

A novel mutation of *PAX6* identified in a Chinese twin family with congenital aniridia complicated with nystagmus

X. Cao^{1*}, X.M. Zhou^{1*}, R. Gan^{2*}, L.Q. Jiang², L. Lu², Y. Wang²,
N. Fan², Y. Yin¹, N.H. Yan¹, W.H. Yu¹ and X.Y. Liu²

¹Ophthalmic Laboratories & Department of Ophthalmology,
West China Hospital, Sichuan University, Chengdu, China

²Shenzhen Key Laboratory of Ophthalmology, Shenzhen Eye Hospital,
Jinan University, Shenzhen, China

*These authors contributed equally to this study.

Corresponding author: X.Y. Liu

E-mail: xliu1213@126.com

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ABSTRACT. Genetic variations within the paired box gene 6 (*PAX6*) gene are associated with congenital aniridia. To detect the genetic defects in a Chinese twin family with congenital aniridia and nystagmus, exons of *PAX6* were amplified by polymerase chain reaction (PCR), sequenced and compared with a reference database. Six members from the family of three generations were included in the study. The twins' father presented with congenital aniridia, nystagmus and cataract at birth, while the twins presented with congenital aniridia and nystagmus. A novel mutation c.888 insA in exon 10 of *PAX6* was identified in all affected individuals. This study suggests that the novel mutation c.888 insA is likely responsible for the pathogenesis of the congenital aniridia and nystagmus in this

pedigree. To the best of our knowledge, this is the first report of this mutation in *PAX6* gene in pedigree with aniridia. Furthermore, no *PAX6* gene defect was reported in twins with congenital aniridia.

Key words: Congenital aniridia; Nystagmus; *PAX6*; Twin

INTRODUCTION

Aniridia is a severe ocular disease with an incidence of 1 in 60,000 to 100,000 people (Song et al., 2005). This disease is a congenital eye disorder characterized by the complete or partial absence of the iris and iris hypoplasia (Lee et al., 2008). Vision may be severely compromised and the disorder is frequently associated with a number of ocular complications: nystagmus, amblyopia, buphthalmos, and cataract (Ramaesh et al., 2005; Kokotas and Petersen, 2010).

PAX6 was originally defined by homology to *Drosophila*, paired as a member of the vertebrate paired box-containing (*PAX*) family and mutations in this gene are known to cause developmental ocular disorders such as aniridia. (Ton et al., 1991; Walther et al., 1991; Chi and Epstein, 2002). It is located on chromosome 11p13, and spans 22 kb and consists of 14 exons and 13 introns (Epstein et al., 1994; Chi and Epstein, 2002). Human *PAX6* is composed of two DNA-binding domains: the paired domain (PD) of 128 amino acids and the homeodomain (HD) of 61 amino acids separated by a linker region of 79 amino acids, and is followed by a proline-, serine-, threonine-rich (PST) domain of 79 amino acids that have a transcriptional transactivation function (Ton et al., 1991; Tang et al., 1998; Li et al., 2008) (Figure 1). Each of the domains seems to be the same important. (Simpson and Price, 2002). Mutation in any one of the domains is equivalent in effect (Simpson and Price, 2002). A large number of *PAX6* mutations have been reported in patients with aniridia. Numerous mutations can be found in the Human *PAX6* Mutation Database (<http://lsdb.hgu.mrc.ac.uk/home.php?selectdb=PAX6>) (Gronskov et al., 1999).

In this study, a novel *PAX6* gene mutation was identified in a Chinese twin family with aniridia. Furthermore, to the best of our knowledge, no *PAX6* gene defect was previously reported in twins with congenital aniridia.

MATERIAL AND METHODS

Patients and clinical examination

The study was approved by the Medical Ethics Committee of the Shenzhen Eye Hospital, Jinan University. Informed consent was obtained from all participants according to the principles of the Declaration of Helsinki. Three patients and three unaffected individuals were enrolled in this study. No consanguineous marriage was noticed in the family. The three patients and three unaffected individuals underwent a complete general ophthalmologic examination, including Snellen best-corrected visual acuity, intraocular pressure (IOP), anterior segment evaluation with slit-lamp microscopy, fundus examination, and B ultrasonic scan.

Mutation screening and sequence analysis

Genomic DNA was extracted from 200 µL venous blood using a Qiamp Blood kit (Qiagen, Hilden, Germany). All procedures were performed according to manufacturer instructions. DNA integrity was detected by 1% agarose gel electrophoresis. Exons of the *PAX6* gene and their adjacent splicing junctions were amplified from genomic DNA by polymerase chain reaction (PCR) using the forward and reverse primers that were modified from previous studies (Brown et al., 1998; Alward, 2000; Yan et al., 2011) (Table 1). PCR consisted of an initial denaturation step at 95°C for 2 min, followed by 35 cycles of denaturation at 94°C for 10 s, 51-56°C for 30 s and extension at 72°C for 1 min, and final extension at 72°C for 5 min. PCR products were directly sequenced using an ABI 377XL automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). Sequence data were compared pair-wise with the published *PAX6* sequence.

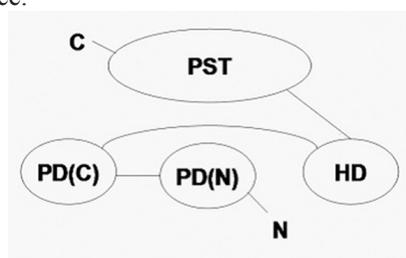


Figure 1. *PAX6* protein contains 3 domain: a paired domain (PD), a homeodomain (HD), and a trans-activation domain (PST).

Table 1. Primers used in polymerase chain reaction for amplification of *PAX6*.

<i>PAX6</i> Exon	Primer direction	Sequence (5' → 3')	Product size (bp)
4	Forward	AAGGGTAGATTTTGTATGCAC	276
	Reverse	GAAGTCCCAGAAAGACCAGA	
5	Forward	CCTCTTCACTCTGCTCTCTT	257
	Reverse	ATGAAGAGAGGGCGTTGAGA	
5a and 6	Forward	TGAAAGTATCATCATATTTGTAG	515
	Reverse	AGGAGAGAGCATTGGGCTTA	
7	Forward	CAGGAGACACTACCAATTGG	265
	Reverse	GACAGGCAAAGGGATGCAC	
8	Forward	GGGAATGTTTTGGTGAGGCT	346
	Reverse	TCTTTGTACTGAAGATGTGGC	
9	Forward	GTAGTTCTGGCACAATATGG	329
	Reverse	GCACTGTGTCTACGTCGAG	
10 and 11	Forward	CTCGACGTAGACACAGTGC	437
	Reverse	TTATGCAGGCCACCACCAGC	
12	Forward	GCTGTGTGATGTGTTCTCTCA	245
	Reverse	AAGAGAGATCGCCTCTGTGC	

RESULTS

Clinical findings

Twin brothers, 12 years of age at the time of analysis, were diagnosed with aniridia and nystagmus at birth. Their corrected visual acuity was 0.1 OD and 0.1 OS. IOP of one twin

(Figure 2, III-1) was 16.1 mmHg OD and 14.4 mmHg OS, while the other (Figure 2, III-2) was 13.7 mmHg OD and 13.4 mmHg OS. Clinical symptoms were identical in both brothers (Figure 3C-F). No abnormalities were detected in the lens, retina and optic nerve. B scanning suggested normal axial lengths.

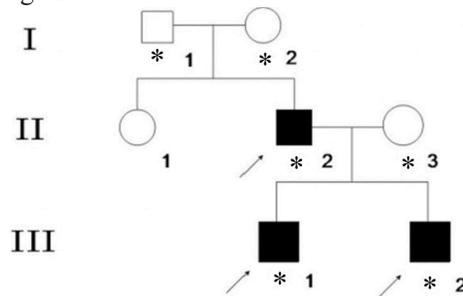


Figure 2. Pedigree of a Chinese family with aniridia and nystagmus. The filled squares and circles indicate affected individuals. Arrow indicates the proband. The asterisks indicate the individuals who had undergone clinical and molecular genetic analyses in the study.

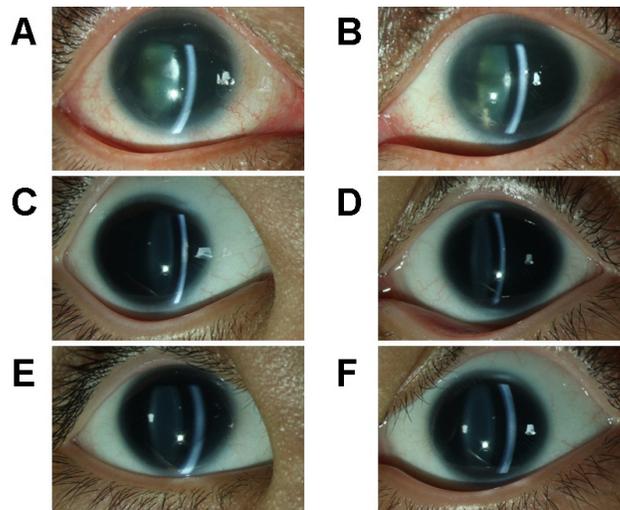


Figure 3. Iris photographs of three patients, **A. B.** the twins' father (II-2); **C. D.** twin (III-1); **E. F.** twin (III-2).

The twins' father (II-2) presented with aniridia, nystagmus, and cataract. His vision was hand motion OU, and IOP was 12.6 mmHg OD and 12.5 mmHg OS. Fundus details could not be seen. He suffered from nystagmus when he was born as noticed by his mother. Several months later, he was diagnosed with aniridia, nystagmus, and cataract (Figure 3 A, B). Ocular abnormalities were not found in the three unaffected members examined in this family.

Mutation screening

All exons of *PAX6* of the affected and unaffected individuals included in this study were analyzed by direct sequencing. A heterozygous mutation, c.888 insA, was identified (Figure 4). The abnormality corresponding to exon 10 was detected in 3 aniridia patients, but was not detected in the 3 unaffected family members and in 100 unrelated Chinese individuals.

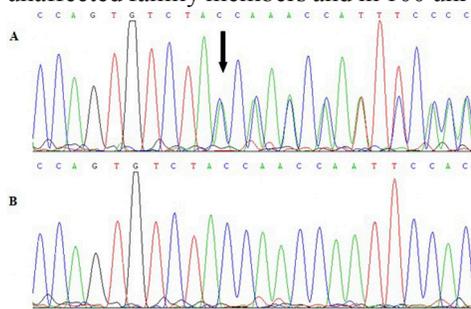


Figure 4. DNA sequence of a part of *PAX6* in the affected patients and unaffected individuals. **A.** A heterozygous insertion at nucleotide 888 (c.888 insA) in exon 10 of *PAX6*. Arrow indicates the location of the mutation. **B.** Normal sequence in control sample.

DISCUSSION

Aniridia is transmitted as an autosomal dominant trait among families in two-thirds of all patients, while other cases are sporadic (Nelson et al., 1984; Zhang et al., 2011). Dysfunction of *PAX6* causes defects during the development of the eye, since *PAX6* mutations are known to cause iris anomalies and Peters' anomaly (Quiring et al., 1994; Yan et al., 2011). Most *PAX6* mutations are found in exons 5-14 (Glaser et al., 1992; Jordan et al., 1992). The *PAX6* gene encodes a highly conserved transcriptional regulatory protein that is expressed in the developing eye, brain, spinal cord and pancreas (Boppana et al., 2012).

In the present study, the novel mutation (c.888 insA) identified generates a frameshift and a premature termination codon (PTC). Most point mutations will lead to a PTC in the *PAX6* open reading frame, which cause over three-quarters of aniridia cases (Prosser and van Heyningen, 1998; Tzoulaki et al., 2005). Nonsense-mediated decay (NMD) is the process by which mRNAs containing PTCs are degraded before they produce large quantities of truncated proteins (Culbertson, 1999; Byers, 2002; Tzoulaki et al., 2005). NMD is a major mechanism acting on *PAX6* mutant alleles and consequently cause most truncated proteins to be produced at significantly lower levels *in vivo* (Tzoulaki et al., 2005).

Congenital aniridia is often correlated with cataract (Gupta et al., 1998; Gronskov et al., 1999; Song et al., 2005;), as occurred in the twins' father in this study. Song et al evaluated 81 different mutations in the *PAX6* gene and 39 of them were accompanied with cataract. There were 18 accompanied with congenital or early cataract in the 39 cases (Song et al., 2005). It was concluded that missense mutations in PD and a reading frameshift in LNK or PST may be primary causes of congenital cataract, while missense mutations in exons 1-6 may cause less severe and more varied phenotypes, especially congenital cataract (Glaser et al., 1994; Azuma et al., 1999; Hanson et al., 1999; Gronskov et al., 1999; Neethirajan et al., 2003; Song et al., 2005).

The twins' father presented with cataract at birth, while the twins did not. The underlying mechanism accounting for the difference between the twins and their father needs to be further explored. The same mutation in *PAX6* has been reported in previous studies to cause different phenotypes (Vincent et al., 2004; Lin et al., 2011), and genotype-phenotype correlations are complicated (Tzoulaki et al., 2005; Lin et al., 2011). The mechanism of aniridia in patients with *PAX6* mutation (c.577_578insG) reported by Bandah et al. (2008) may be either haploinsufficiency of the full-length *PAX6* protein or an aberrant ratio of full-length to paired-less protein isoforms. Various isoforms of *PAX6* protein may be translated due to multiple alternative promoters and splice sites (Kim and Lauderdale, 2006; Bandah et al., 2007; Lakowski et al., 2007; Bandah et al., 2008). The isoforms were expressed together in various tissues and seem to functionally interact to regulate transcription of target genes (Chauhan et al., 2002a,b; Kiselev et al., 2012). Some of these transcripts have a unique expression pattern, which is highly conserved during evolution, but the transcripts have not yet been studied in humans (Lakowski et al., 2007; Bandah et al., 2008).

Conflicts of interest

The authors declare no conflict of interest.

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