Missense Mutation at the C Terminus of the PAX6 Gene in Ocular Anterior Segment Anomalies

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PURPOSE. To report a rare case of ocular anterior segment anomalies including uveal ectropion of the iris, invasion of the conjunctival epithelia into the cornea, and posterior embryotoxon with a missense mutation of the PAX6 gene.

METHODS. The authors performed polymerase chain reaction-single-strand conformation polymorphism analysis and sequencing of the PAX6 gene using genomic DNA of family members and more than 100 control subjects.

RESULTS. The A to G transition at nucleotide 1682 in exon 13 in the patient was identified in an allele that resulted in a Gin to Arg substitution (Q422R) at the C terminus of the protein. The mutation was not found in the parents, a sibling, or control subjects.

CONCLUSIONS. The mutation indicates that the proline-serine-threonine-rich domain at the C terminus of the PAX6 protein plays a role in ocular anterior segment morphogenesis. (Invest Ophthalmol Vis Sci. 1998;39: 828-830)

It is well known that aniridia is caused by mutations of the PAX6 gene, a transcription factor gene in ocular development.¹² Three Because most of these mutations result in premature translational termination on one of the alleles, haploinsufficiency of the gene has been suggested to cause the aniridia phenotype.¹ Other mutations in the PAX6 gene have been detected in patients with Peters' anomaly,² corneal dystrophy,³ and isolated foveal hypoplasia.⁴ Thus, the PAX6 gene acts in the formation of the anterior segments and the neural retina. In mutants of the Small eye (Sey) locus, in which the murine homologue of the PAX6 gene is located,⁷ the inactivation of both alleles causes the eyes not to develop and severe craniofacial disorders to occur; inactivation of one allele results in the development of small eyes and mild central nervous system disorders, the latter of which are detectable only at an embryonic stage. In humans, compound heterozygotes have no eyes and central nervous system disorders.⁷ Thus, the abnormalities depend on the severity of the mutation of the PAX6 gene. Few missense mutations have been identified in the PAX6 gene²,⁴,⁶,⁸ however; the correlation between genotypes and phenotypes, including Peters' anomaly, aniridia, and foveal hypoplasia, provides evidence in support of position-dependent effects of PAX6 mutations in normal ocular development.⁶,⁸

We report a missense mutation at the C-terminal end of the open reading frame of the PAX6 gene in a patient with ocular anterior segment anomalies. This mutation suggests the possibility of another noteworthy function of the PAX6 gene.

CASE REPORT

A 10-year-old girl was diagnosed at a clinic with an abnormality of the iris and was referred to our hospital. Ocular examination revealed corneal opacities without iridocorneal adhesion in the left eye, bilateral invasion of the conjunctival epithelia into the cornea, and uveal ectropion at the pupillary margin (Fig. 1).

Figure 1. Photographs of the anterior segments of the right (A) and left (B) eyes of the patient. The corneoscleral limbus is obscure because of the invasion of the conjunctival epithelia. Uveal ectropion is seen at the pupillary margin. A pigment clump is on the lens surface in the right eye, and the cornea is partially opacified in the left eye.
FIGURE 2. Polymerase chain reaction–single-strand conformation polymorphism analysis of exon 13 reveals a bandshift in the patient, but not in the parents, a sibling, or more than 100 normal control subjects.

The lens, vitreous, and fundus were normal by slit lamp biomicroscopy and ophthalmoscopy. Her visual acuity was 1.0 with slight bilateral myopia.

The ocular position was orthophoric. The electroencephalogram was normal bilaterally; computed tomography failed to show any orbital or cranial abnormality. The patient was normal in size for her age, and she had a normal intelligence level and karyotype (46XX). No ocular abnormality was found in her parents and a sibling.

METHODS

DNA

These studies were conducted in accordance with the tenets of the Declaration of Helsinki. Our use of human subjects was conducted with the patient’s informed consent, was approved by the National Children’s Hospital Experimental Review Board, and was deemed exempt from human subject regulations. After informed consent, blood samples of the patient and family members were collected from the peripheral veins into lithium heparin tubes. Genomic DNA was prepared from isolated leukocytes using a standard phenol/chloroform procedure.9 DNA samples from a normal control subject were also obtained.

Polymerase Chain Reaction–Single-Strand Conformation Polymorphism Assay and Sequencing

Polymerase chain reaction (PCR) primers used for amplification of 14 exons of PAX6 were synthesized using a DNA-RNA synthesizer (model 392; Applied Biosystems, Urayasu, Japan), following a previous report.1 The annealing temperatures were adjusted to 55°C for exons 5a and 13, and to 60°C for all others, and Mg2+ concentration was 1.5 mM. Single-strand conformation polymorphism analyses were performed using automated minigel electrophoresis coupled with silver staining (Phastsystem, Pharmacia, Little Chalfont, UK).9 Because DNA fragments generated by PCR for exon 13 were large, the products were digested into 2 fragments with HindIII before being subjected to electrophoresis. Single-strand conformation polymorphism analyses also were performed after radiolabeling with [α-32P]dATP in the PCR reaction, using conventionally sized gels of 5% polyacrylamide under at least three different conditions in concentrations of glycerol and at running temperature. Nucleotide sequences were determined directly or after cloning on pUC18 using a Sequenase version 2 kit (Amersham, Cleveland, OH) with PCR primers or universal primers in pUC18.

RESULTS

By single-strand conformation polymorphism analysis, an abnormal pattern indicating a heterozygous mutation was detected in the exon 13 product of the patient, but it was not in the unaffected members of the immediate family or in more than 100 normal persons (Fig. 2). Sequencing analysis demonstrated that one of the patient’s alleles had a single A-to-G transition at nucleotide 1682 (Fig. 3), which caused a Gln-to-Arg substitution (Q422R) at the endpoint of the protein (in this study, the number of the nucleotide and amino acid was based on the sequence of accession no. M93650). No other changes in nucleotide sequences were detected in PCR products from the patient’s genomic DNA.

DISCUSSION

We identified a missense mutation at the C-terminal end of the PAX6 protein that causes congenital anterior segment anomalies. The corneal opacity in the left eye of our patient was similar to that in Peters’ anomaly, although obvious iridocorneal adhesion was not seen. Epithelial abnormalities at the corneoscleral limbus and corneal opacities are often seen in eyes with aniridia. It has been reported that the PAX6 gene mutation results in varying degrees of aniridia, which range from an iris of normal size to the absence of one.5 The present case with minute abnormalities of the iris and limbus may be representative of mild aniridia or a related condition.

Most patients with aniridia have either a deletion of the whole gene or mutations of nonsense, a frameshift, or a defect at the splicing junction, that result in premature translational

FIGURE 3. Sequencing of the normal and mutant alleles identifies the heterozygous A-to-G transition at the nucleotide 1682 in exon 13, resulting in a Gln-to-Arg substitution (Q422R) in the protein.
termation on one of the alleles.\textsuperscript{1-3} Only two pedigrees with aniridia have been reported to have missense mutations, respectively, in the paired domain and homeodomain,\textsuperscript{2,3} the DNA-binding motifs of the transcriptional regulators. A single amino acid substitution in an important motif may result in significant protein dysfunction, the same as that in the truncated protein usually seen in aniridia. Other missense mutations found in Peters' anomaly and foveal hypoplasia were also in the paired domain. The paired domain consists of a highly conserved N-terminal subdomain and a more variable C-terminal subdomain. Two subdomains that possess different DNA-binding activities work independently to activate transcription,\textsuperscript{10} and each negatively regulates the function of the other.\textsuperscript{11} Because a missense mutation in the N-terminal subdomain of the paired domain was found in Peters' anomaly\textsuperscript{9} and because those in the C-terminal subdomain were identified in foveal hypoplasia\textsuperscript{6} and aniridia,\textsuperscript{7} the two subdomains play significant roles in ocular anterior and posterior segment formation through development.

The present study provides additional evidence to show the importance of a C-terminal motif of the PAX6 protein for ocular anterior segment formation. The region (152 amino acids) enriched with proline, serine, and threonine residues is similar to that region in CTF-\textsuperscript{12} and Oct-1\textsuperscript{13} genes, which has been indicated to be an activation motif of the transcription factor.\textsuperscript{7} In this region, 17 mutations have been identified so far (they are summarized in the database at http://www.hgu.mrc.ac.uk/Softdata/PAX6), most of which manifested various compound anomalies through the anterior ocular segment to the posterior segment including aniridia, congenital cataracts, autosomal dominant keratitis, and foveal hypoplasia. All mutations but one were nonsense or frameshift, which were predicted to cause premature translational termination or extension out of the frame of the protein. One missense mutation of a single G-to-C transition at nucleotide 1545 that causes G395R in association with −1 out-of-frame deletion of exon 12 has been identified in familial aniridia. However, the missense mutation probably is unimportant, and a mutation at −1 of exon 12 results in an out-of-frame excision of exon 12, removing 50 additional amino acids of the PST-rich domain and resulting in an out-of-frame extension of exon 13. Thus, the Q422R is the first missense mutation in the PST-rich domain that may cause ocular anomaly.

An in vitro study using nonsense mutations in aniridia and Sey\textsubscript{1 Neu} mouse demonstrated that the mutants in the C terminus residues of the PAX6 PST-rich domain decrease their autonomous activation.\textsuperscript{7} The present study also confirms the importance of the C terminus PST-rich domain. The Q422R mutation may alter PAX6 protein function in a relatively subtle way, and the mutation may manifest a mild phenotype compared with previously reported cases, including aniridia, caused by PST-rich domain mutations.

Although the PST-rich domain shows a great variation of amino acid levels among animal species, the C-terminal amino acid, glutamine, is conserved in all known vertebrate PAX6 proteins and some more distant ones, including those of humans (accession no. M93650), mouse (P32117), rat (U69644), chick (P47237), quail (X82151), zebrafish (X63183), Xenopus (U64513), Phallusia (Y09975), squid (U59830), and Caenorhabditis elegans (U29184). The Q422R mutation together with previous studies strongly suggests that a full-length PAX6 protein is necessary for eye morphogenesis.

References