Novel mutations of the *PAX6* gene identified in Chinese patients with aniridia

Panfeng Wang, Xiangming Guo, Xiaoyun Jia, Shiqiang Li, Xueshan Xiao, Qingjiong Zhang

Key Laboratory of Ophthalmology of the Ministry of Education and Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, People’s Republic of China

**Purpose:** Mutations in *PAX6* are the major cause of aniridia. Only a few *PAX6* mutations in Chinese have been reported. This study is to identify novel mutations in *PAX6* of Chinese patients with aniridia.

**Methods:** Aniridia patients were collected from 11 Chinese families, and genomic DNA was prepared from venous leukocytes. The coding regions of *PAX6* were analyzed by direct sequencing of PCR products. Variations detected were further evaluated in available family members as well as in controls by using heteroduplex-SSCP analysis.

**Results:** Four novel mutations including c.141+1G>A, c.184-3C>G, c.542C>A (Ser181X), and c.562C>T (Gln188X) and one known mutation c.120C>A (Cys40X) were identified in *PAX6* of five unrelated patients with aniridia. All five mutations are expected to generate null alleles of *PAX6*. Varied ocular phenotypes were observed in different patients within families.

**Conclusions:** We identified four novel mutations and 1 known mutation in the human *PAX6* gene. These results expand the mutation spectrum in *PAX6* and enrich our knowledge of genotype-phenotype relation due to *PAX6* mutations.

*PAX6* (OMIM 607108) encodes a transcriptional regulator and is expressed in ocular tissues during development, which plays an important role in ocular genesis [1-7]. This gene is situated on 11p13 with 11 coding exons (exon 4 to exon 13 and an extra exon, 5a, that is involved in alternative splicing). Mutations in *PAX6* usually result in aniridia (OMIM 106210) [8] but also, in rare cases, cause other ocular abnormalities such as congenital cataract, Peters anomaly, corneal dystrophy, autosomal dominant keratitis, foveal hypoplasia, microphthalmia, optic nerve malformations including coloboma, morning glory disc anomaly, and optic nerve hypoplasia. A number of mutations have been identified in the *PAX6* mutation database. Reports of *PAX6* mutations in Chinese are limited. Only five novel mutations have been identified [9-11].

In this study, the coding sequences of *PAX6* were analyzed in 11 Chinese families with aniridia. Four novels and one known mutation in *PAX6* were detected, all of which were heterozygous. In two families with novel mutations, affected members from the same family had different clinical phenotypes.

**METHODS**

*Patients and clinical data:* All patients participating in this study were from our Pediatric and Genetic Clinic, Eye Hospital of the Zhongshan Ophthalmic Center. Informed consent conforming to the tenets of the Declaration of Helsinki and following the Guidance of Sample Collection of Human Genetic Diseases (863-Plan) by the Ministry of Public Health of China was obtained from each participant prior to the study. Medical and ophthalmic histories were obtained. Ophthalmologic examinations including visual acuity, slit-lamp and funduscopy examinations were performed by Drs. Zhang and Guo. Initially, 11 probands with aniridia from unrelated families participated in this study.

*Mutation screening:* Genomic DNA was prepared from venous blood. Nine pairs of primers (available on request) [12,13], were used to amplify the 11 coding exons (exon 4 to exon 13 and an extra exon, 5a) and the adjacent intronic sequence of *PAX6* (NCBI human genome build 35.1, NC_000011 for gDNA, NM_001604 for cDNA and NP_001595 for protein). The PCR products of individual exons for each patient were sequenced with the ABI BigDye Terminator cycle sequencing kit v3.1, (ABI Applied Biosystems, Foster City, CA) according to the manufacturer’s recommendations, using an ABI 377 or 3100 sequencer, initially by two commercial sources and finally confirmed by ourselves. Sequencing results from patients as well as *PAX6* consensus sequences from the NCBI human genome database (NM_001604) were imported into the SeqManII program of the Lasergene package (DNASTar Inc., Madison, WI) and aligned to identify variations. Each mutation was confirmed by bidirectional sequencing. Mutation description followed the nomenclature recommended by the Human Genomic Variation Society (HGVS).

*Heteroduplex-SSCP analysis:* Any variation in *PAX6* detected was further evaluated in available family members as well as in 100 normal controls by using heteroduplex-SSCP analysis as previous described in the literature [14]. Two additional pairs of primers (available on request) [12,13], were synthesized for heteroduplex-SSCP analysis of exon 6 and exon 7.

644
RESULTS

Mutation analysis: Direct sequence of PAX6 6 coding regions revealed five heterozygous mutations, including c.120C>A (Cys40X), c.141+1G>A (IVS5+1G>A), c.184-3C>G (IVS5b-3C>G), c.542C>A (Ser181X), and c.562C>T (Gln188X; Figure 1, Table 1). These five mutations were also detected by heteroduplex-SSCP analysis (Figure 2) but none were detected in 100 control samples. Of the five mutations, the first was a known mutation and the other four have not been reported before. For the two families with the c.141+1G>A or c.184-3C>G mutations, the pedigrees are shown in Figure 1. The patient column presents mutation sequence in each family and the normal column shows the corresponding normal sequence. The exact mutations in the five families were labeled under each sequence according to the nomenclature recommended by the HGVS.

Figure 1. Mutations in PAX6 identified in five unrelated families with aniridia. Pedigrees are shown in the left column. The patient column presents mutation sequence in each family and the normal column shows the corresponding normal sequence. The exact mutations in the five families were labeled under each sequence according to the nomenclature recommended by the HGVS.
A 3C>G mutation, one additional affected relative examined also had the same mutation and the disease in the families transmitted as an autosomal dominant trait (Figure 1).

**Genetic information analysis:** The c.542C>A, c.562C>T, and c.120C>A mutations create a premature stop codon and therefore could produce a null allele. The c.141+1G>A and c.184-3C>G mutations involve the splicing sites of PAX6 and would be expected to result in loss of the corresponding splicing sites. To check this possibility, the sequences affected by these two mutations were analyzed for their corresponding information contents by using the Automated Splice Site Analysis (ASSA) server [15,16]. This analysis predicts that the c.141+1G>A mutation will significantly alter the strength of this splicing donor (from 6.8 bits to -6.0 bits) and therefore will abolish this natural splicing site. For the c.184-3C>G mutation, the mutant splicing site of PAX6 in individual AN14 is 6 bits weaker (6.3 bits compared to the natural 12.3 bits) than the natural exon 5a acceptor site, corresponding to about a 62.7 fold decrease in the predicted affinity for this site. Both of these two splicing site mutations would be expected to generate a functionally null allele of PAX6.

**Clinical findings:** Of the five families identified to have causative PAX6 mutations, four probands had complete bilateral defects of the iris and the other one (AN252) just had a partial iris defect (Figure 1, Figure 3). Nystagmus was present in all affected members. One proband (AN233, Figure 3A-C) and two affected family members (AN252M, AN14S) had congenital cataract. Ectopia lentis, corneal dystrophy, or foveal hypoplasia was observed in one proband, respectively (Table 1). The visual acuity of affected individuals was rather poor between 0.1 and 0.2, except for two boys (AN181 and AN252) who were too young to evaluate visual acuity.

AN252M had typical aniridia and congenital cataract but her son (AN252) only had partial iris defect (Figure 1, Figure 3D,E) even though they had the same c.141+1G>A mutation in PAX6. Similarly, AN14S had iris hypoplasia and congenital cataract but his half brother AN14 only had typical aniridia even though both of them inherited the c.184-3C>G mutation from their mother.

**DISCUSSION**

The PAX6 encoded transcription factor contains two DNA binding boxes: a paired box (PB) and a homeobox (HB). A linker region (LNK) situates in between these two boxes and a proline, serine, and threonine-rich (PST) region follows the HB box. The last 30 amino acids constitute a highly conserved COOH-terminal peptide (CT) that regulates DNA binding [17]. In this study, four novel heterozygous mutations and one known mutation were identified in PAX6 of five unrelated Chinese patients with aniridia (Figure 1, Table 1): three (c.120C>A, c.141+1G>A, and c.184-3C>G) involved the PB and the other two (c.542C>A and c.562C>T) affected the LNK.

Of the five mutations, three created a premature termination codon and two are expected to result in loss of splicing sites. The resulting mRNA from the three nonsense mutations should be detected by RNA surveillance and degraded through

---

**Table 1. Sequence variations in PAX6 identified in Chinese patients and the ocular phenotypes**

<table>
<thead>
<tr>
<th>ID</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Inheritance</th>
<th>Clinical manifestation</th>
<th>Exon</th>
<th>HGVS*</th>
<th>Database**</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN233</td>
<td>12</td>
<td>M</td>
<td>Sporadic</td>
<td>Aniridia, congenital cataract, ectopia lentis</td>
<td>e5</td>
<td>c.120C&gt;A</td>
<td>c.482C&gt;A</td>
<td>Cys40X</td>
</tr>
<tr>
<td>AN252</td>
<td>1</td>
<td>M</td>
<td>AD</td>
<td>Partial aniridia</td>
<td>i5</td>
<td>c.141+1G&gt;A</td>
<td>IVS5+1G&gt;A</td>
<td>Loss of splicing donor</td>
</tr>
<tr>
<td>AN14</td>
<td>7</td>
<td>M</td>
<td>AD</td>
<td>aniridia</td>
<td>i5b</td>
<td>c.184-3C&gt;G</td>
<td>IVS5b-3C&gt;G</td>
<td>Loss of splicing acceptor</td>
</tr>
<tr>
<td>AN188</td>
<td>7</td>
<td>M</td>
<td>Sporadic</td>
<td>Aniridia, foveal hypoplasia, strabismus</td>
<td>e7</td>
<td>c.542C&gt;A</td>
<td>c.862C&gt;A</td>
<td>Ser181X</td>
</tr>
<tr>
<td>AN181</td>
<td>1</td>
<td>F</td>
<td>Sporadic</td>
<td>Aniridia, corneal dystrophy#</td>
<td>e7</td>
<td>c.562C&gt;T</td>
<td>c.882C&gt;T</td>
<td>Gln188X</td>
</tr>
</tbody>
</table>

All affected individuals had bilateral nystagmus. Asterisk indicate that the mutations were named according to the nomenclature recommended by Human Genome Variation Society (HGVS) and the double asterisk signifies that the mutations were named according to the human PAX6 allelic variant database. The sharp (hash mark) means all signs were present in both eyes but corneal dystrophy was only observed in the left eye of the individual AN181.
Figure 2. Detection of PAX6 mutations by heteroduplex-SSCP analysis. Two different PAGE gels (A, B) were used. Abnormal migrational SSCP band patterns for each of the five mutations were demonstrated on both gels as compared with normals. A plus sign under a lane indicates a sample with a mutation; a minus sign indicates a normal.

Figure 3. Ocular photographs of two patients with PAX6 mutations. Photos of 12-year-old AN233 taken at the slit lamp with direct (A, B) and retroillumination (C) demonstrate aniridia, ectopia lentis, and congenital cataract. D, E: Photos of 1-year-old AN252 taken at the slit lamp with retroillumination show partial defects of the iris (pupils undilated). The lens equator and shadow of the lens nucleus are visible because of the iris defects.
nonsense-mediated decay before the truncated proteins are produced [18-20]. The two splicing site mutations would result in loss of the corresponding splicing site. Loss of one functional allele will result in 50% reduction of PAX6 function (haploinsufficiency), which has been suggested to contribute to the ocular defects [13,21]. All these five mutations would result in null alleles of PAX6 and therefore contribute to the clinical phenotypes observed in those patients.

In two families with novel mutations, the affected members from the same family had different phenotypes. Different phenotypes caused by the same PAX6 mutation have been reported previously [22]. This makes the analysis of genotype-phenotype correlation more difficult. Correlating phenotype to the site of the nonsense mutation has been unsuccessful as the truncated protein may not really be produced due to nonsense-mediated decay of the mRNA [23]. It is suggested that some hypomorphic alleles with reduced activity may contribute to less severe or variant phenotypes [24].

ACKNOWLEDGEMENTS

The authors thank all patients and family members for their participation. This study was supported in part by the National 863 Plan of China (Z19-01-04-02 to QZ; 04AA104092 to XG), and National Natural Science Foundation of China (30572006 to QZ).

REFERENCES