Abnormal Eye Development Associated with Cat4a, a Dominant Mouse Cataract Mutation on Chromosome 8

Patricia A. Grimes,1 Brigitte Koeberlein,1 Jack Favor,2 Angelika Neubäuser-Klaus,2 and Dwight Stambolian1

PURPOSE. Cat4a, one of four mutant alleles at the mouse Cat4 locus, causes central corneal opacity and anterior polar cataract in heterozygotes and microphthalmia in homozygotes. The Cat4 locus has been mapped to chromosome 8, 31 cM from the centromere. In this study ocular development of Cat4a mutant mice was investigated to characterize the defects in eye morphogenesis.

METHODS. Serial sections from eyes of wild-type, heterozygous, and homozygous littermates were examined by means of light microscopy at selected intervals from embryonic day 11 to postnatal day 1. Eyes of adult heterozygous and homozygous mice were also evaluated histologically.

RESULTS. Failure of separation of the lens vesicle from the surface ectoderm was the earliest structural defect observed. In heterozygous embryos, the abnormality was limited to persistent connection of the anterior pole of the lens to the cornea. Adult heterozygotes had defects in the central corneal stroma and endothelium and anterior polar cataracts with or without keratolenticular adhesion. In homozygous embryos, the persistent connection of lens to surface ectoderm was associated with aborted lens development, failure of closure of the optic fissure, and impairment of growth of the eye cup. Microphthalmic eyes of adult homozygous mice had a poorly developed cornea, and the anterior chamber and vitreous compartment were absent. An extensively folded retina and remnants of a degenerated lens filled the interior of the globe.

CONCLUSIONS. A developmental defect inhibits separation of the lens vesicle from surface ectoderm in mice heterozygous or homozygous for the Cat4a mutation. In homozygous subsequent lens and eye morphogenesis are also severely affected. Cat4a shows phenotypical similarity to several other independent mouse mutations including Small eye, a mutation of the Pax6 gene. Cat4 may be one of several genes involved in a common developmental path and may be part of the Pax6-regulated gene cascade governing eye morphogenesis. (Invest Ophthalmol Vis Sci. 1998;39:1863–1869)

Mouse mutants with congenital ocular abnormalities are an important resource for identification and investigation of genes involved in normal eye development. Characterization of such genes in lower animals can aid in isolation of their human homologues and increase understanding of congenital ocular defects in humans.

The mouse Cat4 locus includes four independent mutant alleles, all of which are expressed in heterozygous carriers as anterior polar cataract and central corneal opacity.1 Three of these mutations (Cat40, Cat4F, and Cat4a) are homozygous lethal, and heterozygotes display moderate microphthalmia in addition to cornea and lens abnormalities. Carriers of the remaining allele (Cat4a) have eyes of normal size whereas homozygotes, which are viable and fertile, show severe microphthalmia with closed eyelids. Although in an earlier study we suggested linkage of Cat4 to chromosome 2,2 more extensive mapping has localized Cat4 to the central region of chromosome 8 at position cM31.3 No other known mutations resulting in cataract or microphthalmia map to chromosome 8, and Cat4 mutants have no visible abnormalities other than eye defects. In this histologic study, we describe the abnormalities of ocular development in heterozygous and homozygous mice expressing the Cat4a mutant allele.

METHODS

The animals used in this study were descendants of congenic C3H-Cat4a/+ mice maintained in Neuherberg. Progeny of mutant animals were examined at intervals between embryonic day (E)11 and birth. The day of vaginal plug detection was designated E0. The heterozygous phenotype was characterized at E11, E13, E15, and E17 in litters of obligate heterozygotes derived from mating homozygous to wild-type mice. The homozygous phenotype was identified by comparison with the established heterozygous phenotype at the same intervals in litters resulting from mating homozygous to wild-type mice. The homozygous phenotype was identified by comparison with the established heterozygous phenotype at the same intervals in litters resulting from mating homozygous to wild-type mice. The homozygous phenotype was identified by comparison with the established heterozygous phenotype at the same intervals in litters resulting from mating homozygous to wild-type mice.
solution (3 parts 95% ethanol:1 part glacial acetic acid) for 24 hours and then transferred to 70% ethanol. The heads were embedded in glycol methacrylate (Historesin; Leica Instruments, Heidelberg, Germany) and sectioned coronally at 3.5 μm to 6 μm. Serial sections through the eyes were collected and stained with toluidine blue. The eyes of six heterozygous and four homozygous adult mice (4–6 months old) were also examined, fixed in 4% paraformaldehyde for 24 hours, and similarly processed. All experimental procedures in this study conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

RESULTS

External Examination of Embryos

On gross examination, heterozygous embryos did not differ from normal littermates during gestation, but homozygous embryos could be unambiguously identified from E13 onward by inspecting the eyes (Fig. 1). In homozygous mutants at E13 the pigmented anterior margin of the optic cup was abnormally heart shaped with a narrow ventral gap at the site of the optic fissure (Fig. 1C). At later stages of development, extension of the dorsal and lateral margins of the optic cup became more prominent, until by E17 the pupillary area was reduced to a narrow Y-shaped opening (Fig. 1D). The diameter of the optic cup was consistently smaller than normal in the homozygous embryos.

Early Development: E11 to E13

At E11, the earliest stage examined, the lens vesicle was normally separated from surface ectoderm (Fig. 2A). In all heterozygous and homozygous mutant embryos, however, a cellular strand connected the surface ectoderm and the anterior wall of the lens vesicle (Fig. 2B, 2C). In homozygous embryos, the lens vesicle and the optic cup were also notably smaller than normal, and the ventral wall of the optic cup was poorly developed.

At E13, the optic cup and lens of normal (Fig. 2A) and heterozygous embryos (Fig. 3B) were similar in size and differentiation despite a persistent connection between corneal and lens epithelium in the heterozygotes. More severe abnormalities were evident in homozygous embryos (Fig. 3C, 3D). A small mass of primary lens fibers formed from the posterior wall of the lens vesicle, but neither an organized anterior lens epithelium nor an equatorial bow region was present. The small optic cup had defects consistent with those observed in intact embryos (Fig. 1C). An abnormally extended and folded region of the dorsal margin of the optic cup was bordered laterally by shortened and blunted regions (Fig. 3C, 3D), and the optic fissure, closed at this stage in normal and heterozygous embryos, persisted as a narrow cleft in the ventral optic cup (Fig. 3C).

Late Development and Adult Phenotype of Heterozygous Mutants

Abnormalities of heterozygous eyes in subsequent development were limited to the site of contact between lens and cornea. At E15 and E17, a full-thickness defect extended through the otherwise normally formed corneal stroma and endothelium. In some eyes the defect was filled with cells connected to and resembling the corneal epithelium with only a small protrusion of the anterior lens pole contacting the internal corneal surface (Fig. 4A). More commonly, the stromal defect was filled with a large conical protrusion of the lens pole (Fig. 4B), and sometimes (E17 only) lens fiber material extruded through the cornea into the conjunctival sac (Fig. 4C). These patterns show that the strand of cells connecting the lens to the surface ectoderm at earlier developmental stages may differentiate into corneal epithelium or lens epithelium. Lenses with a prominent extension into the cornea, in particular those that lost lens fiber material into the conjunctival sac, were smaller at E17 than those attached only to the inner corneal layers. The defect in the surface epithelium and loss of lens mass associated with extrusion of fiber material through the cornea seemed to be repaired before birth, because in all newborn and adult heterozygotes examined, corneal epithelium always covered the central stromal defect, and the lenses were of approximately normal size.
The patterns of lens–corneal attachment evident in E17 heterozygotes presaged the abnormalities observed in mature eyes. The lens remained attached to the cornea in 75% of the adult eyes examined, and in most instances of persistent attachment a small projection of lens epithelium and capsule was entrapped in the central corneal stromal defect (Fig. 4D). Lens tissue incorporated in the cornea was continuous with a multilayered plaque of epithelial cells and capsulelike material overlying an area of anterior cortical fiber disorganization. The structure of the remaining lens was usually normal, but in a few eyes, swelling and disorganization of posterior cortical fibers were evident. Sometimes the lens was only superficially attached to the cornea at the level of the gap in Descemet’s membrane and corneal endothelium. In these cases, the defect in the corneal stroma was filled with a downward growth of corneal epithelium or fibrous scar tissue. The lens was attached by reflection of Descemet’s membrane and corneal endothelium from the lateral margin of the corneal defect onto the intact lens capsule, by fine fibrous connections between the corneal stromal scar and the lens capsule, or by both. In eyes in which the lens was separated from the cornea, the structure of the central corneal lesion showed that the original attachment of the lens and cornea had been of the more superficial type. The stromal defects, which showed no entrapped lens tissue, were filled with a plug of corneal epithelium or fibrous scar tissue (Fig. 4E), and the lens capsule was intact over the region of the anterior polar cataract (Fig. 4F).

**Late Development and Adult Phenotype of Homozygous Mutants**

The lens and eyecup of homozygotes were markedly abnormal at E15 and E17. Although both structures enlarged with increased gestational age, they remained smaller than those of age-matched heterozygous or normal embryos. A thick column of closely packed cells originating from the corneal epithelium joined the lens as a disorganized cluster of abnormal lens epithelium. By E17 the lens epithelium surrounded the lens fiber mass and extended from it in multilayered cords or tubes (Fig. 5A, 5B). In sections stained with the periodic acid–Schiff method, a thin lens capsule surrounded the ectopic cords of epithelial cells (not shown). No organized region of lens fiber formation from epithelial cells was present. Where the epithelial covering of the lens was incomplete, lens fibers bulged from the surface or erupted into the vitreous cavity (Fig. 5B). The eyecup extended anteriorly to occlude the pupillary area and enclose the lens. A layer of condensed mesenchyme extending from the developing choroid fused with the overlying corneal stroma, and no anterior chamber formed. The optic fissure remained partially open (Fig. 5A), but differentiation of the neural retina and pigment epithelium otherwise appeared normal.

Adult homozygous eyes consistently were very small and located deep in the orbit behind permanently closed lids. The average diameter of the fixed globes was 1 mm, approximately one third the diameter of normal adult eyes and approximately the same size as the eyes of homozygous embryos at E17. The microphthalmic eyes were characterized by a poorly developed cornea overlying an almost completely closed eyecup (Fig. 5C). Neither corneal endothelium nor anterior chamber was present. In all eyes, a strand of corneal epithelial tissue penetrated the disorganized corneal stroma and subadjacent densely pigmented uveal tissue to fuse with remnants of the lens lying within the eyecup. Lens elements in the adult eye consisted only of isolated or clustered bladder cells sometimes surrounded by lens capsule. The ectopic strands of lens epithelial cells prominent in developing eyes at E17 were not seen in the adult eyes. The remainder of the eye was filled with an extensively folded but well-differentiated retina. Retinal pigmented epithelium and choroid were normal in appearance except for colobomata near the optic nerve.

**DISCUSSION**

The Cat4 mutation caused a developmental failure of lens vesicle separation from surface ectoderm. In heterozygous embryos, subsequent growth and differentiation of the lens and other ocular structures was not inhibited despite persis-
FIGURE 3. Eye structure of normal (+/+), heterozygous (+/−), and homozygous (−/−) embryos at embryonic day 13. The lens and optic cup of normal (A) and heterozygous (B) embryos are similar in size and organization except for the connection of corneal and lens epithelium (arrows) in the heterozygous mutants. In homozygous embryos (C), the lens consists of a small ball of primary lens fibers capped anteriorly by a long cellular stalk (arrows) continuous with the surface ectoderm. There is no organized anterior lens epithelium or zone of differentiating fibers. The dorsal margin of the optic cup is shortened and blunted in this plane of section, and the open optic fissure is visible as a narrow cleft (arrowheads) in the ventral wall of the optic cup. In a deeper plane of section through the same homozygous eye (D), the optic cup extends anteriorly to encompass the lens; a prominent fold (arrowbead) in the dorsal arm of the optic cup indicates that the eye is not enlarging normally. Toluidine blue; scale bar, 100 μm.

tent ectodermal connection. The resultant abnormalities of the mature eye, including defects in the central corneal stroma and endothelium and anterior polar cataracts with or without keratolenticular adhesion, arose directly from the preexisting epithelial connection between the lens and surface ectoderm. In homozygous embryos, defective lens vesicle separation led to abortive lens development, failure of closure of the optic fissure, and inhibited growth of the optic cup that resulted in microphthalmia.

Lens vesicle–ectoderm separation in mouse eye morphogenesis involves localized cell death in the epithelial layer, but the mechanism triggering this event is unknown. Expression of the Cat4 mutation in the ectodermal cells or adjacent mesenchyme could inhibit necessary interactions between the ectoderm, mesenchymal cells, or extracellular matrix required for separation. In homozygous embryos, the lens vesicle not only remains attached to the surface ectoderm by a long stalk but is positioned deep in the optic cup and does not form an organized anterior epithelium. This defect and the ensuing abnormalities of lens development may arise from a failure of the inductive interactions between the lens and the mesenchymal and neural tissue at the margin of the eyecup that control size, shape, and orientation of the developing lens. The abnormal expansion of the lens epithelial cell population noted in E17 homozygous embryos suggests a transient stimulation of cell proliferation, but the increased epithelial cell population at this stage could also result from a decreased rate of epithelial cell differentiation into lens fibers. The small size of the lens in homozygous embryos during development may account for the associated microphthalmia; normal lens growth is required for appropriate enlargement of other eye structures with the notable exception of the retina. A direct effect of the homozygous mutation on development of the optic cup cannot be excluded.

At least four other independent mutations result in persistent lens–ectoderm connection and microphthalmia in the
FIGURE 4. Embryonic day (E)17 and adult heterozygotes. At E17 (A, B, C), attachment between cornea and lens is always present but varies in extent as indicated in sections from three representative embryos. (A) The intact anterior pole of the lens touches the cornea at the site of a defect in the corneal endothelium. An overlying defect in the corneal stroma is filled by corneal epithelial cells (arrows). (B) A conical protrusion of the anterior lens bulges through the corneal stroma. The lens epithelium is interrupted at the tip of the protrusion, but the overlying corneal epithelium is intact. (C) Lens fibers rupture into the conjunctival sac (asterisks) through a defect in the lens epithelium and all corneal layers. In a representative adult heterozygous mouse, the lens is connected to the cornea in one eye (D) but is separated in the other eye (E, F). (D) At the cornea-lens attachment, lens cells and capsule embedded in the stromal defect are continuous with the anterior lens pole where a multilayered plaque of epithelial cells overlies an area of cortical disintegration. Corneal endothelium and Descemet’s membrane terminate on either side of the fusion point (arrows). (E) In the cornea of the other eye, fibrous scar tissue fills the stromal defect and extends irregularly through the gap in the corneal endothelium and Descemet’s membrane (arrows). (F) The separate and normally positioned lens has an anterior polar cataract similar to that seen in the attached lens. Toluidine blue; scale bar, 100 μm.

Mouse. These include the dominant mutations Small eye (Pax6) and Coloboma, both located on chromosome 2, and the recessive mutations, dysgenic lens and aphakia. The phenotypic similarities suggest that the affected genes function in the same essential steps of eye morphogenesis. The affected gene responsible for the Small eye phenotype in mice has been identified as Pax6, and mutations of the human PAX6 gene have been identified as a cause of congenital aniridia. Pax6 encodes a highly conserved and developmentally expressed transcription factor that has been proposed to regulate the cascade of genes that participate in eye morphogenesis. In mouse development, Cat4, Coloboma, dysgenic lens, and aphakia may all be part of the Pax6 regulatory cascade.

The phenotype of Cat-/+ mice resembles Peters’ anomaly, a human congenital condition characterized by defects in the central corneal stroma, Descemet’s membrane, and corneal endothelium sometimes associated with keratolenticular adhesion and cataract. Identification of PAX6 mutations in two cases of Peters’ anomaly is consistent with the presence of a comparable phenotype in Small eye mice; PAX6 mutations, however, may only be a rare cause of Peters’ anomaly in humans. Peters’ anomaly occurs with and has been grouped with Rieger’s anomaly, Axenfeld’s anomaly, iridogoniodysgenesis, sclerocornea, and congenital endothelial dystrophy, all developmental malformations of the anterior chamber attributed to mesenchymal dysgenesis of the iris and cornea. These disorders show phenotypic overlap, and affected members of the same family may display one or the other malformation suggesting variable expression of the same gene defect. Conversely, genetic heterogeneity is indicated in some phenotypically similar conditions, such as Rieger’s syndrome with linkage to 4q25 and 13q14. The gene involved in 4q25-linked Rieger’s syndrome (RIEG/PITX2) and the mouse homologue (Rieg/Pitx2) have recently been cloned and characterized as a transcription factor belonging to the class of bicoid-related homeo proteins. A mutation of RIGE/PITX2 has also been identified in 4q25-linked autosomal dominant iris hypoplasia, a condition that does not have several of the features characteristic of the Rieger’s syndrome pheno-
The optic fissure (arrowheads) persists as a narrow cleft in the ventral optic cup. (B) A central section through the lens of the same eye shows the absence of any region of lens fiber differentiation and the disorganization of existing fibers. At gaps in the multilayered epithelial covering (arrowheads), lens fiber material erupts into the vitreous cavity. In an adult homozygous mouse (C), the cornea of the microphthalmic globe is pierced centrally by a strand of corneal epithelial cells (arrosw) that connects in adjacent sections with lens remnants composed of bladder cells (le), scattered epithelial cells, and lens capsule. The anterior chamber is absent. Densely pigmented tissue continuous with the choroidal layer extends forward and is fused with the irregularly organized corneal stroma. The vitreous cavity is also absent, and the globe is filled with folded retina (re) that is well differentiated in some areas. Toluidine blue; scale bar, (A, B) 100 μm; (C) 200 μm.

**References**

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