

Original Article

Identification of one novel mutant *PAX6* allele in Chinese congenital aniridia and cataract family

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Abstract: Purpose: to identify the pathogenic gene mutation in a Chinese family with congenital aniridia and lens opacities. Methods: after obtained informed consent, detailed ophthalmic examinations were carried out; genomic DNAs were obtained from seven family members in a three-generation Chinese family. All exons of candidate genes were amplified by polymerase chain reaction (PCR) and were sequenced performed by bidirectional sequencing. Results: By sequencing the encoding regions of the candidate genes, a missense mutation (c.1147A>T) was detected in exon 12 of paired box protein 6 (*PAX6*) gene, which resulted in the substitution of highly conserved serine by arginine at codon 383 (p.S383C). The mutation co-segregated with all patients and was absent in 100 normal Chinese controls. Conclusions: The study identified a novel missense mutation (c.1147A>T) in *PAX6* gene associated with autosomal dominant congenital aniridia and cataract in a Chinese family. It gives further evidence of genotype heterogeneity in congenital aniridia associated with *PAX6*. Our findings expand the mutation spectrum of *PAX6* gene in Chinese.

Keywords: Congenital aniridia, congenital cataract, *PAX6*

Introduction

Aniridia is congenital panocular disease with different range of iris abnormalities from iris hypoplasia to full coloboma, along with abnormalities of cornea, anterior chamber angle, lens, retina and optic nerve. Poor visual ability appears to be correlated with absence of the macular reflex, optic nerve hypoplasia, and the development of cataracts, glaucoma, and corneal opacification [1]. The prevalence of aniridia was estimated to be 1:64,000-1:96,000 based on population studies in Michigan and Denmark [2]. Aniridia occurs as a component of systemic defects WAGR (Wilms tumor, aniridia, genitourinary anomalies, and mental retardation) [3], Gillespie syndrome (aniridia, cerebellar ataxia, and mental retardation) [4] or as isolated forms. Aniridia can be sporadic or familial, commonly inherited in autosomic dominant pattern.

The majority of aniridia cases are caused by mutations in paired box protein 6 (*PAX6*) gene. The *PAX6* gene encodes a highly conserved

transcriptional regulatory protein that is expressed in the developing eye, brain, spinal cord and pancreas [5]. It is active early in ocular morphogenesis, fulfilling multiple roles in development of the retina, lens, cornea and iris [5, 6]. Studying the role of *PAX6* in aniridia can help us understanding the morphologic development of ocular tissues and pathologic mechanisms of aniridia.

Herein, our study identified a novel missense mutation (c.1147A>T) in *PAX6* gene in a Chinese family with autosomal dominant congenital aniridia and cataract, leading to the substitution of serine by cysteine at codon 383 (p.S383C) in the PST domain of the protein.

Materials and methods

Clinical evaluation and DNA specimens

A three-generation family with autosomal dominant congenital aniridia and cataract was ascertained (**Figure 1**). After explanation of the nature and possible consequences of the study,

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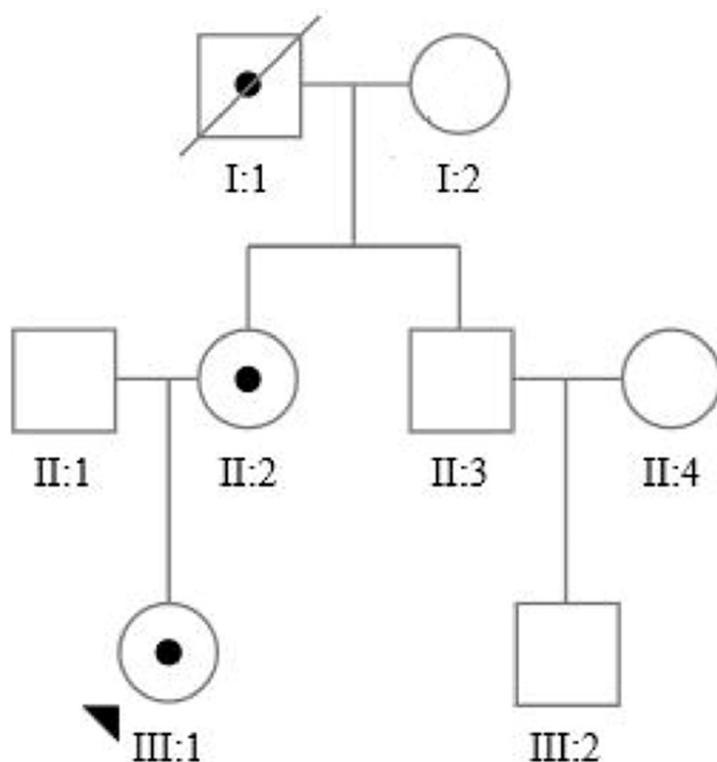


Figure 1. Pedigree of congenital aniridia and cataract. Squares and circles symbolize males and females, respectively. Clear and black dots symbols denote unaffected and affected individuals, respectively. The arrowhead indicates the proband.

Table 1. Primers for PCR amplification of exons in *PAX6* gene

Exons	Forward primer	Reverse primer
1	AGGGAACCGTGGCTCGGC	GGGTGAGGGAAGTGGCTGC
2	TTATCTCTCACTCCTCAGCC	GGAGACCTGTCTGAATATTGC
3	GCTGTTGAACACAGCTTGA	CTGTTTGTGGTTTTGAGCC
4	TTGGGAGTTCAGGCCTACCT	GAAGTCCCAGAAAGACCAGA
5	CTCTTCTCCTTCTCACTCTG	AGAGGGCGTTGAGAGTGG
5a	CTCTACAGTAAGTTCTCATACC	GGAAGTGAGCAGAAAACCAC
6	TGGTTTTCTGTCCACTTCCC	AGGAGAGAGCATTGGGCTTA
7	GTGAGCTGAGATGGGTGA	GACAGGCAAAGGGATGCAC
8	CCCTTTTGGAGGCTCCAAG	TGGCATTACTTGGATTACTGC
9	GTAGTTCTGGCACAATATGG	GTA CTCTGTACAAGCACCTC
10	CTCGACGTAGACACAGTGC	CCCGGAGCAAACAGTTTTAA
11	TTAAACCTGTTTGCTCCGGG	GCCACTCCTCACTTCTCTGG
12	GCTGTGTGATGTGTTCTCA	TAAACACGCCCTCCATAAG
13	CATGTCTGTTTCTCAAAGGG	CGGCTCTAACAGCCATTTTT

seven individuals participated in the study. The study was performed with written informed consent in accordance with the Declaration of Helsinki and following all the guidelines for experimental investigations required by the

Institutional Review Board of Eye and EENT Hospital of Fudan University, Shanghai, China. The Institutional Review Board of Eye and EENT Hospital of Fudan University approved this study. The ophthalmologic examinations, including visual function and dilated slit-lamp examination, were carried out by ophthalmologists. Blood samples were collected and leukocyte genomic DNA was extracted.

Mutation detection

All the exons of candidate genes which associated with autosomal dominant congenital aniridia and cataract were amplified by PCR method, including *PAX6*, *CRYAA*, *CRYBA1/A3*, *CRYBB2*, *CRYBB3*, *CRYGC*, *CRYGD*, *GJA3* and *GJA8* [7]. The primers for *PAX6* gene are listed in **Table 1**. The PCR products were sequenced on both directions with an ABI 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA). The results were analyzed using Chromas (version 2.23) software (<http://www.technelysium.com.au/chromas.html>) and compared with the reference sequences in the NCBI (<http://www.ncbi.nlm.nih.gov/>) gene bank. Mutation naming followed the nomenclature recommended by the Human Genomic Variation Society (HGVS).

Sequences of *PAX6* protein orthologs in human (P26367) and other species were retrieved from the NCBI Protein database. Multiple alignments of *PAX6* protein orthologs from different vertebrate species was conducted using Clustal omega web servers (<http://www.ebi.ac.uk/Tools/msa/clustalo/>).

Bioinformatics analysis

Sequences of *PAX6* protein orthologs in human (P26367) and other species were retrieved from the NCBI Protein database. Multiple alignments of *PAX6* protein orthologs from different vertebrate species was conducted using Clustal omega web servers (<http://www.ebi.ac.uk/Tools/msa/clustalo/>).

Results

Clinical evaluations

We studied a three-generation Chinese pedigree segregating autosomal dominant aniridia and cataract in the absence of systemic defects

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Figure 2. Slit-lamp photograph of right eye in the proband. This photograph showed total opacity of the lens and loss of iris with a remnant iris stump.

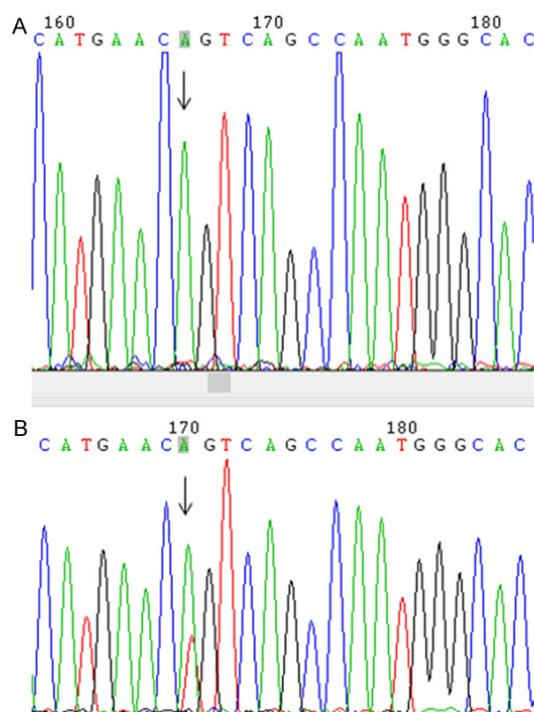


Figure 3. Forward sequence chromatogram of *PAX6* gene. The arrow indicates the A>T transition. The panel A is unaffected, the panel B is affected. The encoded amino acid at codon 383 is AGT encodes serine (S), and TGT encodes cysteine (C).

(**Figure 1**). The affected individual II: 2 had cataract surgery. Ophthalmic records described that the affected individual had loss of iris and bilateral progressive irregular cataract with reduced foveal reflex. Intraocular pressures were not more than 21 mmHg without apparent abnormalities in the optic nerves. The vision acuities in affected II: 2 were less than 20/400 with binocular nystagmus. However, no slit-

lamp images and ocular fundus images pre-surgery were available. The affected individual III: 1 had loss of iris with a remnant iris stump and bilateral cataract (**Figure 2**). Intraocular pressures were less than 21 mmHg in both eyes. The binocular vision acuities in affected III: 1 were hand motion before eyes with binocular nystagmus.

Mutation detection

By bidirectional sequencing of amplified exons of the candidate genes, we found a heterozygous missense mutation, A>T at position 1147 in *PAX6* (NM_001127612.1) in affected individuals, but not in unaffected individuals. The c.1147A>T transition occurred at the first base of codon 383 (AGT>TGT) and was predicted to result in the missense substitution of serine-to-cysteine (p.S383C) at the level of protein translation (**Figure 3**). This mutation was not found in 100 unrelated control individuals. In addition, the identified mutation had not been documented in database of single nucleotide polymorphisms (dbSNP) or in the 1000 genomes project dataset (<http://browser.1000genomes.org>). No other sequence variant was found.

Bioinformatics analysis

Multiple sequence alignment of *PAX6* protein revealed that p.S383 is phylogenetically highly conserved (**Figure 4**).

Discussion

In a Chinese family with congenital aniridia and cataract, we identified a missense mutation c.1147A>T in exon 12 of *PAX6*, leading to the substitution of serine by cysteine (p.S383C). This mutation co-segregated with the phenotype and was not found in 100 unrelated control individuals. It gives further evidence of genotype heterogeneity in congenital aniridia associated with *PAX6*.

The majority of aniridia cases are caused by mutations in paired box 6 gene (*PAX6*). Up to June, 2015, 362 unique *PAX6* DNA variants have been reported in 815 individuals according to *PAX6* mutation database, with more than 80% responsible for aniridia (http://lsdb.hgu.mrc.ac.uk/home.php?select_db=PAX6). *PAX6* protein variants were summarized in **Figure 5**. Nonsense variants and missense substitutions

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Xenopus	ALPPMPSFTMGNNLPMQPPVPSQTSSYSCLPTSPSVNGRSYDITYTPPHMQTHMNSQPMG
Pseudepipalea	ALPPMPSFTMANNLPMQPPVPSQTSSYSCLPTSPSVNGRSYDITYTPPHMQSHMNSQSMG
Homo	ALPPMPSFTMANNLPMQPPVPSQTSSYSCLPTSPSVNGRSYDITYTPPHMQTHMNSQPMG
Mus	ALPPMPSFTMANNLPMQPPVPSQTSSYSCLPTSPSVNGRSYDITYTPPHMQTHMNSQPMG
Rattus	ALPPMPSFTMANNLPMQPPVPSQTSSYSCLPTSPSVNGRSYDITYTPPHMQTHMNSQPMG
Canis	ALPPMPSFTMANNLPMQPPVPSQTSSYSCLPTSPSVNGRSYDITYTPPHMQTHMNSQPMG
Gallus	ALPPMPSFTMANNLPMQPPVPSQTSSYSCLPTSPSVNGRSYDITYTPPHMQTHMNSQPMG
Oryzias	ALPPMPSFTMANNLPMQ---SQTSSYSCLPTSPVNGRSYETITYTPPHMQAHMNSQSMI
Danio	ALPPMPSFTMANNLPMQ---SQTSSYSCLPTSPSVNGRSYDITYTPPHMQAHMNSQSMA
	*****.***** *****:*****:***** *

Figure 4. Multiple alignment of *PAX6* sequences from different species. The box indicated that the p.S383 shares 100% identity among vertebrates.

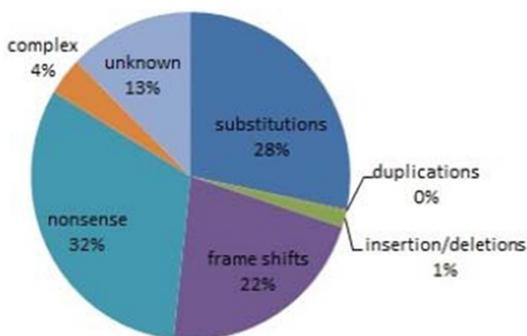


Figure 5. *PAX6* protein variants statistics summary.

account for 60% of protein variants. Over 90% of these variants are predicted to disrupt transcription or translation and likely to be pathological mutations; the remainder are probably neutral polymorphisms.

PAX6 gene with 14 exons, located at 11p13, encodes a highly conserved transcriptional regulator. The 5' terminal of the open reading frame (ORF) encodes two DNA binding domains (a paired domain and a homeodomain) which are separated by a 79-amino acid linker peptide [8]. The 3' terminal of the ORF encodes a transcriptional trans-activation domain (proline, serine and cysteine-rich (PST) region) [8]. *PAX6* protein has transcriptional regulatory function by interactions with target genes through all three of its major function domains [9-12].

The PST region encoded by exons 10-13 of *PAX6* is remarkably conserved among vertebrate species, including human, mouse, quail, zebrafish, sea urchin, squid and ribbon worm [13]. Brain-expressed proteins interact with *PAX6* through the carboxyl -terminus and with the entire PST domain [14]. The last 40 amino acids of the PST domain constitute a highly conserved carboxyl -terminal peptide that has

been implicated in the stabilization of DNA binding by the homeodomain [15]. The dissection study of PST domain in the *GAL4-PAX6* fusion and native *PAX6* expression system showed that all four constituent exons act synergistically to stimulate transcription [13]. Some evidence suggests that conformational structure of the PST domain required for protein-protein interactions play a role in the transcription activity by enhancing the assembly of the transcriptional initiation complex at the promoters of target genes. These complexes may result in either repression or activation of transcription through *PAX6* depending on the complexed partner. Any disruption to the structure of the carboxyl-terminal region could have profound effects on the function of the *PAX6* protein. A reduction in the potency of transactivation of *PAX* genes may lead to a loss-of-function phenotype such as *PAX6* mutations in aniridia. Conversely, an increase in the potency may lead to a gain-of-function phenotype such as overexpression of the human *PAX6* in transgenic mice that have shown an eye phenotype different from small eye [16].

The mutants in the PST region of *PAX6* protein account for 17% of the aniridia phenotype in the variation database (http://lsdb.hgu.mrc.ac.uk/home.php?select_db=PAX6). Substitution, deletion, duplication, insertion mutations in the PST region were predicted to lead to unlikely protein synthesis, abnormally extended protein translation into 3'UTR and reduced transcriptional activation of *PAX6* protein. Missense mutant p.P375Q protein was reported with decreased binding to DNA target and decreased trans-activation activity [17]. Transfection assays demonstrated that p.S363P, p.Q378R, p.M381V, and p.T391A mutant proteins decreased the transcriptional activation potential of *PAX6* protein through the paired

DNA-binding domain in [18]. Missense mutant p. Q422R was reported in a female with aniridia, foveal hypoplasia and cataract and predicted to impair DNA binding activity. Functional studies with expression constructs producing p. Q422R mutant proteins revealed significantly lower transactivation in mutant protein than that in wild-type protein [15]. p. Q422R mutant proteins showed slightly lower target DNA binding than wild-type *PAX6* when a paired domain binding site was used as a probe, however, the mutant proteins failed to bind with target DNA when a homeodomain DNA binding site was used as a probe. This suggested that p. Q422R mutant negatively modulates the function of the homeodomain of *PAX6* protein through conformational alterations and the PST domain participates in modulating the DNA binding function of *PAX6* protein.

The missense mutation p.S383C identified in this study is speculated to change the conformation structure and transcriptional regulatory function of *PAX6* protein, leading the genesis of disease phenotype. Our findings expand the mutation spectrum of *PAX6* gene in Chinese. Further investigation will help us understanding the important role of *PAX6* in the eye development and aniridia formation.

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Disclosure of conflict of interest

None.

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