Early-Onset, Autosomal Recessive, Progressive Retinal Atrophy in Persian Cats

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PURPOSE. An early-onset retinal degenerative disease has been identified in Persian cats. This study genetically, clinically, and histologically characterized the disease. A breeding colony was established to assist with identification of the causative gene and to provide a resource for vision research.

METHODS. Cats were produced from testcross breedings. Kittens underwent serial ophthalmic and neuro-ophthalmic examinations. Globes were harvested from age-matched affected, obligate carrier, and control cats and were evaluated by light microscopy. Fluorescein angiography assessed retinal and choroidal vasculature.

RESULTS. Test breedings confirmed an autosomal recessive mode of inheritance. Rate and extent of disease progression were similar among individual affected cats. The earliest clinical signs (reduced pupillary light reflexes) were seen at 2 to 3 weeks of age. Retinal degeneration was virtually complete by 16 weeks of age. Histologic changes progressed rapidly and paralleled clinical findings. Histologic lesions were limited to the photoreceptors, outer plexiform layer, and retinal pigment epithelium in all but the terminal stages, when subtle changes were noted within the inner nuclear layer.

CONCLUSIONS. Characterized in this study was an autosomal recessive, early-onset, retinal degenerative disease in Persian cats that is likely to be more prevalent in this breed than previously suspected. This feline disease model may identify a new gene or provide biological insight into some forms of early-onset retinitis pigmentosa (RP) in humans and genetic retinal degenerations in other species. A breeding colony that will assist in the identification of the causative gene has been established and is available for studies in vision research. (Invest Ophthalmol Vis Sci. 2005;46:1742–1747) DOI:10.1167/iovs.04-1019

Inherited degenerative retinal diseases form a heterogeneous group of vision disorders associated with photoreceptor dysfunction. Some common subcategories of human retinal degenerations include age-related macular degeneration, retinitis pigmentosa (RP), and Leber’s congenital amaurosis (LCA). Progressive retinal atrophy (PRA) is a similar collective term used to refer to a diverse group of inherited retinal degenerations or dysplasias that cause blindness in animals. Many types of PRA show marked similarities to human retinal diseases and have been developed as animal models for vision research. These animal models have been instrumental in the identification of many of the 150 or more genes associated with retinal degenerations. Animal models continue to help identify new genes responsible for normal retinal function and improve the basic understanding of retinal biology and visual processing and are likely to augment the development of gene and drug therapies.

A variety of species, including the domestic cat (Felis catus), are models for human retinal degenerations. Four types of PRA have been described in cat breeds, and two of them are found in Abyssinian cats. The clinical features of the latter two feline PRAs have been thoroughly characterized, but the causative gene has not yet been identified for either disease. A form of PRA in Persian cats has been briefly described but only at the terminal stages. Affected cats showed marked photoreceptor loss by 15 weeks of age. A recessive mode of inheritance was suggested, but detailed clinical evaluations were not reported and a breeding colony was not established.

Several independently bred Persian cats with an apparently early-onset, heritable retinal degeneration, were identified from different regions of the United States and donated to one of the authors (LAL). A breeding colony was established to characterize the disease and develop a useful model to study retinopathies. Presented data suggest that this is an autosomal recessive, early-onset retinal degeneration in Persian cats that could provide a valuable resource for vision researchers.

METHODS

Subjects and Breeding Studies

A breeding colony was established from five purebred Persian cats (two males and three females) and one male Oriental Shorthair cat donated by breeders. The Persian cats represented four independent catteries, based on pedigree data provided by their owners (Fig. 1). All cats were examined for clinical evidence of retinal degeneration at the University of California, Davis Veterinary Medical Teaching Hospital (VMTH). Examinations were conducted by a board-certified veterinary ophthalmologist (DJM) using slit lamp biomicroscopy (SL-2 slit lamp biomicroscope; Kowa Optomed, Inc., Torrance, CA) and binocular indirect ophthalmoscopy (Video Omega 2C Ophthalmoscope; Heine USA, Ltd., Dover, NH). One male and one female Persian cat had generalized retinal degeneration and were designated as PRA affected. The parents of these two affected cats were reported to behave as if visually normal. The remaining three founder cats were defined as obligate carriers, since they had normal ophthalmic examination findings but when previously bred to cats behaving as if visually normal, they had each produced at least one offspring that developed clinical signs of retinal degeneration before 16 weeks of age. No evidence of
disease transmission was examined by comparing segregating ratios in a pedigree for linkage mapping of the causative gene(s). Sex bias in clinical evaluation, to assess segregation ratios, and to establish a linkage map. Testcross matings between obligate carrier cats and normal control cats were performed to produce cats for a longitudinal clinical studies. Individuals used for linkage mapping were siblings of affected cats; smaller diamonds: stillborn kittens with unknown disease status and unspecified gender; arrows: probands through which lines carrying PRA were ascertainment; solid dots below symbols: founder cats for the colony described in the study; hatched symbol: the Oriental Shorthair breeding cat; open dots above symbols: individuals used for longitudinal clinical studies; underscores: individuals from which globes were obtained for histology.

retinal disease was identified or suspected based on test breedings in the male Oriental Shorthair cat. The established pedigree also segregates for point-restricted coloration, which is a temperature-sensitive mutation that causes cats to have coloration at the extremities (i.e., on the face, ears, tails, and paws). This is the coloration common to Siamese cats. Like Siamese cats, the ‘pointed’ Persians had a subalbinotic fundus.

All animals were housed indoors, under standard laboratory conditions with cyclic fluorescent illumination (14 hours on, 10 hours off) at the Feline Retinoviral Research Laboratory (School of Veterinary Medicine, University of California, Davis [UCD]). Cats were fed standard commercially prepared diets (Iams kitten food; The Iams Company, Dayton, OH; or Science Diet Feline Maintenance; Hill’s Pet Nutrition, Inc., Topeka, KS) appropriate for their age and pregnancy status. All animals were maintained and handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and all experimental procedures were approved by the UCD Institutional Animal Care and Use Committee. Testcross matings between obligate carrier and affected cats were performed to produce cats for clinical evaluation, to assess segregation ratios, and to establish a pedigree for linkage mapping of the causative gene(s). Sex bias in disease transmission was examined by comparing segregating ratios with the $x^2$ test. An outcross breeding of an affected Persian cat and the Oriental Shorthair cat was performed to support the mode of inheritance and to improve genetic variation in the pedigree.

Four domestic shorthair cats (three males, one female) between 3 and 17 weeks of age were obtained from the UCD Feline Nutrition and Pet Care Center. These cats had normal ophthalmic examination findings, no Persian cat ancestry, and served as normal control subjects.

**Clinical Examination**

All kittens produced underwent complete ophthalmic and neuro-ophthalmic examinations beginning soon after eye opening at 10 to 14 days of age. All examinations were performed in the same treatment room at the VMTH to ensure constant and uniform ambient lighting. Ophthalmic examination included slit lamp biomicroscopy and binocular indirect ophthalmoscopy. Findings were recorded using color fundus photography (RC-2 fundus camera; Kowa Optimated Inc., Torrance, CA). The neuro-ophthalmic examination included measurement of horizontal pupil diameter using a Jameson caliper; assessment of direct and consensual pupillary light reflexes (PLRs) with a transilluminator (3.5V Finnoff; Welch Allyn, Skaneateles Falls, NY); assessment of dazzle reflex using the fiberoptic light source from the SL-2 slit lamp biomicroscope; and behavioral testing of vision by menace response, maze testing, visual placing responses, or visual tracking, as appropriate for the age of the animal examined. Vision assessments were subjective and performed in photopic conditions only. Fluorescein angiography was performed on one affected cat (18 months old) and one adult obligate carrier cat (7 years old) with a motorized fundus camera with an excitation filter (Wratten 47; and a barrier filter (Wratten 21; Eastman Kodak, Rochester, NY) after cats were sedated.

**Histologic Examination**

Globes were harvested from affected, age-matched obligate carrier, and control cats by using a transconjunctival enucleation technique performed with the subjects under general anesthesia or immediately after euthanasia with intravenous barbiturate overdose. Enucleated globes were immediately placed in fixative (2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer [pH 7.3]). After fixation for at least 48 hours, the eyes were transacted at the ora ciliaris retinas. The vitreous was gently separated from the posterior eye cup and discarded. The posterior eye cups were sectioned so that representative regions from five areas within the ocular fundus were collected: area centralis (a ganglion-cell-rich, relatively poorly vascularized area temporal to the optic disc and somewhat analogous to the primate macular); midperipheral inferior and superior, and far peripheral inferior and superior. Each region was embedded in glycol methacrylate (Technovit 7100; Energy Beam Sciences, Agawam, MA) and 2-μm sections were stained with toluidine blue for examination with light microscopy (Eclipse E800; Nikon, Osaka, Japan).

**RESULTS**

**Breeding Studies**

A total of 52 kittens from 10 litters were produced by mating obligate carrier and affected cats. A further four kittens from four litters were produced by mating control and affected cats (Fig. 1; Table 1). Three kittens died of causes unrelated to PRA before disease status was determined, and 13 were stillborn. Thus, 40 kittens were available for examination. Disease fre-

**Table 1. Summary of Breeding Results of 14 Matings from which PRA-Affected and Obligate Carrier Cats Were Produced**

<table>
<thead>
<tr>
<th>Mating Type†</th>
<th>Number of Litters</th>
<th>Total</th>
<th>Affected</th>
<th>Unaffected</th>
<th>Unknown‡</th>
<th>Expected Progeny§</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ (A) × ♀ (C)</td>
<td>8</td>
<td>34</td>
<td>16 (11, 5)</td>
<td>15 (9, 6)</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>δ (C) × ♀ (A)</td>
<td>2</td>
<td>18</td>
<td>3 (2, 1)</td>
<td>3 (1, 2)</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>δ (N) × ♀ (A)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2 (1, 1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>δ (A) × ♀ (A)</td>
<td>2</td>
<td>2</td>
<td>1 (0, 1)</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

† A, affected cats; C, obligate carriers; N, normal control cats.

‡ Died of unrelated disease prior to clinical characterization.

§ Assuming that all affected animals were homozygous for mutation. Estimates are based on number of known progeny only.
quency and sex ratios are presented in Table 1. No sex bias was observed in disease transmission ($P = 0.42$). Our breeding pedigree confirms that the form of PRA in these Persian cats followed an autosomal recessive mode of inheritance. Extensive analysis of the pedigree registrations revealed that all founder cats had one common ancestor; however, this ancestor had appeared at least 5 to 11 generations prior.

**Clinical Examination**

Rate and extent of disease progression were remarkably similar between affected individuals of the same litter and between litters. No associations between severity of clinical signs or rate of progression and gender or rate of general physical development were noted. Growth rates between clinically normal obligate carrier and affected cats were similar (data not shown). No clinical evidence of systemic or other ocular disease was noted. Speed and extent of pupillary constriction and dilation were not measured; however, some subjective judgments were possible. Direct and consensual PLRs were present in all animals at 2 weeks of age. The first sign suggestive of retinal disease was a diminution in the extent and speed of direct and consensual PLRs at 2 to 3 weeks of age. This became gradually more notable with increasing age. At 16 weeks of age, PLRs were still present but were dramatically reduced in extent and speed. In addition, pupil dilation (recovery) after direction of the light away from the eye was often subjectively judged as slower in affected than in normal kittens. By 5 weeks of age, horizontal pupil diameter in affected cats (median, 6 mm) was greater than in clinically normal cats (median, 4 mm). Resting pupil size in constant and uniform ambient light gradually increased with age in affected animals until overt mydriasis with sluggish PLR was noted by the terminal stages of the disease at 16 to 17 weeks of age. Dazzle reflex was initially present but was lost in affected animals by 3 weeks of age. Before complete loss of dazzle reflex, some affected individuals ceased to blink in response to the dazzle stimulus, but rather directed their gazes toward the light. Assessment of vision was subjective in very young animals but became less so with increasing age and motor development. Apparent fixation of gaze on objects during behavioral testing of vision in photopic conditions was not detected in any animals for the first 4 weeks of life; however, in most clinically normal obligate carrier animals gaze fixation developed at approximately 5 weeks of age. Gaze fixation did not develop in affected kittens. In some animals, inability to fix gaze was associated with divergent strabismus. Behavioral testing by menace response, visual placing responses, and gaze fixation on moving objects suggested total blindness under photopic conditions in affected individuals by 16 to 17 weeks of age.

Ophthalmoscopic signs of retinal degeneration were progressive and rapid in all affected individuals and paralleled clinical signs. From eye opening until approximately 3 weeks of age, fundus examination was usually limited to observation of optic nerve head and retinal blood vessel size only (Figs. 2A, 2B). Retinal vascular attenuation was first suspected in affected kittens at 4 weeks of age, was usually confirmed at 5 weeks of age, and was unambiguous by 6 to 7 weeks of age (Figs. 2C, 2D). Retinal arterioles and venules appeared to be affected equally. Retinal vessels were rarely visible in affected animals by 16 to 17 weeks of age (Fig. 2E) compared with ophthalmoscopically normal obligate carrier cats (Fig. 2F). However, fluorescein angiography confirmed that retinal vessels were present and patent at this stage and in adult affected cats (Fig. 3).

Subtle, generalized “bronzing” of the tapetal reflection was observed at 6 weeks of age in affected animals. Gradual development of retinal thinning (evident as tapetal hyperreflectivity) was noted in affected cats by 9 weeks of age. Between 9 and 16 to 17 weeks of age, the optic nerve head appeared relatively small and dark compared with that of obligate carrier cats (Figs. 2E, 2F). At 16 to 17 weeks of age, marked generalized retinal thinning (tapetal hyperreflectivity) and retinal vascular attenuation were seen in all affected cats (Fig. 2E) compared with obligate carrier cats (Fig. 2F). The rate, character, and
extent of retinal degeneration did not vary between tapetal and atapetal kittens or between cats with a heavily melanotic retinal pigment epithelium (RPE) and choroid and those with a subalbinotic fundus.

**Histologic Examination**

Twenty-two globes (in brackets) were harvested from 8, 6, and 4 affected, obligate carrier, and control cats, respectively, at 3 \( (n = 3 [1, 1, 1]) \), 5 \( (n = 5 [1, 3, 1]) \), 7 to 8 \( (n = 7 [4, 2, 1]) \), or 16 to 17 \( (n = 7 [3, 3, 1]) \) weeks of age. Histologic changes in affected cats were relatively similar among individuals, progressed rapidly, and paralleled clinical findings (Figs. 4A–D). Lesions were limited to the photoreceptor nuclei and inner and outer segments, outer plexiform layer (OPL), and RPE in all but the terminal stages, when subtle changes were noted within the inner nuclear layer (INL) of affected cats. In affected cats, subtle changes in photoreceptor nuclei alignment and blunting of the photoreceptor inner and outer segments were suspected at the first time point examined (3 weeks of age; Fig. 4A). These changes were confirmed at 5 weeks of age. The photoreceptor nuclei near the area centralis and in inferior and superior mid peripheral regions of the fundus appeared somewhat loosely packed, with more obvious perinuclear space and some reduction in their usual orderly alignment (Fig. 4B). The outer nuclear layer (ONL) near the area centralis was composed of 8 to 10 cell layers in affected animals but was approximately 10 to 12 cells thick in control and obligate carrier cats. Occasional nuclei with morphologic characteristics similar to photoreceptor nuclei were noted among the inner and outer segments. Between 5 and 7 weeks of age, there was a rapid reduction in number and orderly arrangement of photoreceptor nuclei (Fig. 4C). This reduction was most marked near the area centralis, so that the ONL comprised just three to four cell layers. The reduction in photoreceptor nuclei was associated with marked blunting of the inner and outer segments and an increased number of presumed photoreceptor nuclei within this region. Along with the photoreceptor loss, there was marked thinning of the OPL by 7 weeks of age. By 16 to 17 weeks of age, a single layer of photoreceptor nuclei was observed in all regions of the retina of affected cats (Fig. 4D). Some loss of organization and mild vacuolar changes were noted within cells of the INL at the most advanced time point examined (16–17 weeks of age). The inner limiting membrane, nerve fiber layer, ganglion cells, inner plexiform layer, and outer limiting membrane appeared unaffected at all stages of disease. Regardless of layer affected, disease within the neurosensory retina of affected cats increased in severity from the far...
peripheral through the midperipheral regions to the area centralis region near the posterior pole. This gradation was most notable at intermediate time points and became less obvious at terminal stages. When corrected for distance from the posterior pole, tapetal and nontapetal regions appeared to be affected approximately equally. No changes were seen in any layers of the neurosensory retina of obligate carrier (Figs. 4E–H) or control cats (Fig. 4I–L).

Some changes were noted in the RPE of all animals. These changes included cytoplasmic vacuolization, swelling, and occasional cell rupture, sometimes in association with larger, more circular nuclei. Abnormal RPE cells were frequently but not exclusively found overlying the termini of choroidal capillaries as they emerged through the tapetum. Although consistent with autolysis, certain characteristics of these changes were not equivalent among affected, obligate carrier, and control cats. In control cats, these changes were noted at the first time point examined (3 weeks of age), were infrequent and minor, and did not change throughout the course of the study (Fig. 4E). Changes of a similar nature but subjectively greater in severity and frequency also were observed in obligate carrier cats. However, in contrast to the static changes in control cats, changes in obligate carrier cats progressed gradually over all time points examined (Figs. 4F, 4G). Changes within the RPE of affected cats progressed dramatically between 3 (Fig. 4B) and 7 (Fig. 4C) weeks of age but then tended to slow and decrease in severity by the final time point examined (Fig. 4D). Like the changes noted in the photoreceptors, RPE change tended to be more severe in the posterior polar regions than in far peripheral regions at earlier stages of disease.

**DISCUSSION**

A variety of nonhuman animal models of inherited retinal degenerations have long been recognized as counterparts to human retinal degenerations.1 Detailed clinical, histologic, and biochemical characterizations often are necessary to match the phenotypes and to implicate or exclude causative genes of nonhuman animal models with those in humans. Only two forms of feline PRA have been evaluated extensively: an autosomal recessive rod–cone degenerative disorder in one strain of Abyssinian cat found mainly in northern Europe and an autosomal dominant disease described as a rod-cone dysplasia (rcd), so far found only in the United Kingdom.6–15 In 1973, Rubin and Lipton described a PRA in Persian cats that is similar in its terminal clinical and histologic appearance to the Persian cat disease we observed in the current study.5 However, the presented disease appears more clinically and histologically advanced at equivalent time points. Because breeding records were not available from the previously reported cats, a relationship with the current Persians could not be established. However, it is feasible that the same disease has been reascertained. The three other forms of feline PRA that have been described have either a different age of onset or mode of inheritance,5–7 suggesting that these diseases represent at least different alleles, if not different genes, for PRA in the domestic cat.

The presented Persian cats have an early-onset PRA with an autosomal recessive mode of inheritance. Supporting evidence for this mode of inheritance is as follows: (1) Affected kittens were produced from visually and ophthalmoscopically normal cats; (2) probands had evidence of consanguinity; (3) the proportion of affected and unaffected progeny from matings of obligate carrier to affected cats was consistent with an autosomal recessive trait; (4) affected kittens of both genders were observed in similar proportions; and (5) affected-to-unaffected matings produced clinically normal animals that were subsequently proven by further breeding to be obligate carrier cats. The common ancestry suggests that the mutation causing PRA in the founders of the colony is identical by descent; however, any randomly selected groups of cats within a breed could show similar kinship. The identification of several independent lines of Persians affected with PRA and the extended generations required to identify a common ancestor suggest that this disease should be found throughout the breed and that many cats are not being ascertained. The popularity of the Persian breed among cat fanciers suggests that this disease should be of marked concern for the cat fancy and Persian breeders and owners.

Clinical and histologic evidence confirms that this disease begins very early in life—possibly at or before 3 weeks of age—and progresses rapidly, particularly between 5 and 7 weeks of age, until there is almost complete loss of photoreceptors by 16 to 17 weeks of age. The disease is characterized by generalized outer retinal (especially photoreceptor) degeneration with relatively minor involvement of the RPE. Considering that postnatal development of the feline retina continues for at least the first 9 weeks16 and possibly up to 5 months17 of life, the age of disease onset we describe in these cats suggests that this form of PRA can be characterized as a novel form of rcd. However, further characterization of the nature of photoreceptor degeneration using electrophysiologic, immunohistochemical, and electron microscopic studies is needed. We conducted preliminary electroretinograms (ERGs) on selected cats in this pedigree. The scotopic and photopic ERG recordings in obligate carrier cats at the youngest age available for examination using this method (9 weeks of age) were normal, whereas those of affected cats were already nonrecordable at this age (data not shown). The nonrecordable ERG responses in clinically affected kittens show that the disease is an early-onset disorder affecting both rod and cone photoreceptors. Further studies in groups of younger kittens are needed to describe more precisely the electrophysiologic sequence of events.

The histologic changes in the RPE were unexpected, especially when they occurred without associated photoreceptor loss in obligate carrier cats. The nature of these changes and their static presence in control animals suggests that autolysis may have played a role in their pathogenesis. However, the regional distribution, temporal progression, and increased severity and frequency of changes in affected versus obligate carrier cats suggest a genetic component. It is possible that an underlying predisposition to autolytic changes was present in affected and, to a lesser extent, in obligate carrier cats. Failure to open globes before immersion may then have led to variable fixation and facilitated autolytic tendencies in obligate carrier and affected cats. Elucidation of the pathogenesis of these changes requires further investigation with different fixation techniques.

Many forms of PRA, including some forms of rcd, have been reported in a variety of dog breeds. Clinical and histologic features and the age of onset of some forms of canine rcd, such as rcd1 in Irish setter dogs, rcd2 in collie dogs, and rcd3 in Cardigan Welsh corgi dogs are similar to those described herein in Persian cats.18–20 The loss of photoreceptor inner and outer segments and nuclei within the ONL, along with reduction in the OPL in 5-week-old PRA-affected Persians appears similar to histologic changes described in 4-week-old rcd2-affected dogs.19 Furthermore, the notable reduction in ONL nuclei and OPL thickness in affected Persian cats at 7 weeks of age appears similar to that observed in 8-week-old rcd2-affected and 18.5-week-old rcd1-affected dogs.18,19 In addition, rate of disease progression is faster in rcd2 than in rcd1.21 The PRA we describe in Persian cats can be detected
ophthalmoscopy at an earlier age and progresses more rapidly than both rcd1 and rcd2 in dogs.18,19

Adding domestic cat models of heritable blindness will add the number of useful resources for genetically heterogeneous degenerative retinal diseases. Domestic cats have manageable-sized eyes for examination and surgical and therapeutic manipulations, are one of the most highly characterized animals for visual neurophysiology,15 and have sufficient genetic resources for efficient genetic studies.22–25 Furthermore, the form of PRA described in the present study has a very early onset, cat globes are physiologically and anatomically similar to human globes,26 and cats experience less severe intraocular inflammation after surgery than do dogs.27 Combined with low housing costs of cats, these features suggest these cats could be an efficient model for research investigating transplantation, gene therapy, and drug therapy. The availability of a feline model of retinopathy will also permit assessment of efficacy and safety of treatment in an additional nonhuman animal species before clinical trials in human beings.28

We have described an autosomal recessive, early-onset PRA in Persian cats. This form of PRA shares some clinical and histologic features with canine rcd1 and rcd2, but is more rapidly progressive than both. The earlier onset and rapid progression to total blindness may suggest that it is analogous to an early form of RP. Identification of this disease in diverse Persian cat bloodlines suggests that this disease should be of marked concern for the cat fancy and Persian cat breeders, and owners and veterinarians should be vigilant for this condition. Further evaluation of this disease with electrophoretography, electron microscopy, and immunohistochemistry will further characterize the disease, determine the order and relative severity of rod and cone degeneration, and identify the most appropriate comparison to disease in human beings. Additional breedings are proceeding, to extend the pedigree for linkage studies and to provide an animal resource for the vision research community.

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