Corneal Abnormalities in Pax6⁺⁻ Small Eye Mice Mimic Human Aniridia-Related Keratopathy

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PURPOSE. To investigate corneal abnormalities in heterozygous Pax6⁺⁻/Sey-Neu (Pax6⁺⁻, small eye) mice and compare them with aniridia-related keratopathy in Pax6⁺⁻/⁻ patients.

METHODS. Fetal and postnatal corneal histopathology, adult corneal thickness, and the distribution of K12-immunostained cells were compared in wild-type and Pax6⁺⁻/⁻ mice.

RESULTS. Prenatally, the corneal epithelium was thinner in Pax6⁺⁻/⁻ fetuses than wild-type littersmates, but the stroma appeared irregular, hypercellular, and thickened. The anterior chamber angle was obliterated, and the iris was hypoplastic from early developmental stages. The adult Pax6⁺⁻/⁻ corneal epithelium was thinner, had fewer layers, and included goblet cells, indicating repopulation from conjunctival epithelium. The ocular surface was often roughened, with epithelial vacuolation and lens tissue within the stroma. The corneal stroma was thicker centrally, with an irregular lamellar alignment. Many adult Pax6⁺⁻/⁻ corneas were vascularized or contained cellular infiltrates, but some remained clear. Corneal degeneration was age-related: Older Pax6⁺⁻/⁻ mice had prominent subepithelial pannus and more goblet cells in the peripheral corneal epithelium. Cytokeratin 12 stained very weakly in the subepithelial pannus and more goblet cells in the peripheral corneal epithelium. Cytokeratin 12 stained very weakly in the midsuprabasal and parabasal epithelium layers, but more intensely in the parabasal and suprabasal epithelium layers. In control mice, Pax6 was expressed in the basal epithelial layer, limbal stem cells, and in the ciliary body, and all layers of the retina. Two mouse Pax6 mutations, Pax6Sey and Pax6Sey-Neu, produce truncated non-functional Pax6 proteins. Pax6⁺⁻/⁻ mice that are homozygous for either of these mutant alleles are without functional Pax6 protein and die at birth with no eyes, whereas heterozygous Pax6⁺⁻/⁻ mice are viable and fertile but have small eyes and aniridia.11 These heterozygous Pax6⁺⁻/⁻ (small eye) mice share many of the ocular features of human aniridia and have been widely accepted as an animal model for this condition11–18 (Jordan TL, Fleck B, Van Heyningen V, ARVO Abstract 2072, 1994).

Conclusions. Corneal abnormalities in Pax6⁺⁻/⁻ mice are similar to those in aniridia-related keratopathy in Pax6⁺⁻/⁻ patients. This extends the relevance of this mouse model of human aniridia to include corneal abnormalities. Infiltration of goblet cells suggests impaired function of Pax6⁺⁻/⁻ limbal stem cells, abnormal expression of cytokeratin 12 may result in greater epithelial fragility, and corneal opacities in older mice may reflect poor wound-healing responses to accumulated environmental insults. (Invest Ophthalmol Vis Sci. 2003;44: 1871-1878) DOI:10.1167/iovs.02-0576

Mutations in the Pax6 gene underlie many cases of human aniridia.1–3 This is a bilateral, panocular condition affecting not only the iris, but also the cornea, anterior chamber angle, lens, retina, and optic nerve. Although iris hypoplasia is the most apparent feature, it is not the major determinant of visual loss. Corneal opacification, cataract, optic nerve hypoplasia, glaucoma, and macular hypoplasia all contribute to the eventual visual loss. Aniridia-related keratopathy (ARK) is a term used to encompass all corneal opacification changes observed in aniridic eyes. These abnormalities develop later in life, and the underlying mechanisms are not understood.

The most commonly recognized manifestation of ARK is peripheral vascularized epitheliopathy, which has been attributed to stem cell failure.1–5 A less well-recognized feature of ARK is corneal stromal opacification (CSO), which appears to arise later in life and may be triggered by corneal surgery. In a recent study, a series of nine patients with aniridia underwent surgery involving corneal incisions and later showed a marked stromal scarring response that we consider to be part of the ARK spectrum (Ramaesh K, Dhillon B, unpublished data, 2002).

The Pax6 gene encodes the Pax6 transcription factor,1 and its sequence and expression pattern are highly conserved in evolution. The mouse Pax6 gene is homologous to the human Pax6 gene, and both are widely expressed in developing eye tissues, including the lens, corneal epithelium, iris, ciliary body, and all layers of the retina.7–10 Two mouse Pax6 mutations, Pax6Sey and Pax6Sey-Neu, produce truncated non-functional Pax6 proteins. Pax6⁺⁻/⁻ mice that are homozygous for either of these mutant alleles are without functional Pax6 protein and die at birth with no eyes, whereas heterozygous Pax6⁺⁻/⁻ mice are viable and fertile but have small eyes and aniridia.11 These heterozygous Pax6⁺⁻/⁻ (small eye) mice share many of the ocular features of human aniridia and have been widely accepted as an animal model for this condition11–18 (Jordan TL, Fleck B, Van Heyningen V, ARVO Abstract 2072, 1994).

However, significant visual morbidity in human aniridia arises from corneal abnormalities, and an animal model of these features would facilitate further investigation. Although iris hypoplasia, cataracts, and incomplete separation of the lens from the cornea have been described in Pax6⁺⁻/⁻ mice and are comparable to defects seen in Pax6⁺⁻/⁻ patients, the corneal anomalies have not been fully characterized. We now report a detailed histologic and morphometric study of the cornea in heterozygous Pax6⁺⁻/⁻ and wild-type (Pax6⁺⁻/⁻) mice. We found the corneal abnormalities of Pax6⁺⁻/⁻ mice to be an excellent model for ARK in Pax6⁺⁻/⁻ humans.

MATERIALS AND METHODS

Mice

Heterozygous Pax6⁺⁻/⁻ mice and Pax6⁺⁻/⁻ wild-type littersmates were produced from Pax6⁺⁻/⁺ female x Pax6⁺⁻/Sey-Neu male crosses and...
distinguished by eye size, and their genotypes were confirmed by polymerase chain reaction (PCR). All animal procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Fetuses were staged from the day of the vaginal plug (defined as embryonic day E0.5) and E14.5 and E16.5 fetal heads were fixed (fixation of specimens is described in the following sections). E18.5 fetuses were decapitated and eyes removed and fixed. Most of the postnatal mice were examined at 8 to 17 weeks, but some animals were examined at 2 weeks or 9 months, to test for age-related changes, and 12-month-old mice were used for immunohistochemistry (described later). Postnatal day (P)14 juvenile mice and adults were killed by cervical dislocation and their eyes removed and fixed. Adult eyes were weighed before fixation.

**Histologic Analysis**

The specimens were fixed in Bouin fixative overnight and, to facilitate sectioning, lenses were removed from all 8- to 17-week-old adult eyes and most of the 9-month-old eyes but not from the fetal or postnatal 2-week-old eyes. Eyes were embedded in paraffin wax, and 7-μm serial sections cut. Fetal heads were sectioned transversely, and eyes were sectioned in an anterior–posterior plane to include the cornea, iris, and retina. Serial sections of the ocular region of the head of the embryos, and the central part of the adult cornea were stained with hematoxylin and eosin. For PAS staining of the adult eyes, slides were treated with periodic acid and then stained with Schiff reagent. The histologic features of the developing cornea, iris, and anterior chamber of heterozygous Pax6+/− (small eye) and Pax6+/− (wild-type) littermates were compared.

**Morphometric Measurements**

Images of sections of adult corneas were captured by a video camera attached to a microscope (×40 objective; BH2; Olympus, Tokyo, Japan), and measurements made with image-analysis software (Color Vision; Improvision, Coventry, UK) on a computer (Macintosh; Apple, Cupertino, CA). The measuring scale was calibrated, and the thicknesses of the corneal epithelium and the whole cornea were measured in four places (two close to the center and two at the periphery) in five of the middle 13 serial sections of each adult eye (sections mid-6, mid-3, mid, mid+3, and mid+6). The thickness of the corneal stroma plus endothelium was calculated by subtracting the thickness of the epithelium from the thickness of the whole cornea. Mean thicknesses were calculated for the central and peripheral regions of each section and used to calculate mean thicknesses in each eye.

**Cytokeratin 12 Immunohistochemical Staining**

Eyes of 12-month-old adult heterozygous Pax6+/− and Pax6+/− (wild-type) mice were fixed in 4% paraformaldehyde and embedded in paraffin. Sections cut at 7-μm were mounted on poly-i-lysine-coated slides. Deparaffinized sections were treated with 100% ethanol, incubated with 3% hydrogen peroxide for 20 minutes to block endogenous peroxidase activity, rehydrated with 70% ethanol, and washed in PBS. The sections were treated with normal rabbit serum for 30 minutes to block the nonspecific binding of antibodies and then incubated overnight at 4°C with cytokeratin 12 (K12) antibody (goat polyclonal IgG; Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:500 in blocking serum. Slides were washed in PBS and then treated for 30 minutes with secondary antibody (biotinylated, anti-goat IgG; Vectastain ABC Kit; Vector Laboratories, Burlingame, CA) diluted 1:200 in blocking serum, washed in PBS and then incubated for 30 minutes with avidin-biotin complex (ABC). After sections were washed in PBS, the binding antibody was visualized with 3,3′-diaminobenzidine (DAB; Sigma, Poole, UK). All the sections were then lightly counterstained with hematoxylin. Control slides were incubated with normal rabbit serum without K12 antibody, but otherwise treated normally.

**Statistics**

Unpaired t-tests were used to compare the thickness of the corneal epithelium, the corneal stroma plus endothelium, and the whole cornea in Pax6+/− and Pax6+/− mice, separately for the central and peripheral regions. Paired t-tests were used to compare the thickness of the central and peripheral regions from the same samples.

**RESULTS**

**Histologic Analysis of the Anterior Segment in E14.5 to E18.5 Fetuses**

As previously reported,17,18 one striking abnormality of all the heterozygous Pax6+/− fetuses was that the lens vesicle remained attached to the surface ectoderm (corneal epithelium) by a persistent lens stalk (Figs. 1B, 1D, 1F). Other, previously undescribed abnormalities of the anterior segments of Pax6+/− fetuses are documented later.

Both eyes of five Pax6+/− and five wild-type E14.5 fetuses were examined. By E14.5 the anterior chamber had not started to form in eyes of Pax6+/− heterozygotes, whereas in wild-type eyes the vascular pupillary membrane had already separated the lens and the developing cornea, thereby forming the anterior chamber (Figs. 1A, 1B). In both wild-type and Pax6+/− corneas, the surface ectoderm consisted of a basal layer of cuboidal cells and superficially flattened perilimbal cells. Mesenchymal cells had migrated between the surface ectoderm and the lens to form the corneal stroma. Mesenchymal cells were mostly arranged parallel to the surface ectoderm in both genotypes but the stroma was thicker and hypercellular in the heterozygotes (Fig. 1B).

Both eyes of five Pax6+/− and six wild-type E16.5 fetuses were examined. By E16.5, the eyelids were closed, and the anterior chamber had developed in both Pax6+/− and wild-type fetuses, but, in Pax6+/− heterozygotes, the center of the anterior chamber was interrupted by the persistent lens stalk. Differentiation of the iris had started in both genotypes, and the iridocorneal angle had formed in wild-type eyes, but, in Pax6+/− eyes, the angle was filled with mesenchyme (Figs. 1C, 1D). The corneal epithelium appeared similar in both genotypes, comprising a basal cuboidal layer and superficially flattened perilimbal cells. In wild-type eyes the mesenchymal cells of the stroma were arranged parallel to the corneal surface throughout, but in Pax6+/− fetuses the stroma appeared thicker, and the parallel arrangement was distorted toward the center of the cornea.

Both eyes from two Pax6+/− and two wild-type E18.5 fetuses were examined. By E18.5, the wild-type anterior chamber was well developed, and a definitive iridocorneal angle had formed with the elongation of the iris and the differentiation of the ciliary body (Fig. 1E). In contrast, in Pax6+/− heterozygotes, the iris was hypoplastic, and the iridocorneal angle was obliterated by cells (Fig. 1F). In wild-types, the corneal epithelium consisted of basal cuboidal and superficially flattened cells, whereas in heterozygotes it appeared thin and consisted entirely of flattened cells. Conversely, the stroma appeared thicker in Pax6+/− eyes than in the control eyes. The corneal endothelial cells had started to differentiate from the mesenchymal cells, but, in the Pax6+/− heterozygotes, they were not clearly distinguishable at the center where the cornea and lens failed to separate.

**Histologic Analysis of the Anterior Segment in Young Mice**

Both eyes from two Pax6+/− and four wild-type mice were examined at P14, approximately the time of eyelid opening. Pax6+/− heterozygotes had further anomalies in addition to those present at fetal stages. The corneal epithelium remained thinner (two to three cell layers instead of three to four) and Bowman’s layer (anterior limiting lamina) was interrupted in
some areas. The epithelial surface, which was sometimes smooth but was often rough, and the superficial cells either contained vesicles or appeared to be sloughing off.

As at the fetal stages, the anterior stroma was hypercellular in Pax6+/− eyes, and there were irregularities in lamellar alignment. In wild-type mice the stroma was avascular, comprising extremely flattened spindle-shaped keratocytes scattered between the regularly arranged lamellae. In Pax6+/− eyes, the posterior stroma appeared normal, with regular lamellar alignment, but, anteriorly, the stroma sometimes appeared edematous and, in some cases (two of four eyes), formation of pannus occurred in the superficial stroma. In Pax6+/− heterozygotes the persistent lens stalk appeared as an epithelial plug in the cornea, and, in one case, lens material was present within this plug (Fig. 2E).

In Pax6+/− heterozygotes, the iris was hypoplastic and often involved in adhesions. Keratolenticular adhesions were present in the central cornea, and there were also adhesions between the anterior iris stroma and the trabecular meshwork (where the iridocorneal angle was narrow; Fig. 3A), between the iris stroma and the peripheral cornea (Fig. 3B), and between extensions of abnormal iris processes and the cornea (Fig. 3B). In wild-type mice the posterior surface of the cornea was covered by a layer of distinctive endothelial cells. This was absent in areas of keratolenticular (Fig. 3C) and iridokeratotic adhesions in Pax6+/− heterozygotes but normal elsewhere.

**Histologic Analysis of the Anterior Segment in Adult Mice**

Both eyes from 10 Pax6+/− and 10 wild-type mice were examined at 8 to 17 weeks. The eyes from adult Pax6+/− heterozygotes were significantly lighter than their wild-type littermates (mean ± SEM: 16.0 ± 0.3 mg versus 21.4 ± 0.5 mg), and many of the histologic features were similar to those at P14. In most cases the iris was hypoplastic, but this condition varied from mild to severe hypoplasia, the latter resulting in development of only a rudimentary iris.

In most heterozygous eyes, the corneal epithelium was thin and irregular, and the superficial layer of cells either had vesicles or appeared to slough off the surface. The epithelium usually consisted of three to four layers (rather than the normal...
or in the eyes of the 2-week-old present in the corneal epithelium in any of the wild-type eyes the central region were noted (Fig. 2C). Goblet cells were not occurred in the peripheral corneal epithelium, but two cases in Pax6 (Fig. 2F).

Of lens material within this plug was observed in some corneas as a partial plug in the super extending the full thickness of the central cornea (Fig. 2D) or persistent lens stalk appeared either as a full epithelial plug, (D) extending the full thickness of the cornea. (E, F) Presence of lens material within the epithelial plug of a 2-week-old eye (E) and an adult eye (F). In both cases, the corneal epithelium was irregular and atrophic. (G, I) Superficial corneal pannus. (G) Presence of vesicles in the superficial corneal epithelium and the fibrovascular tissue in the superficial corneal stroma replacing Bowman’s layer. (H) Pannus consisting of large blood vessels with intact Bowman’s layer and (I) cellular infiltration in the super- ficial corneal stroma. (J, K) Nine- month-old adult eyes: (J) PAS staining showed high frequency of goblet cells in the peripheral cornea and (K) extensive superficial pannus re- placed Bowman’s layer. (A, B, E–I, K) H&E staining. (In mice, Bowman’s layer is easily visualized by electron microscopy but is less obvious with light microscopy.27) Scale bars, 50 μm.

The appearance of Bowman’s layer varied among adult Pax6+/− heterozygotes, being intact in some (e.g., Figs. 2C, 2F, 2H, 2I) and interrupted in others (e.g., absent from regions shown in Fig. 2G). In most cases the stroma appeared thicker toward the center, and the lamellar alignment was irregular in the anterior stroma. Superficial formation of corneal pannus was present in all cases (Figs. 2G, H), whereas it formed in only some Pax6+/− eyes at 14 days (as just described). The degree of vascularization varied from superficially placed small blood vessels to large feeder vessels extending from the periphery toward the center. In some areas, the pannus appeared to be degenerative (sub- epithelial fibrovascular tissue formation and preservation of Bowman’s layer, Fig. 2H), whereas elsewhere the pannus was inflammatory (Bowman’s layer was replaced by vascularized connective tissue with infiltration of inflammatory cells, Fig. 2G). Infiltration of inflammatory cells occurred in the superficial stroma (Fig. 2D).

Corneal endothelial cells were absent from areas of kerato- lenticular and iridocorneal adhesions. In one Pax6+/− heterozygous mouse, the lenses were abnormally located toward the front of both eyes. The lenses were not removed from these two eyes, and histology revealed coloboma of the iris and ciliary body and persistent hyperplastic primary vitreous (PHPV) that was continuous with the retina through the coloboma of the ciliary body (Figs. 3D–F). Lens material was
detected in abnormal locations (Fig. 3E), including the epithelial plugging of the cornea (Fig. 2F).

**Histologic Analysis of the Anterior Segment in Older Mice**

One eye from each of three Pax6+/− and three wild-type mice were examined at 9 months. As at 8 to 17 weeks, the eyes of 9-month-old adult Pax6+/− heterozygotes had hypoplasia of the iris, a persistent lens stalk, and an irregular corneal epithelium with three to four cell layers. Other abnormalities were more severe at 9 months. Two of three 9-month-old heterozygous eyes had goblet cells in the peripheral corneal epithelium that were far more numerous than those seen in any of the 20 heterozygous eyes at 8 to 17 weeks (Fig. 2J). The superficial stroma contained thick fibrovascular pannus that was more extensive than in the 8- to 17-week-old mice, and it replaced Bowman’s layer in most areas (Fig. 2K). Cellular infiltration was visible in the posterior stroma.

**Morphometry of the Adult Cornea**

The measurements of corneal thickness in 8- to 17-week-old adults support the above qualitative descriptions, implying that in Pax6+/− mutant mice the corneal epithelium is thinner, because it has fewer rows of cells. Figure 4 shows

**Figure 3.** Ocular developmental abnormalities of 2-week-old (A, B, C) and adult (D, E, F) Pax6+/− mice (H&E staining). (A) Iridokeratotic adhesion obliterating the iridocorneal angle. (B) Iridokeratotic adhesion and abnormal iris process. (C) Keratolenticular adhesion; corneal endothelium is absent in the area of adhesion (between arrows). (D, E, F) Serial sections showing forward positioning of the lens and PHPV with iris and ciliary body coloboma. (D) PHPV continuous with sclera through the coloboma of the ciliary body. (E) Retrolenticular fibrovascular mass and presence of lens material in abnormal locations (arrowbeads). (F) Retrolenticular fibrovascular mass eroding the posterior lens capsule (short arrow) and the ciliary body coloboma. Scale bars, 100 μm.

**Figure 4.** Comparison of thickness of central (C) and peripheral (P) regions of the cornea between adult Pax6+/− heterozygotes and Pax6+/+ mice. *P = 0.003; **P < 0.0001 by unpaired t-test.
that the corneal epithelium was significantly thinner in Pax6<sup>+/−</sup> heterozygous mice than wild-type Pax6<sup>+/+</sup> mice in both the central and peripheral regions of the cornea (P < 0.0001 by unpaired t-tests). For both genotypes, the epithelium was thicker in the central region than at the periphery and this was statistically significant by paired t-tests (P < 0.01 in Pax6<sup>+/−</sup> heterozygotes and P < 0.0001 in wild-type). Unlike the corneal epithelium, the stromal thickness varied from section to section in both wild-type and Pax6<sup>+/−</sup> mice. Some of this variability could have been caused by compression or stretching of the lamellar structure during removal of a Pax6<sup>+/−</sup> lens with a lens–corneal bridge or during histologic processing. Thus, the biological significance of any variation in thickness of the stroma or whole cornea remains unclear.

### K12 Immunohistochemical Staining

In 12-month-old wild-type mice, K12 immunohistochemical staining was positive in all cell layers of the corneal epithelium (Fig. 5B), but was negative in the basal and suprabasal cells of the limbal and conjunctival epithelium (not shown). The non-immune serum control was completely negative (Fig. 5A). In 12-month-old heterozygous Pax6<sup>+/−</sup> mice, K12 staining was positive in the central corneal epithelium (Fig. 5C), whereas the peripheral corneal epithelium stained very weakly with the antibody (Fig. 5D). The staining pattern was uniform in wild-type corneal epithelia but, in some areas of Pax6<sup>+/−</sup> corneal epithelia, the superficial cells stained more weakly than the basal cells (Fig. 5B, 5C). Localized areas of weakly stained epithelium (basal and suprabasal) were also observed in the central cornea of Pax6<sup>+/−</sup> eyes.

### DISCUSSION

**Abnormalities of the Corneal Epithelium in Pax6<sup>+/−</sup> Mice**

During development, the corneal epithelia of the Pax6<sup>+/−</sup> mice failed to undergo proper differentiation and maturation. In adults the epithelium was thinner, and the superficial layers were fragile. Our observations of the atrophic corneal epithelium and the presence of goblet cells in Pax6<sup>+/−</sup> mice are similar to the histopathologic and cytologic findings of human aniridia. The presence of goblet cells indicated incursion of conjunctival epithelial cells onto the cornea of aniridic eyes and possibly reflected impaired functioning of Pax6<sup>+/−</sup> limbal stem cells.

Although corneal opacities have been observed in human aniridia, the presence of vesicles in the corneal epithelium has not been reported. Our histologic finding of epithelial vesicles in the mouse cornea could be due to the corneal edema that occurs in conjunction with trauma and inflammation. Reduction in the epithelial thickness probably impairs the protection against environmental insults and may be the cause of the inflammatory changes observed in the Pax6<sup>+/−</sup> mice.

Margo suggested that the focal absence of Bowman's layer could be a developmental defect in human aniridia. In the mouse, Bowman's layer develops during the postnatal period and focal absence of Bowman's layer, not accompanied by degenerative pannus, was noticeable from P14 in the Pax6<sup>+/−</sup> mice. The acellular matrix of Bowman's layer is secreted by the corneal epithelium during development, and its absence could be due to a dysfunction of the epithelial cells.

**FIGURE 5.** Immunohistochemical staining with anti-K12 antibody in the corneal epithelium of wild-type and Pax6<sup>+/−</sup> mice. (A) Control section incubated with nonimmune rabbit serum. (B) K12 staining of the corneal epithelium from a wild-type mouse. (C, D) In Pax6<sup>+/−</sup> mice, the central corneal epithelium showed reduced K12 staining (C), and the peripheral corneal epithelium stained very weakly (D). Scale bars, 50 μm.
Abnormalities of the Corneal Stroma and Endothelium

In human aniridia there is a tendency to form corneal opacification and vascular pannus, and a histopathologic study reported formation of pannus at the age of 19 months. Irregular lamellae alignment, neovascularization, and cellular infiltration were also observed in the corneal stroma of Pax6+/− mice. These were detected in the superficial stroma even at P14, indicating an early onset of pannus formation in the mice as well. Furthermore, Pax6+/− mice had central corneal nebulae similar to human aniridic cornea.

The Pax6+/− mice did not show any apparent endothelial abnormalities except in areas where there were corneal adhesions. This is consistent with histologic studies of human aniridia but this aspect of corneal function in aniridia has not been studied in vivo. Both tissues are derived from the cranial neural crest. Migration of cranial neural crest cells has been shown to be impaired in Pax6+/− rats, a possibly nonautonomous phenotype that is thought to result from deficient signaling from the olfactory epithelium. It is possible, therefore, that the deficiencies of the Pax6+/− endothelium and stroma are a function of a subtle defect in neural crest migration. Alternatively, they may be a primary consequence of defective development of the anterior segment or the result of the failure of the affected corneal epithelium to insulate them fully from normal environmental insults.

Other Abnormalities of the Anterior Segment of Pax6+/− Eyes

In the Pax6+/− mice, in addition to aniridia and corneal abnormalities, we observed some features of Peters’ anomaly (lens–corneal bridge and other keratolenticular adhesions), and persistent hyperplastic primary vitreus. Peters’ anomaly can occur as a result of mutation in the human PAX6 gene, and the Pax6+/− mouse model is also relevant to this condition.

Age-Related Corneal Abnormalities in Pax6+/− Mice and Human Aniridia

Age-related progressive changes in the corneas of aniridic eyes have been reported in a clinical investigation. Our study of Pax6+/− mice also suggests age-related progression in corneal abnormalities, and in mice these progressive changes seem to be very rapid. Vascular pannus formation was observed at P14 and was more extensive and more frequent in older mice. Ectopic goblet cells were not present at 2 weeks but were observed in 8-week-old Pax6+/− adults and were more numerous at 9 months. This implies progressive invasion of the corneal epithelium by conjunctival epithelium and may reflect a progressive decline in Pax6+/− limbal stem cell function. The higher frequency of corneal opacities in older mice may reflect a poor wound-healing response to accumulated environmental insults.

Pax6+/− Mice as a Model of Aniridia-Related Keratopathy

An essential initial step in understanding the molecular pathology of an inherited disorder is identification of the mutant gene(s) responsible. An animal model that is similar in genotype and phenotype can provide a significant contribution toward understanding how the pathogenesis occurs. Mutations in PAX6 cause human aniridia but the role of Pax6 mutations in the molecular pathology of human aniridia (which affects the cornea, anterior chamber angle, lens, retina, and optic nerve as well as the iris) is not understood. Mice that are heterozygous null for Pax6 have already been used as a model for lens and iris abnormalities associated with aniridic human patients that are Pax6+/−. Our results show that heterozygous Pax6+/− mice have corneal abnormalities similar to those in human Pax6+/− aniridia, and this study extends the relevance of this mouse model to include aniridia-related keratopathy. In our study, there was a time-dependent progression in corneal disease severity that parallels the ARK observed in humans.

Factors Influencing the Etiology of Aniridia-Related Keratopathy in Pax6+/− Mice and PAX6+/− Humans

The limbus contains stem cells, and the clonal progeny of those cells migrate inward and maintain the corneal epithelium. The abnormalities in human aniridic corneas are usually presumed to be due to the paucity or malfunction of limbal stem cells, and incursion of conjunctival epithelial cells onto the cornea of the aniridic eyes. However, studies in animal models have offered novel alternative explanations for the development of ARK.

Indirectly, experimental evidence from animal models suggests that the fragility of the epithelium in the Pax6 mutation may be related to a deficiency of epithelial cytokeratins. These intermediate filaments play a major role in the corneal epithelium, binding neighboring epithelial cells together and anchoring basal epithelial cells to the underlying basal lamina at desmosomes and hemidesmosomes, respectively. Corneal-specific K12 and K3 keratin pairs are expressed in differentiated epithelium, and their presence is essential for the maintenance of corneal epithelial integrity. Regulation of K12 is Pax6 dependent, and in K12-knockout mice, the superficial corneal epithelium is fragile and often detached from the surface. Compared with the wild-type mice, the corneal epithelium of the heterozygous Pax6+/− mice showed decreased K12 staining, especially at the peripheral and superficial corneal epithelium. Tseng and Li reported decreased K12 staining in human aniridic corneal epithelium and the corneal findings in the murine model of aniridia are in keeping with those observed in the human cornea. It is notable that the superficial corneal epithelium of the heterozygous Pax6+/− mouse had vesicles and appeared to be fragile. This fragility of the Pax6+/− corneal epithelium may be attributed to the deficiency of K12, a key factor in the maintenance of epithelial integrity. It is tempting to speculate that the low levels of Pax6 expressed in Pax6+/− mice and patients with aniridia leads to dysfunctional K12 and a fragile corneal epithelium.

During corneal wound healing, matrix metalloproteinase (MMP)-9, (also known as gelatinase-B, or Gel-B) is upregulated and plays a major role in remodeling of the extracellular matrix. Uregulation of Mmp9 depends on the expression of Pax6, which is also elevated during corneal wound healing. It has been shown in a mouse model that deficiency of MMP-9 leads to corneal abnormalities similar to ARK, and expression of Mmp9 is essential for extracellular matrix remodeling during corneal wound healing. This suggests that expression of Mmp9 may be abnormal in Pax6+/− mice and may lead to an abnormal corneal wound-healing response. Abnormal corneal wound healing may contribute to ARK, and the natural history of this condition may also be determined by abnormal epithelial–anterior stromal interactions. Further studies of the cellular mechanisms underlying these epithelial–stromal relationships will further our understanding of corneal transparency and may lead to strategies to minimize aniridia-related keratopathy.

Now that we have established that heterozygous Pax6+/− mice provide a good model of human aniridia-related keratopathy, it should be possible to test whether the etiology of this condition involves an intrinsic deficiency of limbal stem cells,
epithelial fragility related to cytokeratin deficiency, and/or an abnormal wound-healing response.

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Note Added in Proof

Anterior segment abnormalities, that are comparable to some of those reported here, have been described recently for mice heterozygous for a different null allele of Pax6. (Baulmann DC, Ohlmann A, Flügel-Koch C, Goswami S, Cvekl A, Tamm ER. Pax6 heterozygous eyes show defects in chamber angle differentiation that are associated with a wide spectrum of other anterior segment abnormalities. Mech Dev. 2002;118:3–17.)

References


