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A novel 4.25 kb heterozygous deletion in *PAX6* in a Chinese Han family with congenital aniridia combined with cataract and nystagmus

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Abstract

Background: The aim of this study is to identify the genetic defect in a Chinese family with congenital aniridia combined with cataract and nystagmus.

Methods: Complete ophthalmic examinations, including slit-lamp biomicroscopy, dilated indirect ophthalmoscopy, anterior segment photography, and anterior segment optical coherence tomography (OCT) were performed. Blood samples were collected from all family members and genomic DNA was extracted. Genome sequencing was performed in all family members and Sanger sequencing was used to verify variant breakpoints.

Results: All the thirteen members in this Chinese family, including seven patients and six normal people, were recruited in this study. The ophthalmic examination of affected patients in this family was consistent with congenital aniridia combined with cataract and nystagmus. A novel heterozygous deletion (NC_000011.10:g.31802307_31806556del) containing the 5′ region of *PAX6* gene was detected that segregated with the disease.

Conclusion: We detected a novel deletion in *PAX6* responsible for congenital aniridia in the affected individuals of this Chinese family. The novel 4.25 kb deletion in *PAX6* gene of our study would further broaden the genetic defects of *PAX6* associated with congenital aniridia.

Keywords: Congenital aniridia, PAX6, Deletion, Copy number variant

Background

Aniridia (Online Mendelian Inheritance in Man identifier, OMIM, 106210) is a rare, congenital ocular disorder with the characteristics of partial or complete absence of the iris. Aniridia occurs in approximately 1/64,000 to 1/96,000 live births and is primarily characterized by iris hypoplasia [1, 2]. Two-thirds of aniridia cases have an obvious hereditary history with autosomal dominant inheritance, complete penetrance and variable expressivity, while the remaining cases refer to sporadic cases [3–5]. Aniridia can occur isolated, as part of WAGR (Wilms tumor, aniridia, genitourinary disorders, and retardation) syndrome, WAGRO syndrome (WAGR and obesity), or other associated syndromes [6, 7]. In addition to the variable iris hypoplasia, congenital aniridia is usually accompanied with lens opacity or dislocation, nystagmus, glaucoma, aniridia-related keratopathy.

Paired box gene-6 (*PAX6*, OMIM: 607108), a member of the paired box gene family located on chromosome 11p13, was identified as a candidate gene for aniridia, spanning about 22 kb and encoding a transcription factor

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that contains two conserved DNA binding domains (a paired box and a paired type homeobox) [8–11]. This gene plays an essential role in eye development, as well as brain, spinal cord and pancreas [12]. Most congenital aniridia cases are caused by variants in *PAX6* [13–15]. Prior to the current study, according to the Human PAX6 Allelic Variant Database (LOVD PAX6 database, version 180,804) (http://lsdb.hgu.mrc.ac.uk/home.php?select_db=PAX6), 491 unique variants of *PAX6* have been identified. Most of these variants are frameshift variants, splice site variants, or nonsense variants, which have been considered to produce truncated proteins or result in loss-of-function due to nonsense mediated decay, while other variants were missense [16, 17].

In this study, we performed genome sequencing to identify the molecular cause of congenital aniridia in a Han Chinese family to further investigate the genetic and phenotypic spectrum of congenital aniridias.

Methods

Subject recruitment and clinical examination

A four-generation family with aniridia was recruited in the Shanghai General Hospital in Shanghai, China. Thirteen family members of this family (Fig. 1) took part in this study. This study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of Shanghai General Hospital. Informed consent was obtained from each participant. Seven of the 13 family members were diagnosed with congenital aniridia. No consanguinity was present in the family. Each family member received complete and comprehensive clinical and ophthalmic examination, including visual acuity test, intraocular pressure (IOP) measurement, anterior segment examination, slit lamp examination, fundus exam and orthoptic evaluation, as well as the examination of physical malformations and neurological deficits. In addition, 300 ethnically matched healthy individuals with no direct or collateral ties and no related phenotypes and systemic underlying diseases were recruited.

DNA preparation

Genomic DNA was extracted from peripheral blood using the TruSeq DNA LT Sample Prep kit (Illumina, San Diego, CA) according to the manufacturer's protocol. DNA samples were stored at − 20 °C until used, and DNA integrity was evaluated by 1% agarose gel electrophoresis.

Whole-genome sequencing

Whole-genome sequencing (WGS) was performed in all 13 family members. The libraries were constructed with TruSeq Nano DNA LT Sample Prepararion Kit (Illumina, San Diego, CA, USA). Briefly, the genomic DNA was sheared into fragments with length ~ 350 bp using S220 Focused-ultrasonicators (Covaris, USA). Adapters were ligated onto the 3′ end of the sheared fragments. After polymerase chain reaction (PCR) amplification and purification, the final libraries were sequenced on the Illumina sequencing platform HiSeq X Ten platform (Illumina Inc., San Diego, CA, USA)

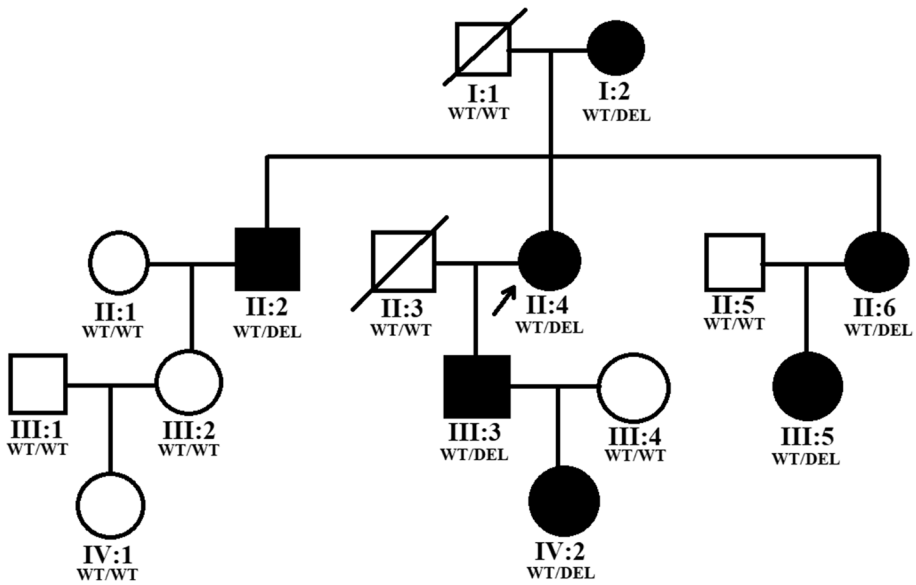

Fig. 1 Pedigree of the four-generation family with congenital aniridia. Solid symbols indicate affected individuals, and open symbols indicate unaffected individuals. Arrow indicates the proband of this family

and 150 bp paired-end reads were generated. The average sequencing depth was at least 30 × .

Routine whole-genome sequencing analysis

The raw reads were subjected to a quality check and then filtered by fastp (https://github.com/OpenGene/fastp). Reads were aligned to hg38 using SpeedSeq [18]. Single nucleotide variants and insertions/deletions (indels) calling were performed using Genome Analysis Toolkit v2.1 [19]. Structural variants and copy number variants were analyzed in SpeedSeq [18]. Annotations of single nucleotide variants, indels, structural variants and copy number variants were performed with ANNOVAR [20]. Variant filtering was performed as illustrated in Supplementary 1.

Real time-polymerase chain reaction

RT-PCR was accomplished using the FastStart Universal SYBR Green Master (Rox) (Roche) in the ABI PRISM® 7300 real time-PCR system (Applied Biosystem, Foster City, CA, USA). POLR2A, RPP14 and TBX15 were used as endogenous controls. We used melting curves to monitor non-specific amplifications. Relative expression level was computed using 2-ΔΔCt method. The primer sequences used were 5′-TCCACG GGGCTCGAATATGG-3′ (forward) and 5′-ACCTCG GTTGGGAGTTCAGG-3 (reverse) for Exon 3, and 5′-AATCTTCTGCCGGGTGGAGT-3′ (forward) and 5′- TTTCCTCAGGTCACAGCGGA-3 (reverse) for Exon 4, separately.

Variant validation

In order to identify the exact breakpoints of the deletion in *PAX6* gene after WGS analysis, primers were designed in the region surrounding the deletion using Primer3 software (version 4.0, http://bioinfo.ut.ee/primer3-0.4.0/). PCR primer pairs and amplification conditions are available upon request. PCR products were checked by 1% agarose gel electrophoresis and purified with SAP-Exon I kit (USB, USA). Purified PCR products were directly sequenced in both forward and reverse directions using an ABI 3730xl genetic analyzer (Applied Biosystems, Foster City, CA, USA) per manufacturer's instructions. DNA sequences were analyzed using Chromas (version 2.22) and DNAMAN (version 7) software. The primer sequences were 5′-TAAATTTATTTTTGT GCTGACCTTG-3′ (forward) and 5′- ATTTCAGGC AAGTTCTGTGGTG − 3 (reverse) for the *PAX6* gene.

Results

Clinical findings

The family investigated in this study shows an autosomal dominant mode of inheritance and is shown in Fig. 1. As illustrated in Table 1, seven affected patients (I:2, II:2, II:4, II:6, III:3, III:5, IV:2) presented with severe visual impairment and glare in both eyes since their early childhood. They received ophthalmic examination and showed similar clinical symptoms, including low visual acuity, aniridia, significant photophobia,

Table 1 Clinical characteristics of the seven patients in this Chinese Han family

Patients	Age, y	Gender	Eye	BCVA	IOP, mmHg	Keratopathy	Aniridia	Nystagmus	Crystalline lens	Glaucoma
I:2	96	F	OD	LP	13	+	+	+	Cataract, Dislocation	–
			OS	LP	15	+	+	+	Cataract, Dislocation	–
II:2	68	M	OD	20/200	15	+	+	+	Absence[a]	–
			OS	HM	17	+	+	+	Absence	–
II:4	64	F	OD	LP	16	+	+	+	Absence	+
			OS	20/200	14	–	+	+	Absence	+
II:6	61	F	OD	20/160	13	–	+	+	Absence	–
			OS	20/120	15	–	+	+	Absence	–
III:3	37	M	OD	HM	22	+	+	+	IOL	+
			OS	HM	23	+	+	+	IOL	+
III:5	34	F	OD	20/120	15	+	+	+	IOL	–
			OS	20/200	16	–	+	+	Cataract	–
IV:2	12	F	OD	20/80	13	–	+	+	Cataract	–
			OS	20/100	11	–	+	+	Cataract	–

M male, *F* female, *OD* the right eye, *OS* the left eye *BCVA* best corrected visual acuity; *LP* light perception, *HM* hand movement, *IOP* intraocular pressure, *IOL* Intraocular lens. All of the other family members have complete iris, without nystagmus or other major eye diseases, and thus are not listed in the table

[a] Absence means the patients had history of phacoemulsification

nystagmus, cataract (or aphasias, intraocular lens). Furthermore, five patients (I:2, II:2, II:4, III:3, III:5) presented with keratopathy and two patients (II:4, III:3) were found to have binocular glaucoma. Representative photos from anterior segment photography, and anterior segment optical coherence tomography (OCT) of the patients with aniridia are shown in Fig. 2. Some non-ocular symptoms, such as intellectual disability, kidney disease, neurological deficits were not found in the patients. All of the other family members did not have an aniridia phenotype or other major eye diseases.

Genome sequencing

Filtered variants identified via whole genome sequencing in the affected members were compared with those present in the six healthy individuals. Annotations and filtering of single nucleotide variants (SNVs), indels, structural variants (SVs) and copy number variants (CNVs) were performed shown in Supplementary 1. All the PAX6 variants were provided as Supplementary 2 and no other rare SNV/Indel or SV/Indel was found that is likely to be involved in disease. A ~ 4.25 kb deletion region in *PAX6* gene was detected in affected members that spanned exons 3–4 (NM_000280.5), likely causing abnormal gene

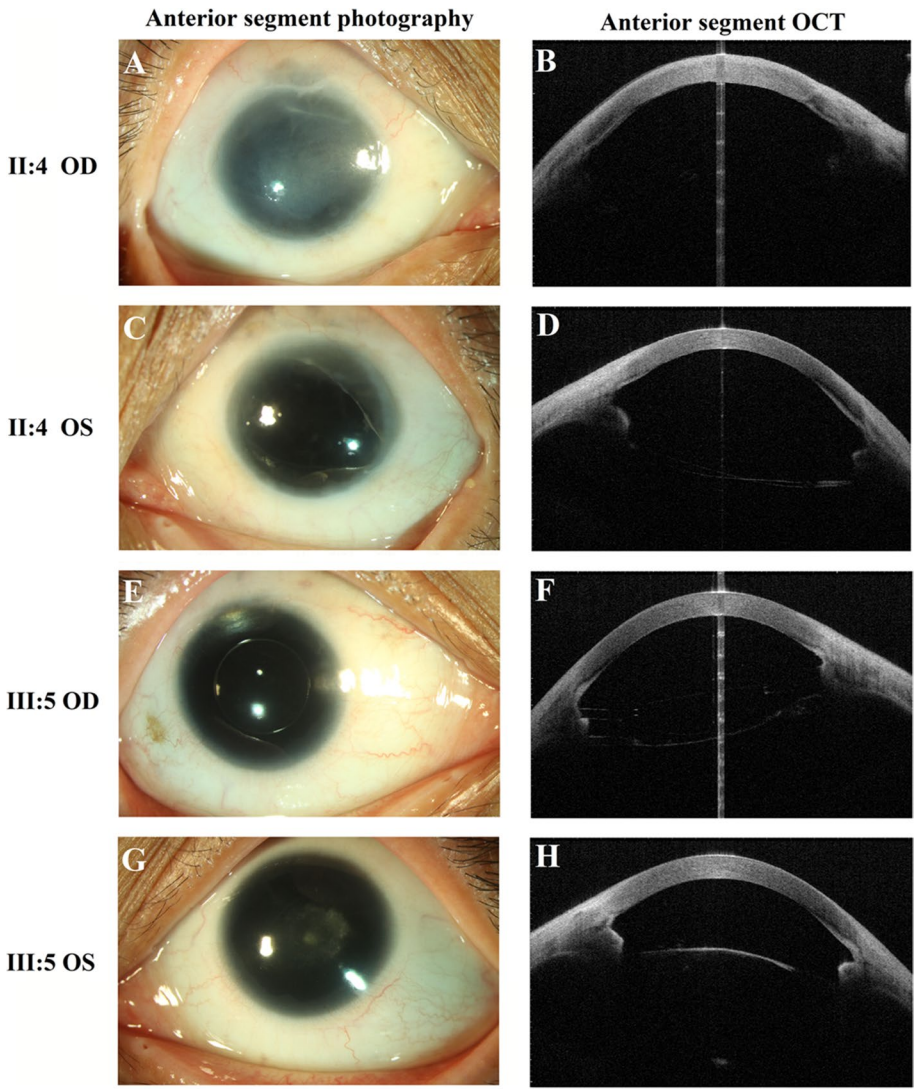

Fig. 2 Representative photos of the patients in the family with aniridia. **A**, **C** Anterior segment photography of the proband (II:4) displayed complete aniridia and aphasias in both eyes and corneal leukoplakia in the right eye. **B**, **D** Anterior segment OCT of the proband (II:4) also exhibited total iris absence in both eyes. **E**, **G** Anterior segment photography of the patient III:5 displayed complete aniridia in both eyes, intraocular lens in her right eye and cataract in her left eye. **F**, **H** Anterior segment OCT of the patient III:5 also exhibited total absence of the iris in both eyes

translation and/or nonsense mediated decay. This variant is absent from the Database of Genomic variants [21]. *PAX6* variants have previously been shown to be implicated in aniridia [22–24], and as this variant co-segregated with the phenotype, it was considered as causative for disease in the patients. Figure 3 shows comparison of high throughput sequencing between affected and unaffected members by Integrative Genomics Viewer (IGV).

Validation of a large deletion in PAX6

The remaining DNA of the family was verified by real-time PCR and the results confirmed skipping of exons 3 and 4 (Fig. 4).

To determine the exact breakpoints of the *PAX6* deletion, we performed PCR and Sanger sequencing using primers flanking the deletion in the patients and unaffected individuals. We verified the presence of a novel 4250 bp heterozygous deletion within the *PAX6* gene, NC_000011.10:g.31802307_31806556del, was identified in all the affected family members (Fig. 5), but not in any of the unaffected members and in the 300 unrelated controls from the same ethnic background. The two breakpoints are located at Intron 4 and the 5′ Untranslated Region (UTR) respectively. The variant was classified as pathogenic based on the guidelines from the American College of Medical Genetics [25, 26].

Discussion

Congenital aniridia, with or without cataract and nystagmus, is a kind of clinically ocular malformation inherited in an autosomal dominant mode of inheritance with variable expression. *PAX6*, which is located on chromosome 11p13, plays an important role in eye development process by regulating the tissue specific expression of various molecules, structural proteins and related hormones [17]. Most of aniridia cases occur due to a genetic defect of *PAX6* no matter what in sporadic and familial forms. A lot of studies [23, 27–31] have reported that variants in the *PAX6* gene can lead to the clinical symptom of aniridia. Furthermore, *FOXC1* and *PITX2* variants were also associated to aniridia-like phenotypes [32]. The present study identified a novel deletion variant in *PAX6* gene in this Chinese Han family. This finding expands the spectrum of the *PAX6* variants resulting in congenital aniridia.

According to the Leiden Open Variation Database (LOVD, https://www.lovd.nl/) *PAX6* gene database, nearly 90% of disease-causing variants lead to the aniridia phenotype, while the remaining 10% causes follicular dysplasia, Peters Syndrome and small eyeballs [33]. Among these aniridia patients, the clinical manifestations are diverse and aniridia can accompanied with other ocular abnormalities. In this family, corneal

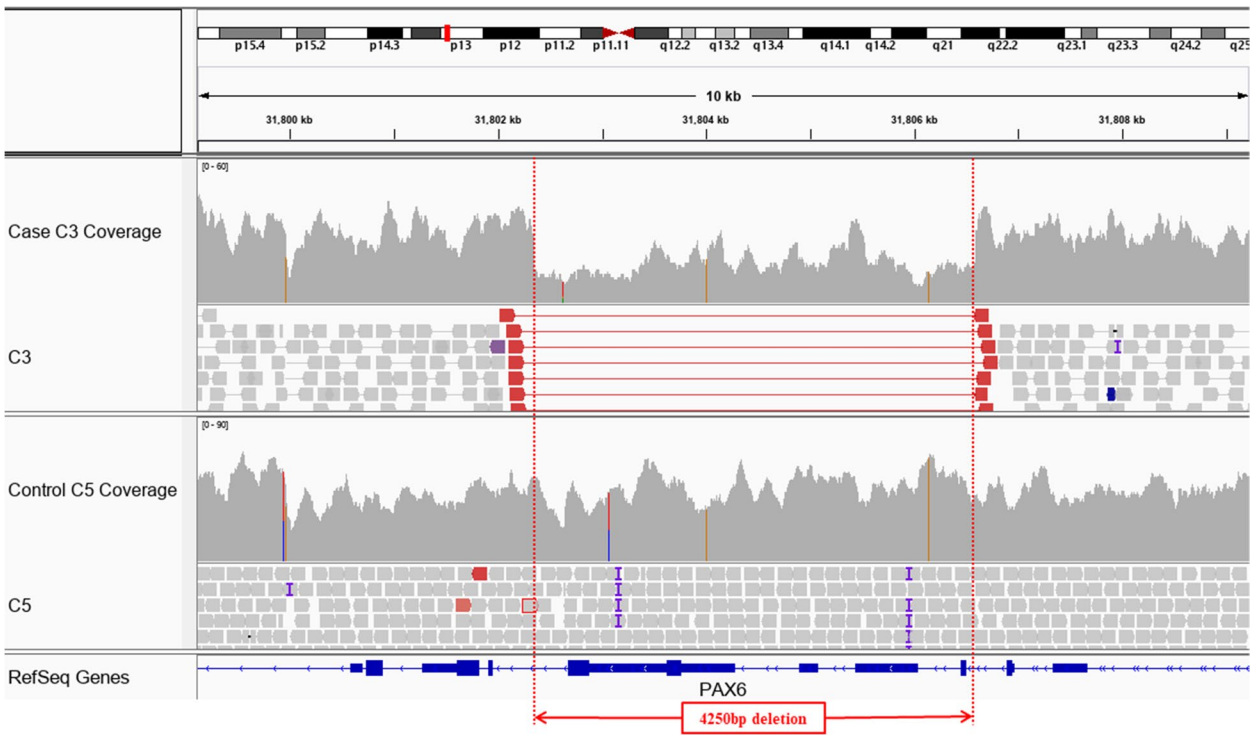

Fig. 3 Comparison of high throughput sequencing between affected (and unaffected members by Integrative Genomics Viewer

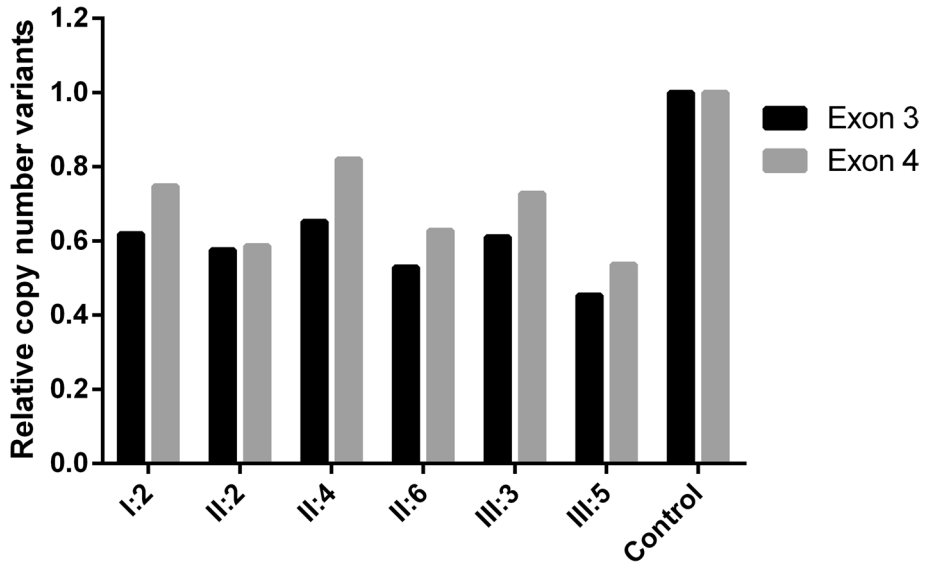

Fig. 4 Quantities of exons 3 and 4 by RT-PCR

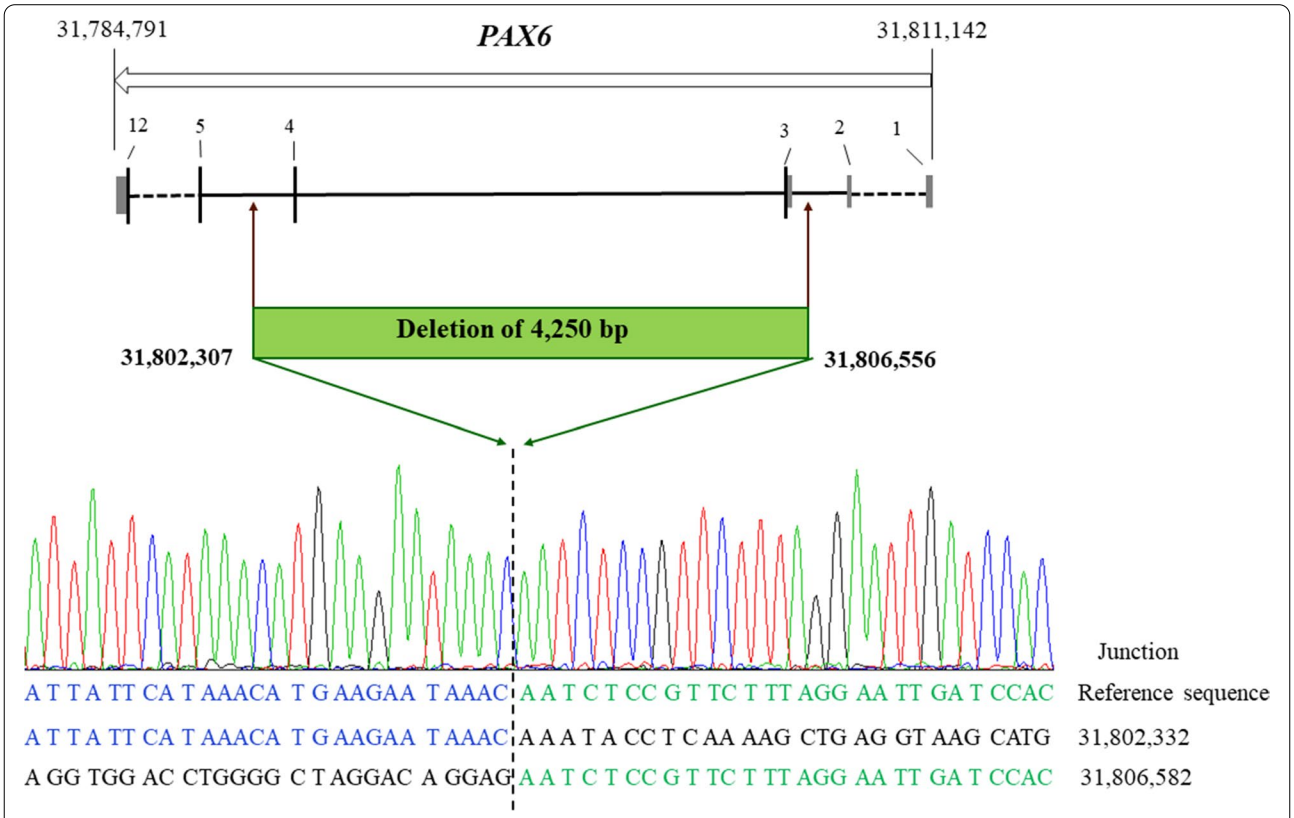

Fig. 5 Sequence chromatograms showing the PAX6 deletion mutation identified in this study. The numbers (from 1 to 12) represent exons; black boxes represent coding region and grey boxes represent UTR; Solid lines exons represent introns; intermittent lines represent introns with unequally proportional length

leukoplakia or nebula could be found in eight eyes of all fourteen affected eyes (57.1%). The condition of the cornea of patients with aniridia also needs regular examination. Corneal opacification and pannus began peripherally and spreads centrally early in life, which could lead to total opacification of the cornea called aniridia-associated keratopathy (AAK) [34, 35]. Abnormalities of the lens were found in all fourteen affected eyes (100%), manifested cataract and dislocation in this family. Reports of the incidence of glaucoma in cases of aniridia is widely variable, from 6 to 75% [36, 37], and 28.6% in our study. In the early stage, the angle appears open in most cases of aniridia and glaucoma is not present. However, tissue strands containing blood vessels form connections between the iris stroma and the angle wall as time goes on. Once this abnormal iris tissue migrates forward, it might obscure the posterior trabecular meshwork and scleral spur, obstructing the angle and blocking aqueous outflow [38].

The *PAX6* gene spans for approximately 22 kb on chromosome 11p13, contains 14 exons, and encodes two major protein isoforms with either 422 (canonical isoform) or 436 (5a isoform) amino acids [39, 40]. *PAX6* is regulated by multiple enhancers located up to hundreds of kilobases from this locus. Variants in this gene or in the enhancer regions can cause ocular phenotypes and the activity of this protein is essential for the development of neural tissues, particularly the eye [8, 30]. In all affected individuals, the 4.25 kb heterozygous deletion encompasses exons 3 and 4, where the transcription starting site (TSS) is located, which can totally destroy translation initiation or initiation from cryptic sites. Some deletions located downstream of *PAX6* without affecting the coding region are also known to cause aniridia [30, 41], likely affecting downstream regulatory regions.

Conclusion

In conclusion, a novel 4.25 kb deletion in the *PAX6* gene was found in this Han Chinese family with congenital aniridia combined with cataract and nystagmus. This result expands the mutation spectrum and provides new genetic defects of *PAX6* gene. With the development of genetic analysis, more detailed attention should be required in the clinical consequence of diverse *PAX6* variants.

Abbreviations

PAX6: Paired box gene-6; BCVA: Best corrected visual acuity; IOP: Intraocular pressure; LP: Light perception; HM: Hand movement; IOL: Intraocular lens; OCT: Optical coherence tomography; WGS: Whole genome sequencing; PCR: Polymerase chain reaction; SNV: Single nucleotide variant; SV: Structural variant; CNV: Copy number variant.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12886-021-02120-0.

Additional file 1.

Additional file 2.

Acknowledgments

We thank the all family members for their participation. All authors attest that they meet the current ICMJE criteria for authorship.

Authors' contributions

X.X. and I.S had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Conception and design: T.Q and C. C. Data collection: T.Q, C.C, C.L, Q.G and K.L. Analysis and interpretation: T.Q, C.C, C.L, G.W, I.S and X.X. Drafting the manuscript: T.Q, C.C and I.S. Critical revision of the manuscript: T.Q, C.L, I.S and X.X. Supervision: X.X and I.S. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated during the current study are available in the National Genomics Data Center (NGDC) repository, the accession number is HRA000707 and the persistent web link is https://bigd.big.ac.cn/gsa-human/s/dGFiEmrB.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of Shanghai General Hospital. A written informed consent was obtained from each participant.

Consent for publication

A written informed consent was obtained from each participant for the publication of this research and any accompanying images.

Competing interests

The authors declare that they have no competing interests.

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