to confirm these results.

Results: A novel mutation (c.114_119delinsAATTTCC;p.Pro39fs) in the *PAX6* gene was identified in subjects III-2 and III-3 in these family, and both of these subjects exhibited complete

aniridia, cataracts, glaucoma, high myopia, and foveal hypoplasia.

Conclusions: We identified a novel *PAX6* frameshift heterozygous deletion mutation in a Chinese family and determined that this mutation was a probable cause of various eye abnormalities in carriers.

Background

Aniridia is a condition that is defined by a near or total absence of an iris, and can be either acquired or congenital. Congenital aniridia is a rare condition that affects 1:64,000–1:96,000 people and is often sporadic^[1], although up to two-thirds of patients exhibit an autosomal dominant form of aniridia^[2].

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

To date, over 500 mutations in the *PAX6* gene and its regulatory regions have been characterized, with many of these mutations resulting in *PAX6* haploinsufficiency that can cause significant ocular and systemic abnormalities^[5]. To date, however, few studies have characterized hybrid frameshift deletion insertion *PAX6* mutations associated with ocular diseases in Chinese families. In the present report, 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 brother 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, other members of the family had no eye symptoms (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 of the proband and her brother were performed, 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).

2.3. Mutation screening

2.3.1. Sequencing: About 4 ml of venous blood was sampled from proband and proband's brother. Sequence samples were prepared based on appropriate protocols for the Sure Select Target Enrichment System Capture Process (Illumina HiSeq X), after which exome sequencing was conducted.

2.3.2. Analysis: Raw reads that were of low quality were removed, after which remaining reads were mapped to the UCSC (University of California Santa Cruz) hg19 reference genome (<http://genome.ucsc.edu/>), and 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_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>).

2.3.3. Interpretation: The guidelines of the American College of Medical Genetics and Genomics (ACMG) were used to facilitate appropriate data analysis. Briefly, only those genetic variations with known, definitive genetic associations were analyzed, and 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.

2.3.4. Sanger sequencing was used to validate identified mutations.

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.Goldmann tonometry revealed her IOP to be 44 mmHg OD and 38mmHg OS,with her best-corrected visual acuity(BCVA)being 20/100 OD and 20/125 OS.Further evaluation revealed refractive errors of-8.5 D OD and-6.0 D OS,with eyeball axis length(AL)values of 26.3 mm OD and 26.0 mm OS.The anterior chamber in both eyes appeared normal,with peripheral angles being open in both eyes.A slit lamp revealed the presence of bilateral peripheral cataracts and posterior capsular opacification,while UBM examination revealed iris coloboma.A further fundus examination a large optic disc with bilateral glaucomatous cupping and peripapillary atrophy,while OCTA examination revealed diffuse superior and inferior RNFL(retinal nerve fibre layer)thinning,reduced wVD(whole image vessel density),idVD(inside disc vessel density),and ppVD(peripapillary vessel density)vessel density,and significant foveal hypoplasia.Visualfield tests highlighted bilateral glaucomatous visual field defects(Table 1,Figure 2).The proband had no other discomfort, especially hearing loss or abnormal olfaction.

Brother of the Proband

The 23-year-old brother of the proband reported a history of glaucoma that had been diagnosed at a different hospital one year prior,and was managing this condition with IOP-lowering eye drops.With glasses,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 did his sister,including complete aniridia,cataracts,glaucoma,high myopia,and foveal hypoplasia.In addition,the brother exhibited a decreased VD relative to that normally observed in healthy eyes.The superior and inferior RNFL of the brother's eyes were thicker relative to his sister's eyes,and he exhibited less pronounced bilateral glaucomatous visual field defects(Table 1,Figure 3). Proband's brother had no hearing loss or abnormal olfaction either.

3.2 Mutation analysis

Second-generation sequencing analyses revealed the presence of a heterozygous frameshift deletion mutation(c.114_119delinsaatttcc:p.P ro39fs)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).According to the ACMG guidelines, the mutation is a possible pathogenic variant (Table 2).

Discussion

By analyzing a Chinese family with a history of congenital aniridia,we herein identified a novel hybrid mutation(C.114_119delinsAATTTCC:p.Pro39fs)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],and is 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 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.Pro39fs)resulted in a frameshift from the 39th codon of this gene,resulting in 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.

This study has multiple limitations.For one,per the ACMG guidelines,the identified mutation can at present only be identified as a possible pathogenic or clinically unknown mutation that may be linked to phenotypic findings in study subjects.In addition,we identified other mutations that were considered to be beyond the scope of the present study.This study was also unable to identify certain structural mutations(including inversions,rearrangements,and ectopic mutations),dynamic mutations,low prevalence chimeric mutations,regulatory region mutations,or epigenetic variability.Furthermore,owing to the existence of highly repetitive low-complexity regions and homologous sequences,certain exonic regions have little to no coverage in our analyses.

Conclusions

In summary,we identified the novel heterozygous c.114_119delinsAATTTC:p.Pro39fs 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.Our findings further have the potential to aid in the genetic diagnosis of aniridia.

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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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

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Table

Table 1.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(μ m)		wiVD%		idVD%		PPVD%	
	OD	OS	OD	OS	OD	OS	OD	OS
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

Table 2

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 .	Possible pathogenicity
PM2	It is a rare variant, not included in the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/about) East Asian database.	

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 Humphrey 750 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 Humphrey 750 visual field test (i,j). Vertically scanned ultrasonic B images of the eyeball.

Figure 4

Next Generation Sequencing revealed the presence of a frameshift heterozygous deletion mutation (red box) in the proband (a) and the proband's brother (b). Sanger Sequencing confirmed the mutation sites (red arrow), with the dual peaks corresponding to the presence of the indicated heterozygous insertion variants in the proband (c) and the proband's brother (d).