



A rare *PAX6* mutation in a Chinese family with congenital aniridia

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ABSTRACT. Aniridia is an autosomal dominant disorder characterized by the complete or partial loss of the iris and is almost associated with mutations in the paired box gene 6 (*PAX6*). We examined three generations of a Chinese family with congenital aniridia and observed genetic defects. Exons of *PAX6* from 12 family members were amplified by polymerase chain reaction, sequenced, and compared with reference sequences in NCBI reference sequence database (http://www.ncbi.nlm.nih.gov/nuccore/NG_008679.1?from=5001&to=38170&report=genbank). A rare mutation c.2T>A (M1K) in exon 4 of *PAX6* was identified in all affected family members but not in unaffected family members. Our results suggest that the c.2T>A (M1K) mutation may be responsible for the pathogenesis of congenital aniridia in this family. To our knowledge, this is the first report of the M1K mutation in *PAX6* in a Chinese family with this disease and the second report worldwide.

Key words: Congenital aniridia; *PAX6*; Gene mutation; Eye development

INTRODUCTION

Aniridia is characterized by the complete or partial absence of the iris, and it commonly affects both eyes. In addition, aniridia can result in other complex conditions such as corneal disorder, cataract, lens dislocation, foveal hypoplasia, and optic nerve abnormalities in the affected eyes, which can severely compromise vision. Aniridia is associated with several ocular complications, including nystagmus, amblyopia, and buphthalmos, and systemic disorders, including Wilms tumor (Ramaesh et al., 2005; Lang, 2007; Kokotas et al., 2010). Hereditary aniridia is usually inherited in an autosomal dominant manner (Chen et al., 2013).

Paired box gene 6 (*PAX6*; OMIM 607108) is located on chromosome 11p13 and contains 14 exons and 13 introns. It is a member of the paired box gene family and encodes a transcriptional regulator that plays a role in oculogenesis and several developmental processes (Grindley et al., 1995; Collinson et al., 2003; Noriko et al., 2013). Defects (deletions or mutations) in *PAX6* have been identified in patients with anomalies in the iris, indicating a very important role of *PAX6* in eye development (Ton et al., 1991; Jordan et al., 1992; Yan et al., 2011). This gene is expressed in the developing eyes as well as in the nervous system. Mutations in *PAX6* result in defective ocular development in different species, including eyeless phenotype in *Drosophila*, small eye phenotype in mouse, and iris anomalies and Peters' anomaly in humans (Yan et al., 2011). *PAX6* is responsible for ocular disorders, including aniridia and anterior segment anomalies (Jordan et al., 1992; Hanson et al., 1994). To date, more than 800 mutations have been reported in the *PAX6* mutation database (<http://pax6.hgu.mrc.ac.uk>).

In this study, we performed clinical evaluation of a large Chinese family with aniridia and analyzed mutations in *PAX6* in the affected and unaffected family members of this family. We identified a rare c.2T>A (M1K) mutation in exon 4 of *PAX6* in all affected family members. To the best of our knowledge, this is the first report of the M1K mutation in *PAX6* in a family with congenital aniridia in Asia.

MATERIAL AND METHODS

Patients and clinical examination

This study was approved by the Institutional Review Board of Shenzhen Eye Hospital, Jinan University. Written informed consent was obtained from each participant according to the principles of the Declaration of Helsinki. Eight affected and four unaffected family members were enrolled in this study (Figure 1). Non-consanguineous marriages were found in the family. All the individuals underwent complete general ophthalmologic examination, including Snellen best-corrected visual acuity test, anterior segment examination, slit-lamp microscopy, intraocular pressure (IOP) measurement, and fundus examination.

DNA extraction

Samples of peripheral venous blood were obtained from the family members included in the study. Genomic DNA was isolated and purified from 200 μ L venous blood by using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), according to a standard procedure. The integrity of the DNA samples was evaluated by performing electrophoresis on 1% agarose gel.

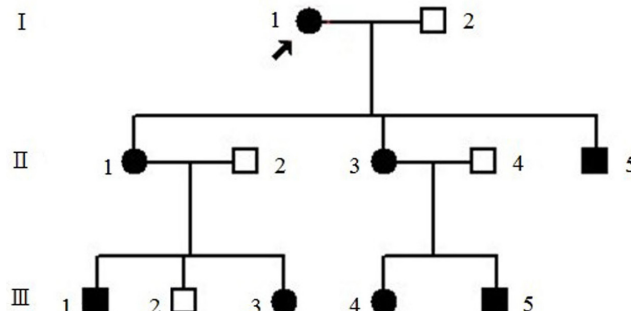


Figure 1. Pedigree of a Chinese family with aniridia and nystagmus. The filled squares and circles indicate affected family members. Arrowhead indicates the proband. All the members indicated in the figure underwent clinical and molecular analyses in this study.

Mutation screening and sequence analysis

Exons of PAX6 and their adjacent splicing junctions were amplified from the genomic DNA by polymerase chain reaction (PCR) with primers that were modified from those used in a previous study (Yan et al., 2011; Table 1). The PCR amplification procedure included initial denaturation at 94°C for 4 min; 35 cycles of denaturation at 94°C for 10 s, annealing at 51-56°C for 30 s, and extension at 72°C for 30 s; and final extension at 72°C for 5 min.

Table 1. Primers used for amplifying PAX6.

Exon	Primer direction	Sequence (5'→3')	Annealing temperature (°C)	Product size (bp)
4	Forward	AAGGGTAGATTTTGTATGCAC	54	276
	Reverse	GAAGTCCCAGAAAGACCAGA		
5	Forward	CCTCTTCACTCTGCTCTCTT	54	257
	Reverse	ATGAAGAGAGGGCGTTGAGA		
5a and 6	Forward	TGAAAGTATCATCATATTTGTAG	54	515
	Reverse	AGGAGAGAGCATTGGGCTTA		
7	Forward	CAGGAGACACTACCATTG	56	265
	Reverse	GACAGGCAAAGGGATGCAC		
8	Forward	GGGAATGTTTTGGTGAGGCT	54	346
	Reverse	TCTTTGACTGAAGATGTGGC		
9	Forward	GTAGTTCTGGCACAATATGG	51	329
	Reverse	GCACTGTGTCTACGTGCGAG		
10 and 11	Forward	CTCGACGTAGACACAGTGC	54	437
	Reverse	TTATGCAGGCCACCACCAGC		
12	Forward	GCTGTGTGATGTGTTCTCTCA	54	245
	Reverse	AAGAGAGATCGCCTCTGTGC		

The PCR products were directly sequenced using an ABI 377XL automated DNA sequencer (Applied Biosystems, Foster City, CA, USA) at the Beijing Genomics Institute. The sequencing data were compared with the published sequence of PAX6 in a pairwise manner. The identified mutation was named based on the nomenclature established by the Human Genomic Variation Society.

Bioinformatics analysis

To analyze the evolutionary conservation of the mutant region, homologous sequences from nine species were aligned using ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). The

potential effect of the c.2T>A (M1K) missense mutation was predicted using SIFT, a tool which predicts whether an amino acid substitution affects protein function on line (<http://sift.jcvi.org/>).

RESULTS

Clinical findings

The proband (patient I: 1; age, 50 years; Figure 1) had aniridia, aniridia-related keratopathy (ARK), glaucoma, nystagmus, and ptosis. Her visual acuity was no light perception (NLP) in both the eyes. Ocular hypertension was noticed in both eyes. Other intraocular structures were not visible because of ARK (Figure 2A).

The proband's first daughter (patient II: 1; age, 27 years; Figure 1) had aniridia, ARK, cataract, superior subluxation of the lens, glaucoma, nystagmus, and ptosis (Figure 2B, Figures 3A-B). Her visual acuity was NLP OD and HM/10 cm OS. IOP was 32 mmHg OD and 27 mmHg OS. Cup/Disc (C/D) ratio was approximately 1.0, and choroidal atrophy was noticed in the fundus of the right eye (Figure 4A).

The proband's second daughter (patient II: 2; age, 24 years; Figure 1) had aniridia, ARK, cataract, temporal subluxation of the lens, glaucoma, nystagmus, and ptosis (Figure 2C-D). Her visual acuity was FC/20 cm OD and HM/10 cm OS. IOP was normal in both the eyes. Her fundus could not be checked because of severe nystagmus.

The proband's son (patient II: 3; age, 18 years; Figure 1) had aniridia, superior subluxation of the lens, nystagmus, and ptosis (Figure 3C). His visual acuity was 0.02 OD and 0.04 OS. IOP was normal in both the eyes. No disorders were noticed in both the fundi.

All the grandchildren of the proband (patient III: 1, III:3, III:4, and III:5; two girls and two boys; age, 2-6 years; Figure 1) had aniridia, nystagmus, and ptosis. Their visual acuities were poor, but IOPs were normal. Retinal abnormalities were not noticed (Figure 4B) in both the eyes of all the grandchildren, except in one child (patient III: 3, Figure 1) who had hypogenetic optic disc in both the eyes and choroidal atrophy in the right eye (Figure 4C-D).

No ocular abnormalities were observed in the four unaffected family members.

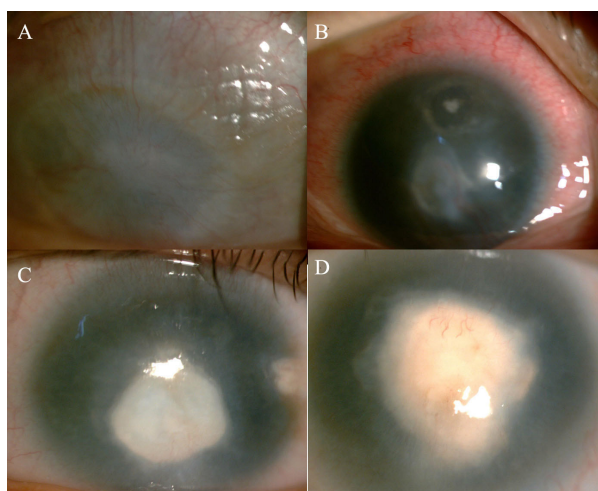


Figure 2. Photographs of the corneal anterior segment of the affected family members. A-D: aniridia-related keratopathy.

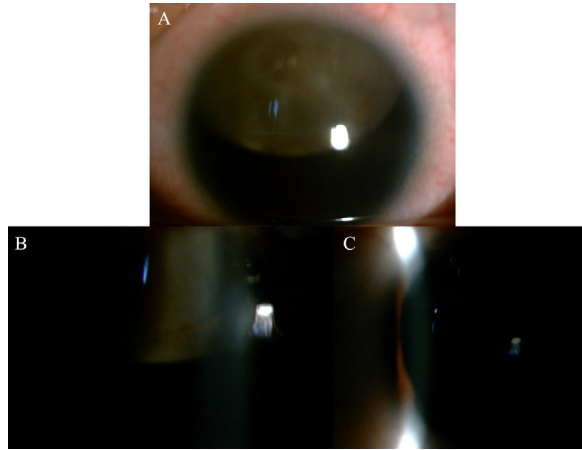


Figure 3. Photographs of the iris and lens of the affected family members. **A.** Superior subluxation of the lens; **B.** Cataract; **C.** Aniridia.

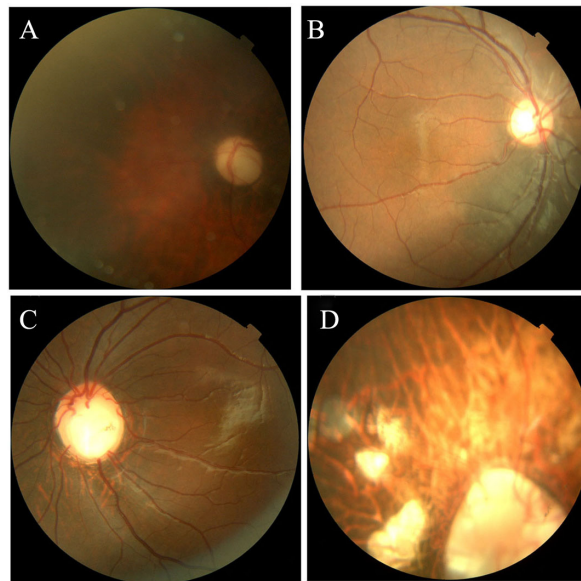


Figure 4. Photographs of the fundus of the affected family members. **A.** C/D = 1.0 and choroidal atrophy; **B.** C/D = 0.3 and boundary of optic disc is clearly; **C-D:** hypogenetic optic disc and choroidal atrophy.

Sequencing of *PAX6*

All the exons of *PAX6* were screened in the affected and asymptomatic family members included in this study. A rare mutation c.2T>A (M1K) was identified in exon 4 of *PAX6* in all the affected family members (Figure 5). This mutation, which was previously reported as a disease-causing mutation in an Icelandic family with congenital aniridia (Gronskov et al., 1999), was co-segregated with the phenotype in the family included in the present study.

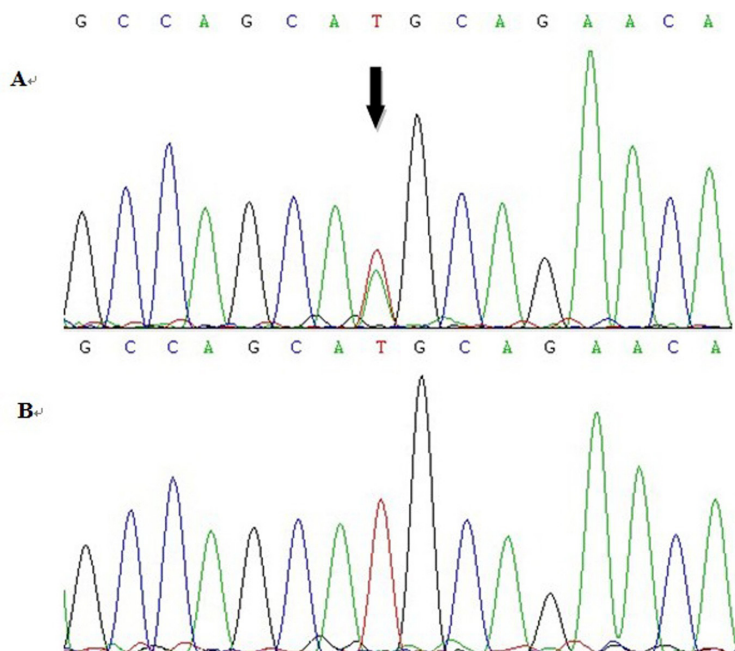


Figure 5. Heterozygous mutation in PAX6 in the Chinese family with aniridia. **A.** Heterozygous missense mutation c.2T>A (M1K) in exon 4 of PAX6 (indicated by the arrow); **B.** Wild type sequence in the control sample.

Bioinformatics analysis

Analysis of orthologs from nine species by using ClustalW2 showed that amino acids in the mutation site of PAX6 were highly conserved (Figure 6). SIFT predicted that the c.2T>A(M1K) mutation was a damaging mutation, with a reliable score of 0.00.

[<i>Gallus gallus</i>]	MQNSHSGVNQLGGVFNAGRPLPDS TRQKIVELAHSGARPCDISRILQ
[<i>Homo sapiens</i>]	MQNSHSGVNQLGGVFNAGRPLPDS TRQKIVELAHSGARPCDISRILQ
[<i>Bos taurus</i>]	MQNSHSGVNQLGGVFNAGRPLPDS TRQKIVELAHSGARPCDISRILQ
[<i>Macaca mulatta</i>]	MQNSHSGVNQLGGVFNAGRPLPDSTRQKIVELAHSGARPCDISRILQ
[<i>Sus scrofa</i>]	MQNSHSGVNQLGGVFNAGRPLPDS TRQKIVELAHSGARPCDISRILQ
[<i>Rattus norvegicus</i>]	MQNSHSGVNQLGGVFNAGRPLPDS TRQKIVELAHSGARPCDISRILQ
[<i>Oryctolagus cuniculus</i>]	MQNSHSGVNQLGGVFNAGRPLPDS TRQKIVELAHSGARPCDISRILQ
[<i>Mus musculus</i>]	MQNSHSGVNQLGGVFNAGRPLPDS TRQKIVELAHSGARPCDISRILQ
[<i>Canis lupus familiaris</i>]	MQNSHSGVNQLGGVFNAGRPLPDS TRQKIVELAHSGARPCDISRILQ

Figure 6. Mutation in a highly conserved residue. Bioinformatics analysis of PAX6 from nine species showing that the codon encoding N-formylmethionine at position 1 is highly conserved.

DISCUSSION

Aniridia is caused by mutations in PAX6 and affects the cornea, anterior chamber, iris, lens, retina, macula, and optic nerve. Symptoms of aniridia, including ARK, glaucoma, cataract, and lens subluxation, may be secondary to anomalies of the anterior segment. Mutations in PAX6, which is a highly conserved gene across evolutionary lineages, have been recognized as the genetic cause of aniridia (Gehring et al., 2001; Yan et al., 2011). PAX6 is essential for the morphogenesis of the eye, brain, nose, and other olfactory organs (Prosser et al., 1998; Gehring et al., 2001). Mice with a homozygous Sey mutation (expressed in the developing pancreas and olfactory cavity epithelium) die at birth because they lack eyes and noses and have severely malformed brains. The only identified patient with presumed homozygous mutations in PAX6 died before 37 weeks of gestation and completely lacked eyes, nose, and adrenal glands (Glaser et al., 1994). The diversity and severity of clinical phenotypes may be correlated with gene dosage effect. Crucial phenotypic variations are associated with PAX6 mutations. To date, more than 800 mutations in PAX6 have been identified (most in exons 5-14) in patients with anomalies of the iris. These mutations have been detected in patients belonging to different ethnicities (Glaser et al., 1992; Jordan et al., 1992).

In this study, affected individuals were present in every generation of the pedigree, and there was no difference in genders with respect to morbidity. In addition, severities of the anterior segment anomalies were different in each affected family member. The main clinical features of the affected family members included in this study were aniridia, nystagmus, and ptosis. The affected family members in the second generation (II: 1, II: 2, II: 3) had lens subluxation, and the two daughters of the proband (II: 1, II: 2) had cataract. The proband (I: 1) and her two daughters (II: 1, II: 2) also had glaucoma and ARK. A 4-year-old affected family member in the third generation (III: 3) had abnormal fundus (optic nerve hypoplasia) in both the eyes. Interestingly, the affected family members in the third generation did not have ARK, glaucoma, cataract, and lens subluxation, which were detected in the affected family members belonging to the first and second generations. These family members showed mild clinical manifestations compared with their elders. Mutations in different domains of PAX6 result in diverse phenotypes (Sale et al., 2002), implying that the diversity of the disease phenotype is related to specific mutations and the resulting gene dosage.

In the present study, genetic analysis of the affected and asymptomatic family members identified a rare heterozygous mutation in exon 4 that changed the ATG codon for methionine to AAG codon for lysine (M1K). This mutation was detected in all the affected family members but not in the asymptomatic family members. Clinical features of the affected family members ranged from total aniridia to other findings such as nystagmus, ptosis, ARK, glaucoma, cataract, and lens subluxation. In 1999, the M1K missense mutation was reported as the cause of aniridia in one Danish patient who had intermediate nystagmus, lens ectopia, late cataract, mild corneal dystrophy, moderate photophobia, and glaucoma (Gronskov et al., 1999). SIFT predicted the deleterious effects of this mutation. Bioinformatics analysis performed in the present study by using ClustalW2 showed that the mutation site was completely conserved in the nine species studied (Figure 6), indicating that this site may play a role in the structure and function of the protein. Therefore, the M1K mutation was considered as the causative mutation of aniridia in this family. To the best of our knowledge, this is the first report of the M1K mutation in PAX6 in patients with aniridia in Asia. This is a rare mutation that occurred in exon 4 and not in exons 5-14, which generally carry most aniridia-causing mutations.

In humans, aniridia and related ocular disorders were caused by heterozygous mutations in the PAX6 gene. While PAX6 haploinsufficiency is loss of function of one allele which leads

heterozygous mutations to no avail (Vincent et al., 2003; Tzoulaki et al., 2005), suggesting that aniridia is a heterozygous disease and that a homozygous condition results in fatality, with almost complete failure of eye development. The mutation M1K is special because it involves the translation initiation codon. This mutation is predicted to abolish translation and is thought to be associated with the complete loss of function of the mutant allele that leads to severe structural and functional changes in the protein. In addition, this mutation is expected to result in a more severe and diversified phenotype. Therefore, this mutation was considered as the cause of variability and severity in phenotypes in the family included in this study and even among members having an identical mutation.

PAX6 mutations resulting in the immature termination of protein translation are often associated with serious symptoms of aniridia. This was evident in the affected family members included in this study who showed severe symptoms. Treatment of aniridia is difficult and is often only partially successful. Early and accurate treatment of young patients can prevent the progression of ocular anomalies. For instance, artificial tears may be useful for delaying problems associated with the ocular surface (Lee et al., 2008). Ophthalmic surgery is useful for the patient with congenital aniridia to improve visual function effectively. In addition, eye surgery can be used to separately assess the risk of several postoperative complications.

In conclusion, we reported the results of the clinical and molecular evaluation of a three-generation Chinese family with aniridia and identified a rare heterozygous M1K mutation in PAX6. This mutation was thought to be associated with considerable variability and severity in phenotypes even among family members having an identical mutation. Similar cases of aniridia should be examined to elucidate the correlation between gene dosage effect and disease phenotype.

Conflicts of interest

The authors declare no conflicts of interest.

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