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

Jianfu Zhuang1,2*, Xiaole Chen3*, Zhihua Tan3, Yihua Zhu4, Kanxing Zhao2 & Juhua Yang3

1Xiamen Eye Center of Xiamen University, Xiamen, Fujian, China, 2Clinical College of Ophthalmology, Tianjin Medical University, Tianjin, China, 3Biomedical Engineering Center, Fujian Medical University, Fuzhou, Fujian, China, 4Department of Ophthalmology, The First Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian, China.

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.

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.
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 (D15S18, 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.13. 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 mutation 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 phenotypes7,13. 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 novo10, but there are a few reports of the parental origin of them15,16. 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 gene4,17,18. 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 allele15,16, 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). 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%), which reflects a hypermutability state of the PAX6 gene, but the potentially...
breakpoints and junction occurred exactly at the mutation site. Between non-sister chromatids during spermatogenesis, when the underlying mechanism remains unclear. The present proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. The proband (II-1) transmitted it to his affected son (III-1) by crossing-over. However, such coincidence appears to be rare and unequal crossing over should often lead to relatively large duplications or deletions. 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 replication. 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 due to unequal crossing over.

**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 conditions (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 | TGTAAAAACGACGCGCAGTCCTATTCCTCCGGCGCTGTGTTT | 472 |
| 3–4 | PAX6-2MR | CAGGAAAAACGCTATGACCAGCAGAAAGAAGGCGG | 625 |
| 5–5a | PAX6-5MF | TGTAAAAACGACGCGCAGTCCTATTCCTCCGGCGCTGTGTTT | 1117 |
| 6–7 | PAX6-6MF | CAGGAAAAACGCTATGACCAGCAGAAAGAAGGCGG | 1209 |
| 8–9 | PAX6-7MR | CAGGAAAAACGCTATGACCAGCAGAAAGAAGGCGG | 960 |
| 10–11 | PAX6-8MF | TGTAAAAACGACGCGCAGTCCTATTCCTCCGGCGCTGTGTTT | 1152 |

DNA sequencing primers: M13 forward primer (TGTTAAAACGACGGCGGTGTC) and M13 reverse primer (CAGGAAAAACGCTATGACCAGG).

PCR conditions: 94°C/30; 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. and X.L.C. Data interpretation and analysis: J.H.Y. 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
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