A novel PAX6 gene mutation in a Chinese family with aniridia

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Purpose: The PAX6 gene mutation in aniridia has been studied in various ethnic patients, but not well studied in the Chinese population. In the present study, we have investigated the PAX6 gene mutation in a Chinese family with congenital aniridia.

Methods: Total genomic DNA was isolated from peripheral blood of three aniridia patients (who also suffered from bilateral congenital cataracts) and two non-carriers in a Chinese family. Fourteen exons of the PAX6 gene were amplified by polymerase chain reaction (PCR). PCR products of each exon were analyzed by single strand conformational polymorphism (SSCP). The PCR products with abnormal SSCP patterns were subcloned and sequenced to identify the mutation.

Results: Abnormal SSCP patterns were found in all affected patients but not in non-carrier family members. A novel mutation (c.857delG) in exon 7 was detected by sequencing analysis. This frame shift mutation was predicted to lead to a pre-stop codon in exon 8, and generate a novel 40 amino acid peptide from codon 165.

Conclusions: A novel PAX6 gene mutation was identified in a Chinese aniridia family. This mutation may also contribute to congenital cataracts in these aniridia patients.

Aniridia is a severe eye disease with an incidence of 1 in 60,000 to 100,000 people. This disease is characterized by a lack of iris and accompanied by severe age related corneal degeneration. The disease is also associated with other eye abnormalities such as cataracts. Most of aniridia patients result from genetic disorders with an autosomal dominant inheritance. A mutation in the PAX6 gene has been identified to be the major genetic factor for congenital aniridia.

The PAX6 protein is a transcription factor essential for the development of the eye [1]. The human PAX6 gene spans 22 kb on chromosome 11 and consists of 14 exons and 13 introns [2,3]. The PAX6 protein has a paired domain (PD), a homeodomain (HD), and a trans-activation domain (PST) from the N-terminal to the C-terminal end of the molecule. The PD and the HD, which are separated by a linker region (LNK), are the structural basis for the binding activity of PAX6 protein. The PST domain is able to activate the expression of downstream genes in cells [4,5].

Mutations in the PAX6 gene have been reported in various ethnic patients (Human Genetics Unit PAX6 Allelic Variant database), but this mutation has not been well studied in the Chinese population. In the present study, a novel PAX6 gene mutation was identified in a Chinese family with aniridia.

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METHODOLOGY

The aniridia family: The Chinese family studied in this report originated from Yunnan province, the southwestern area of China. Four individuals in three successive generations were found to have the same congenital ocular disease (Figure 1). Three live patients (Figure 1, subjects II-2, II-3, and III-1) were 31, 26, and 5 years old, respectively. All patients suffered from aniridia and bilateral cataracts at birth. They showed abnormal vision even from their early childhood. They could detect only hand movement when this study was performed. Other clinical symptoms included slight eyeball horizontal tremor, severe iris hypoplasia, and lens opacity. For all three patients, application tonometry revealed normal intraocular pressures in both eyes. Corneas were normal in size and transparency. No abnormalities were detected in the retina, choroids, and optic nerve. Subject II-2 underwent cataract surgery at the age of 31, and his visual acuity was improved to 0.08 in both eyes. Subject II-3 underwent cataract surgery at the age of 26 and her visual acuity was improved to 0.1 in both eyes.

DNA samples: This study was performed with informed consent and in accordance to the tenets of the Declaration of Helsinki. The blood samples were collected from 3 aniridia patients (Figure 1, subjects II-2, II-3, and III-1) and 2 non-carriers (Figure 1, subjects I-2 and I-1) of a Chinese family. Healthy normal controls (100) were recruited for this study as well. Total genomic DNA was extracted from peripheral blood using the QiAmp Blood kit (Qiagen, Hilden, Germany).

Mutation analysis: Mutation analysis in the PAX6 gene was performed by SSCP on PCR fragments of each exon amplified from genomic DNA using previously reported PCR...
primers and PCR conditions [3,6]. The superimposed mutant PCR products were subcloned into pGEM-T vector (Promega, Madison, WI) and sequenced to identify the mutation.

RESULTS

Based on SSCP analysis, abnormal patterns corresponding to exon 7 of the \( \text{PAX6} \) gene were detected in 3 aniridia patients, but were not detected in 2 non-carrier family members, nor in 100 unrelated Chinese individuals (data not shown). In all 3 affected cases, a heterozygous G deletion at nucleotide 857 (c.857delG) in exon 7 of the \( \text{PAX6} \) gene (M93650) was confirmed by sequencing (Figure 2). This frame shift mutation was predicted to lead to a pre-stop codon in exon 8 of the \( \text{PAX6} \) gene, and generate a novel 40 amino acid peptide from codon 165.

DISCUSSION

Mutations in the \( \text{PAX6} \) gene have been demonstrated to be the genetic cause for congenital aniridia in various ethnic patients [7,8]. In the present study, we described a novel mutation (c.857delG) in the \( \text{PAX6} \) gene in a Chinese family with aniridia. This frame shift mutation was similar to a previously reported mutation c.853delC excepting 2 different amino acids in the predicted coding polypeptide [9]. Both of the mutations were accompanied with congenital cataracts in these aniridia patients.

Gupta [9] and Gronskov [10] reported that congenital aniridia was often associated with cataracts. We evaluated 81 different mutations in the \( \text{PAX6} \) gene that had been reported previously with detailed clinical data and 39 were accompanied with cataract. Only 18 of these 39 were accompanied with congenital or early cataract [6-15]. What was the correlation between the site effect of \( \text{PAX6} \) mutations and the congenital cataract? It appeared that congenital cataract was mainly correlated with missense mutations in PD (7 in 18 stated above) and a reading frame shift in LNK or PST (9 in 18 stated above) [6-15]. Missense mutations in exons 1-6, which involved the PD domain, may cause less severe and more varied phenotypes, especially congenital cataract [9,11-13]. Although frame shift mutations (which were mainly predicted to generate truncated protein) have always been proposed to be associated with complete loss of function of the mutant allele [16,17], such frame shift mutations related above were associated with congenital cataract phenotype and were therefore likely to have a partial gain of function aspect. This may at least partly ex-
plain why certain frame shift mutations in the LNK and PST domain can cause congenital cataract in these aniridia patients.

In summary, our data add a novel mutation to the existing spectrum of PAX6 gene mutations. This mutation may also contribute to congenital cataracts.

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REFERENCES