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A novel *de novo* duplication mutation of *PAX6* in a Chinese family with aniridia and other ocular abnormalities

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Aniridia is a congenital panocular disorder caused by the mutations of the paired box gene-6 (*PAX6*). To investigate the clinical characterization and the underlying genetic defect in a Chinese family with aniridia and other ocular abnormalities, we recruited the family members who underwent ophthalmic examination. Two patients in this family, the proband and his affected son, both have bilateral aniridia, foveal hypoplasia and nystagmus. Moreover, the proband also had presenile cataracts, but his affected son did not show cataracts at the time of examination. Sequencing *PAX6* revealed that a heterozygous duplication mutation c.95_105dup11, predicted to generate non-functional truncated protein at position Gly36 (p.G36X), was found in the affected individuals but not in any of the unaffected family members including the parents of the proband. Haplotype analysis showed that the proband and his affected son shared a common disease-related haplotype, which was arisen from the proband's unaffected father through crossing-over. In conclusion, we identified a novel *de novo* duplication mutation of *PAX6* in the aniridia and other ocular abnormalities family. This mutation has occurred *de novo* on a paternal chromosome by direct duplication, which presumably results from replication slippage or unequal non-sister chromatids exchange during spermatogenesis.

Aniridia (OMIM#106210) is a rare congenital, autosomal dominant hereditary, bilateral, panocular disorder affecting not only the iris but also the cornea, anterior chamber, lens, retina and optic nerve. About two-thirds of cases are familial with dominant inheritance, high penetrance and variable expressivity. The remaining one-third of cases is sporadic and expected to be transmitted to the next generation in an autosomal dominant fashion^{1–3}.

The paired box gene-6 (*PAX6*) (OMIM#607108) on chromosome 11p13 was described as a candidate for human aniridia by positional cloning⁴. The *PAX6* gene encodes a highly conserved transcriptional regulator involved in oculogenesis and other developmental processes^{5,6}. The *PAX6* protein has two DNA binding domains, a paired domain (PD) and a homeodomain (HD), which separated by a glycine rich linker segment (LNK). The C-terminus, a domain rich in proline, serine, and threonine (PST), acts as transactivator⁷. *PAX6* mutations cause small eyes in mice⁸ and eyeless phenotype in *Drosophila*⁹. In humans, heterozygous mutations of the *PAX6* gene cause aniridia as well as other various congenital abnormalities such as Peters' anomaly, foveal hyperplasia, corneal opacification, congenital cataracts, keratitis and microphthalmia^{3,7}. So far, more than three hundred mutations of *PAX6* have been identified in patients with ocular malformations, which were archived in Human *PAX6* Allelic Variant Database¹⁰.

Here, we reported the clinical characterization of a Chinese family with aniridia and other ocular abnormalities, where a novel *de novo* duplication mutation of *PAX6* was identified in the patients of this family. This duplication mutation was presumably derived from paternal chromosome by replication slippage or unequal non-sister chromatids exchange during spermatogenesis.

Results

Two individuals were affected with aniridia and other ocular abnormalities in Family AN-11. The proband (II: 1) was a 40-year-old man with complete absence of the iris, and congenital nystagmus in both eyes. He also suffered from bilateral progressive cataracts at the age of 32 years (Fig. 1-A, B). His visual acuity was very poor (0.15 in left eye and 0.12 in right eye). Using the direct ophthalmoscope, his central fovea of macula area was not observed.

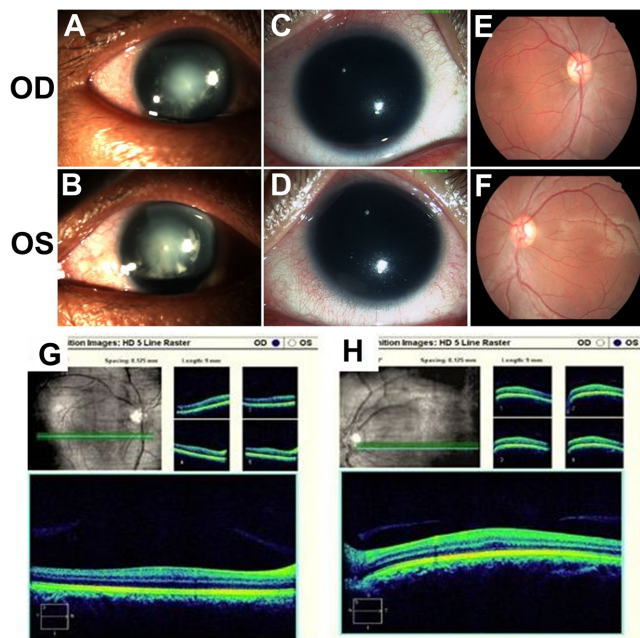


Figure 1 | Clinical features of the affected patients. The proband (II:1) with complete aniridia and presenile cataracts (A, B) and his affected son (III:1) with aniridia (C, D) by slit lamp examination; the foveal hypoplasia of the proband's son (III:1) by funduscopy photographs (E, F) and by optical coherence tomography (G, H). OD and OS stand for right eye and left eye, respectively.

The 18-year-old son (III:1) of the proband had bilaterally no iris (Fig. 1-C, D) accompanied with congenital nystagmus, foveal hypoplasia (Fig. 1-E, F, G, H), and poor visual acuity (0.4 in left eye and 0.3 in right eye), but without cataracts. The proband's parents (I:1 and I:2) were clinically normal in both eyes.

Mutation analysis of *PAX6* in the proband revealed a heterozygous duplication mutation c.95_105dup11TAGCTCACAGC in exon 5 (Fig. 2), which resulted in the introduction of a premature termination codon (PTC) into the N-terminal subdomain of paired domain of *PAX6* (p.G36X). The mutation was also detected in his affected son, but not in his parents, suggesting that it represents a *de novo* and inheritable mutation. This mutation was not detected in other unaffected members of this family and 103 unrelated normal controls (Fig. 2). 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>). Since this duplication mutation has not been reported previously, we deposited it in Human *PAX6* Allelic Variant Database (ID No. PAX6_00668)¹⁰.

To establish the parental origin of the *de novo* *PAX6* mutation, we performed the genotyping with four selected microsatellite markers (D11S904, D11S914, D11S1751 and D11S935) flanking *PAX6* gene in available family members. Both the proband (II:1) and his affected son (III:1) shared the same disease-related haplotype, which was arisen from non-sister chromatids of his unaffected father (I:1) by crossing-over (Fig. 3). To verify paternity, we genotyped additional microsatellite markers located on different autosomes (D1S218, D2S177, D5S2501, D10S1216 and D22S1167) and confirmed that individual I:1 is indeed the biological father of the proband (II:1).

Discussion

We here reported two members in Family AN-11 who were affected with aniridia, foveal hypoplasia and congenital nystagmus. Moreover, the proband was also affected with presenile cataract (onset before age 40 years). Except for aniridia, these clinical features were similar to those described by Thomas et al.¹². The affected son of the proband has not been found to have cataracts at the time of examination, but the risk of developing cataracts is supposed to take place later in his life. Our patients were caused by a heterozygous duplication insertion (c.95_105dup11), leading to a PTC mutation within the paired domain of *PAX6* protein (p.G36X), which consistent with most PTC mutations tend to generate relatively severe phenotypes^{7,13}. The PTC mutant mRNAs are generally detected and degraded by the nonsense mediated decay (NMD)^{7,14} and therefore we predict that our duplication mutation is probably functionally null.

More than one-third of *PAX6* mutations are *de novo*¹⁰, but there are a few reports of the parental origin of them^{15,16}. In this study, we determined that the duplication mutation c.95_105dup11 of *PAX6* has occurred *de novo* on a chromosome inherited from the proband's father and transmitted to his son (Fig. 3). The paternity was unequivocally confirmed by testing with four independent microsatellite markers. The WAGR syndrome (Wilms tumor, aniridia, genital anomalies and mental retardation) is caused by deletion of band 11p13, which involved in *WT1* tumor-suppressor gene and *PAX6* gene^{4,17,18}. Approximately 90% of these deletions are *de novo*, most frequently of paternal origin¹⁹. Thus we supposed that *de novo* insertion and/or deletion mutations in *PAX6* were preferential susceptibility of paternal origin in aniridia. In fact, all *PAX6* *de novo* mutations reported to date occur exclusively on the paternal allele^{15,16}, which also supported the above inference. However, it needs further verification in more cases.

Microdeletions and microinsertions causing inherited disease account for 24% logged mutations in the Human Gene Mutation Database (www.hgmd.org)²⁰. Interestingly, of all the reported mutation types in *PAX6*, both deletions and insertions were found with a considerably high frequency i.e., 145 of 346 (41.9%)⁸, which reflects a hypermutability state of the *PAX6* gene, but the potentially

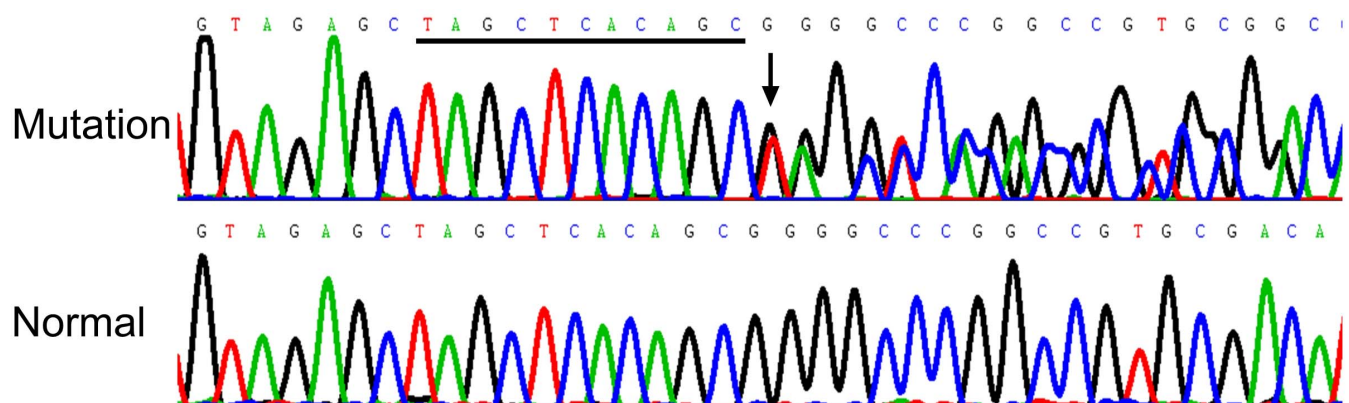


Figure 2 | DNA sequence chromatograms of the c.95_105dup11 mutation in exon5 of human *PAX6* gene.

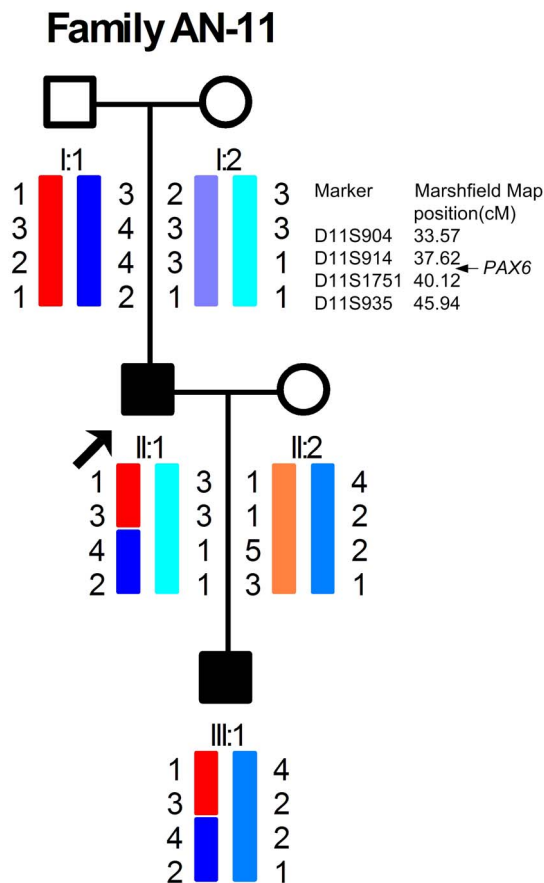


Figure 3 | Pedigree and haplotype analysis of Family AN-11 with aniridia and other ocular abnormalities. Squares and circles symbolize males and females, respectively. Filled symbols denote affected status. The proband is indicated by an arrow. Four selected microsatellite markers (D11S904, D11S914, D11S1751 and D11S935) flanking *PAX6* gene listed in descending order from the centromeric end. *PAX6* gene is located between D11S914 and D11S1751 on 11q13. The disease-related haplotype is arisen from non-sister chromatids of the proband's father (I: 1) by crossing-over. The proband (II: 1) transmitted it to his affected son (III: 1).

underlying mechanism remains unclear. The present *de novo* duplication mutation may result from an unequal crossing-over between non-sister chromatids during spermatogenesis, when the breakpoints and junction occurred exactly at the mutation site.

However, such coincidence appears to be rare and unequal crossing over should often lead to relatively large duplications or deletions²¹. Thus, the most likely explanation is that putative mechanism appears to be occur non-sister chromatid exchange and following slipped mispairing mediated by runs of repeat element (AGC) surrounding the mutational position during DNA replication²⁰. However, we are not able to rule out the occurrence of the other mechanisms due to the small number of patients in our recruited family.

In conclusion, we found a novel *de novo* duplication mutation of *PAX6* in a Chinese family with aniridia and other ocular abnormalities. The *de novo* mutation was of paternal origin, mostly resulting from unequal non-sister chromatids by cross-over during spermatogenesis or slipped-strand mispairing of a direct repeat during replication.

Methods

Subjects and DNA specimens. This study followed the tenets of the Declaration of Helsinki, and was approved by the Ethics Committee of Fujian Medical University. The methods were carried out in accordance with the approved guidelines. Written informed consent was obtained from all participants or parents/legal guardians of all the subjects who were studied.

A three-generation family (Family AN-11) with aniridia and other ocular conditions was recruited, and all of five individuals (2 affected and 3 unaffected individuals) took part in this study (Fig. 3). Clinical and ophthalmological examinations were performed on the affected individuals, as well as on the unaffected family members. Phenotype was documented by slit lamp photography, funduscopy and optical coherence tomography. Blood samples were obtained from the above subjects and 103 unrelated normal controls from the same ethnic background prior to the study. Genomic DNA was extracted from peripheral blood leukocytes using the Wizard Genomic DNA Purification Kit (Promega, Beijing, China), according to manufacturer's instructions.

Mutation screening. The entire coding exons and splice junctions of the human *PAX6* gene were amplified by PCR using previously reported PCR primers and conditions¹¹, which were listed in Table 1. PCR products were purified using Wizard SV Gel and PCR Clean-Up System (Promega, Beijing, China) according to the manufacturer's instructions, and were directly sequenced using M13 forward primer and M13 reverse primer (Table 1). When a suspected mutation is found in the proband, it was further confirmed in all of available other family members as well as in 103 normal unrelated individuals from the same ethnic background. Mutation descriptions follow the nomenclature recommended by the Human Genomic Variation Society.

Haplotyping analysis. To determine the parental origin of the *de novo* mutation, the genotyping was performed with four selected microsatellite markers (D11S904, D11S914, D11S1751 and D11S935) flanking *PAX6* gene in available family members. The additional microsatellite markers located on different autosomes (D1S218, D2S177, D5S2501, D10S1216 and D22S1167) were performed haplotyping analysis for verification of paternity. Briefly, PCR products from each DNA sample were separated by gel electrophoresis with a fluorescence-based on ABI 3730 automated sequencer (Applied Biosystems) using ROX-500 as the internal lane size standard. The amplified DNA fragment lengths were assigned to allelic sizes with GeneMarker Version 2.4.0 software (SoftGenetics, State College, Pennsylvania, USA). Pedigree and haplotype data were managed using Cyrillic (version 2.1) software.

Table 1 | PCR primers used for amplification of *PAX6* gene

Exon	Primer Name	M13 forward primer or reverse primer + specific sequence 5'-3'	Product size(bp)
1 ~ 2	PAX6-1MF	TGTAACACGACGGCCAGTCTCATTTCCTGCTGCTG	472
	PAX6-2MR	CAGGAAACAGCTATGACCAAGCGAGAAGAAAGCGG	
3 ~ 4	PAX6-3MF	TGTAACACGACGGCCAGTTCAGAGAGCCCATGGACGTAT	625
	PAX6-4MR	CAGGAAACAGCTATGACCGAAGTCCCAGAAAGACCAGA	
5 ~ 5a	PAX6-5MF	TGTAACACGACGGCCAGTCTCTTCTCTCTCTCACTCTG	1117
	PAX6-5aMR	CAGGAAACAGCTATGACCGGGAAGTGGACAGAAACC	
6 ~ 7	PAX6-6MF	TGTAACACGACGGCCAGTGGTTTCTGTCCACTTCCC	1209
	PAX6-7MR	CAGGAAACAGCTATGACCGAGCATGGAAGCCCTGAGAGGA	
8 ~ 9	PAX6-8MF	TGTAACACGACGGCCAGTGGGAATGTTTGGTGAGGCT	960
	PAX6-9MR	CAGGAAACAGCTATGACCGTACTCTGTACAAGCACCTC	
10 ~ 11	PAX6-10MF	TGTAACACGACGGCCAGTGTAGACACAGTGCTAACCTG	431
	PAX6-11MR	CAGGAAACAGCTATGACCTTATGCAGGCCACCAACGAGC	
12 ~ 13	PAX6-12MF	TGTAACACGACGGCCAGTGAATGATCAGACTTGTGGCAG	1152
	PAX6-13MR	CAGGAAACAGCTATGACCGAAGTGAAGCGGCTCTAACA	

DNA sequencing primers: M13 forward primer (TGTAACACGACGGCCAGT) and M13 reverse primer (CAGGAAACAGCTATGACC; PCR conditions: 94°C/5'; 94°C/30", 58°C/45", 72°C/1 ~ 2', 10 cycles; 94°C/30", 61°C/45", 72°C/1 ~ 2', 25 cycles; 72°C/5'; 4°C/∞.



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Author contributions

Study design: J.H.Y. and K.X.Z. collected the samples and performed the experiments: J.F.Z., X.L.C., Z.H.T. and Y.H.Z. Data interpretation and analysis: J.H.Y., J.F.Z. and X.L.C. Wrote the manuscript: J.H.Y. and X.L.C. All authors have read and approved the final manuscript.

Additional information

Competing financial interests: The authors declare no competing financial interests.

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