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Characterization of an Early-Onset, Autosomal Recessive, Progressive Retinal Degeneration in Bengal Cats

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Purpose. A form of retinal degeneration suspected to be hereditary was discovered in a family of Bengal cats. A breeding colony was established to characterize disease progression clinically, electrophysiologically, and morphologically, and to investigate the mode of inheritance.

Methods. Affected and related cats were donated by owners for breeding trials and pedigree analysis. Kittens from test and complementation breedings underwent ophthalmic and neuro-ophthalmic examinations and ERG, and globes were evaluated using light microscopy.

Results. Pedigree analysis, along with test and complementation breedings, indicated autosomal recessive inheritance and suggested that this disease is nonallelic to a retinal degeneration found in Persian cats. Mutation analysis confirmed the disease is not caused by *CEP290* or *CRX* variants found predominantly in Abyssinian and Siamese cats. Ophthalmoscopic signs of retinal degeneration were noted at 9 weeks of age and became more noticeable over the next 4 months. Visual deficits were behaviorally evident by 1 year of age. Electroretinogram demonstrated reduced rod and cone function at 7 and 9 weeks of age, respectively. Rod responses were mostly extinguished at 14 weeks of age; cone responses were minimal by 26 weeks. Histologic degeneration was first observed at 8 weeks, evidenced by reduced photoreceptor numbers, then rapid deterioration of the photoreceptor layer and, subsequently, severe outer retinal degeneration.

Conclusions. A recessively inherited primary photoreceptor degeneration was characterized in the Bengal cat. The disease is characterized by early onset, with histologic, ophthalmoscopic, and electrophysiological signs evident by 2 months of age, and rapid progression to blindness.

Keywords: blindness, domestic cat, electroretinogram, heritable, photoreceptor, PRA

The domestic cat (*Felis silvestris catus*) is a popular companion animal[1,2] as well as an important model for human disease.[3–5] Due to their more recent domestication,[6–8] cats have relatively fewer breeds than dogs,[9,10] and, accordingly, fewer inherited diseases have been identified in the domestic cat.[4,11] However, several ophthalmic diseases in domestic cats have a genetic basis.[12] Three distinct inherited forms of progressive retinal atrophy (PRA) are well documented in domestic cats: an early-onset, dominantly-inherited rod–cone dysplasia (*rdy*)[13] and a late-onset recessively-inherited rod–cone degeneration (*rdAc*),[14] both in Abyssinian cats; and an early-onset, recessively-inherited rod–cone dysplasia in Persian cats.[15] The different modes of inheritance and clinical presentations seen among these retinopathies make affected cats excellent models for various human retinal diseases, including forms of retinitis pigmentosa (RP) and Leber congenital amaurosis (LCA).[12,16] The life span of the domestic cat and the size of its globe make this species an effective model for evaluation of gene therapies, as well as functional retinal prostheses.[12,17] Therefore, detailed clinical, morphologic, and genetic descriptions of feline forms of PRA will support their use as naturally-occurring, large animal models for inherited retinal disease in humans.

The Bengal cat breed was founded in the late 1960's by hybridization of domestic cats and a wild felid species, the Asian leopard (*Prionailurus bengalensis*).[10,18] Predominantly two domestic cat breeds, Abyssinians and the Egyptian Mau, were used to found the Bengal breed and their genetic

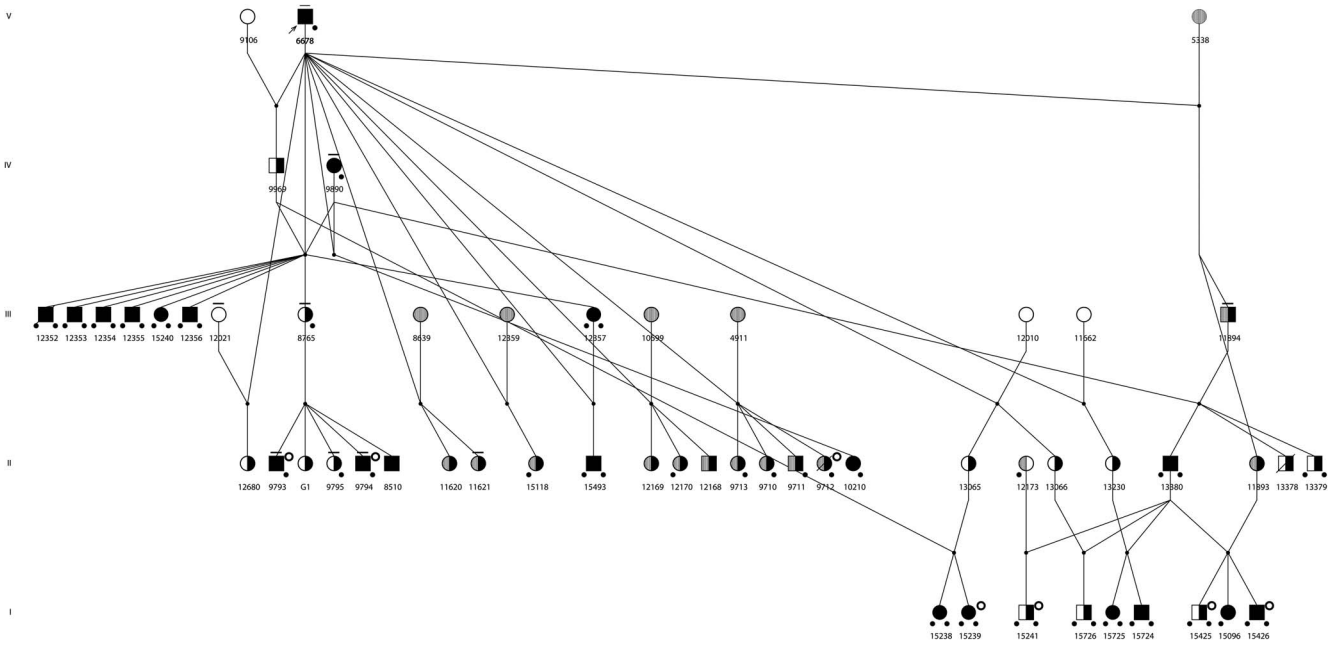

Figure 1. Pedigree of Bengal cats examined for PRA. Only examined cats and their sibships are presented. *Circles* represent females, *squares* represent males, *open symbols* indicate phenotypically normal cats including founder cats of other breeds; *solid symbols* indicate Bengal PRA-affected cats as determined by final diagnosis of fundus and or ERG examinations, *half-filled symbols* indicate obligate carrier cats; *hatched symbols* indicate Persian cats with Persian PRA; *smaller symbols* indicate stillborn kittens of unknown disease status, also marked with a *question mark*, *diamonds* indicate cats of unspecified sex. The *arrow* indicates the proband. A *solid dot below right* of the symbol indicates cats that were examined funduscopically, three cats in the study are not included on the pedigree as they were age-matched controls from unrelated domestic shorthair cats. An *open dot above right* of the symbol denotes cats from which one or both globes were enucleated for histologic examination; cats with a *left dot below* the symbol were evaluated by ERG, the same three cats not included on the pedigree for funduscopic examination were examined by ERG; cats with a *line above* their symbol were diagnosed by ERG, but the data are not included in the report as the recording were performed using a different protocol. One female affected cat was not successfully bred, however, funduscopic examinations were performed and globes were used for histology. For breedings of the affected proband to wild-type cats, only cats examined are presented.

contribution is still evident.[19] This study describes a fourth heritable retinal degeneration in domestic cats, specifically in the Bengal breed. Clinical, behavioral, electrophysiological, morphologic, and genetic evaluations of this PRA in Bengal cats suggest this disease could serve as a feline model for human autosomal recessive RP. The early-onset but gradual progression suggest this form of PRA may be a valuable cat model for evaluating gene therapies, pharmaceutical interventions, and visual prostheses.

Materials and Methods

Research Colony

All animals were maintained and handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and all colony management procedures and animal experiments were approved by the University of California (UC) Davis (Davis, CA, USA) Institutional Animal Care and Use Committee, protocols 11977, 15117, and 16691.

The proband was a 29 weeks of age intact male Bengal that presented to the Ophthalmology Service of the UC Davis Veterinary Medical Teaching Hospital (VMTH) with a complaint of "unusual eye shine." Examination revealed bilaterally widely dilated and nonresponsive pupils, with bilateral generalized retinal vascular attenuation and marked pantapetal hyperreflectivity in an otherwise normal eye. The diagnosis was retinal degeneration, likely progressive retinal atrophy (PRA). At our request, additional cats from the breeder's lines were presented for ophthalmic examination to determine if the condition was heritable.

The proband, three additional affected adult cats (Fig. 1, Cat 9890 and two not on pedigree) and a 5-month-old neutered male from the breeder's line of Bengal cats (Fig. 1, Cat 8510) were identified as affected with PRA. The proband, two related adult intact affected female cat (Fig. 1, Cat 9890, one not on pedigree) and one funduscopically normal dam of affected cats (suspected to be an obligate carrier, Fig. 1, Cat 8765) were donated to establish a breeding colony to characterize this disease (Fig. 1). Once the breeding program was established, cats were examined periodically to provide a clinical description of disease progression.

Ophthalmic and Neuro-Ophthalmic Examinations

Neuro-ophthalmic examinations included measurement of horizontal pupil diameter using a Jameson caliper, assessment of direct and consensual pupillary light reflexes (PLR) using a 3.5 V Finnoff transilluminator (Welch Allyn, Skaneateles Falls, NY, USA), assessment of dazzle reflex using white light directly from the fiberoptic cable of a slit lamp biomicroscope (SL-2; Kowa Optimed, Inc., Torrance, CA, USA), and behavioral testing of vision using menace response, visual placing responses, or visual tracking as appropriate for the age of the animal examined. Vision assessments were subjective and were always performed in photopic conditions with occasional, additional evaluations in scotopic conditions. Ophthalmic examination included slit-lamp biomicroscopy before and after pupil dilation with 1% tropicamide, and binocular indirect ophthalmoscopy after pupil dilation. All clinical examinations were performed by board-certified veterinary ophthalmologists

at the UC Davis VMTH and were conducted in the same examination rooms to ensure constant and uniform ambient lighting. Representative findings were recorded using color fundus photography.

Electroretinography

The investigators conducting the clinical examinations and ERG were masked regarding disease status and regarding each other's findings. To avoid any confounding effects, ERG and clinical examinations were conducted on different days.[20] For ERG, cats were premedicated with subcutaneously administered atropine (0.02 mg/kg), and induced with 3% to 4% isoflurane delivered in oxygen via a tight-fitting mask. Animals were intubated and maintained with 1.0% to 2.5% isoflurane in oxygen. Body temperature was maintained with a forced, air warming system, and pulse rate, respiratory rate, rectal temperature, and systolic blood pressure were monitored.

All animal preparation for ERG was conducted in ambient room light with illuminance of 2 lux (Sekonic Studio Deluxe II model L-398 photometer; Sekonic Co. Ltd., Tokyo, Japan). All ERG recordings were conducted on the left eye, with cats positioned in sternal recumbency. Maximal pupillary dilatation was achieved using 1% tropicamide and the cornea was anesthetized using 0.5% proparacaine. Eyelids were retracted with a Barraquer eyelid speculum, and globes were centered using 1 to 2 subconjunctival stay sutures. To improve conduction, the recorded eye was kept moist with a drop of 2% methylcellulose. Signals were recorded using a contact lens electrode (ERG-Jet; Universo Plastique, LKC Technologies, Inc., Gaithersburg, MD, USA). Subcutaneous needles (E2; Astro-Med, Inc., West Warwick, RI, USA) served as reference and ground electrodes, and were placed at the ipsilateral lateral canthus and aural pinna, respectively. Electrode impedance was maintained at less than 5 kΩ.

Recordings were conducted using a Handheld Multi-species ERG system (HMsERG; Ocuscience, Henderson, NV, USA) with a bandpass of 0.3 to 300 Hz. Background adaptation light and stimuli were delivered using a handheld mini Ganzfeld dome positioned approximately 1 cm from the tested eye. Recordings were conducted using the Dog Diagnostic Protocol preprogrammed into the HMsERG, which is recommended for evaluating retinal function in animals suspected to have PRA.[21,22] The recording protocol began with a 20-minute dark adaptation period, during which rod function was tested every 4 minutes (DA1–DA5) using a dim 10-mcd*s/m^2 stimulus. Subsequently, mixed rod–cone responses to standard, 3 cd*s/m^2, and high, 10 cd*s/m^2, intensity stimuli were recorded. Cone function was assessed following 10 minutes of light adaptation (background luminance of 30 cd/m^2) using a high-intensity flash and the cone flicker test (3 cd*s/m^2).

Mean ± SD (median) ages of affected ($n = 6$) and unaffected ($n = 5$) cats undergoing ERG were 25.0 ± 2.8 (25) and 26.3 ± 3.6 (28) weeks, respectively, ages at which feline electroretinographic recordings reach adult values.[23,24] A- and b-wave amplitudes were measured from baseline to the first trough and from that trough to next positive peak, respectively. Implicit times were measured from stimulus onset to the trough or peak. For flicker tests, the frequency domain amplitude of the first harmonic was extracted (using HMsERG Fourier analysis software) and analyzed. Electroretinographic data from the two groups were compared using Student's *t*-test (two-tailed distribution) or, for nonnormally distributed data, the Mann-Whitney *U* test. To document functional progression of disease, two affected and two unaffected cats were each recorded at 5, 7, 9, 11, and 14 weeks of age. These results are presented descriptively.

Histologic Examination

Immediately following euthanasia with intravenous barbiturate overdose, globes from nine cats at various ages, which had been studied clinically and electrophysiologically, were harvested for morphologic evaluation. 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, eyes were frozen and sectioned approximately at the level of the ora ciliaris retinae. The vitreous was gently separated from the posterior eye cup and discarded. The posterior eye cups then were sectioned such that representative regions from five areas were collected: area centralis; mid-peripheral inferior and superior; and far peripheral inferior and superior fundus. Each region was embedded in glycol methacrylate and 2-μm sections were stained with toluidine blue for examination by light microscopy. Retinas were compared descriptively.

Genetic and Breeding Studies

To genetically characterize this disease, an extended pedigree was produced from the four Bengal founder cats at the UC Davis cat colony. One female affected cat was not successfully bred. A variety of test and complementation breedings with Persian cats affected with PRA[15] were undertaken to determine if the form of PRA noted in these Bengal cats was allelic with the disease described in Persian cats. Parentage of all kittens was confirmed by DNA testing (data not shown).[25] Founders of the Bengal PRA colony were evaluated for mutations responsible for known forms of PRA in Abyssinian cats.[26,27] For *CRX*, PCR primers flanking the n.546delC variant (CRXF: GCAC AAACCAAGGCTCGTCC, CRXR: GATCCAGGCCACTGAAATAG GAG) produced a 331-bp fragment and the PCR primers flanking the *CEP290* IVS50 + 9T > G variant (CEP290F: CTTTTAGAATGTATGAAACCAAGCTGAA, CEP290R: AGATAA TGCCAAGGAGTGGCTTGA) produced a 217-bp fragment. The PCR used 1.5 mM $MgCl_2$ and the PCR cycles were as follows: initial denaturation at 94°C for 4 minutes followed by 40 cycles: 94°C × 30 seconds, 56°C or 64°C × 30 seconds, 72°C × 30 seconds, and final extension at 72°C for 20 minutes. Denaturation was 56°C for *CEP290* and 64°C for *CRX*. Both variants were genotyped by direct Sanger sequencing as previously described.[28]

Results

Ophthalmic and Neuro-Ophthalmic Examinations

Over a period of 6 years, 33 cats, including controls, were clinically examined (Fig. 1) on 89 occasions. Twenty-nine are presented in the figure; the three unrelated normal domestic shorthair controls and one affected female that did not breed are not represented. Cats examined ranged from 4 weeks to 2.75 years of age, and comprised 21 males and 12 females. The median (range) number of examinations per cat was 3 (1–5). Obligate carrier cats were never observed with evidence of retinal degeneration, therefore they were not all examined in subsequent generations. Funduscopic evidence of retinal degeneration is described and depicted in Figure 2. In affected animals, initial funduscopic signs usually became evident between 8 and 20 weeks of age and comprised a generalized increase in granularity and subsequently reflectivity of the tapetum lucidum, along with mild retinal vascular attenuation evident as reduction in vessel diameter and loss of overt quaternary, and sometimes tertiary branching. These signs are considered pathognomonic for widespread retinal thinning in felines. Funduscopic changes became gradually more apparent with increasing age such that

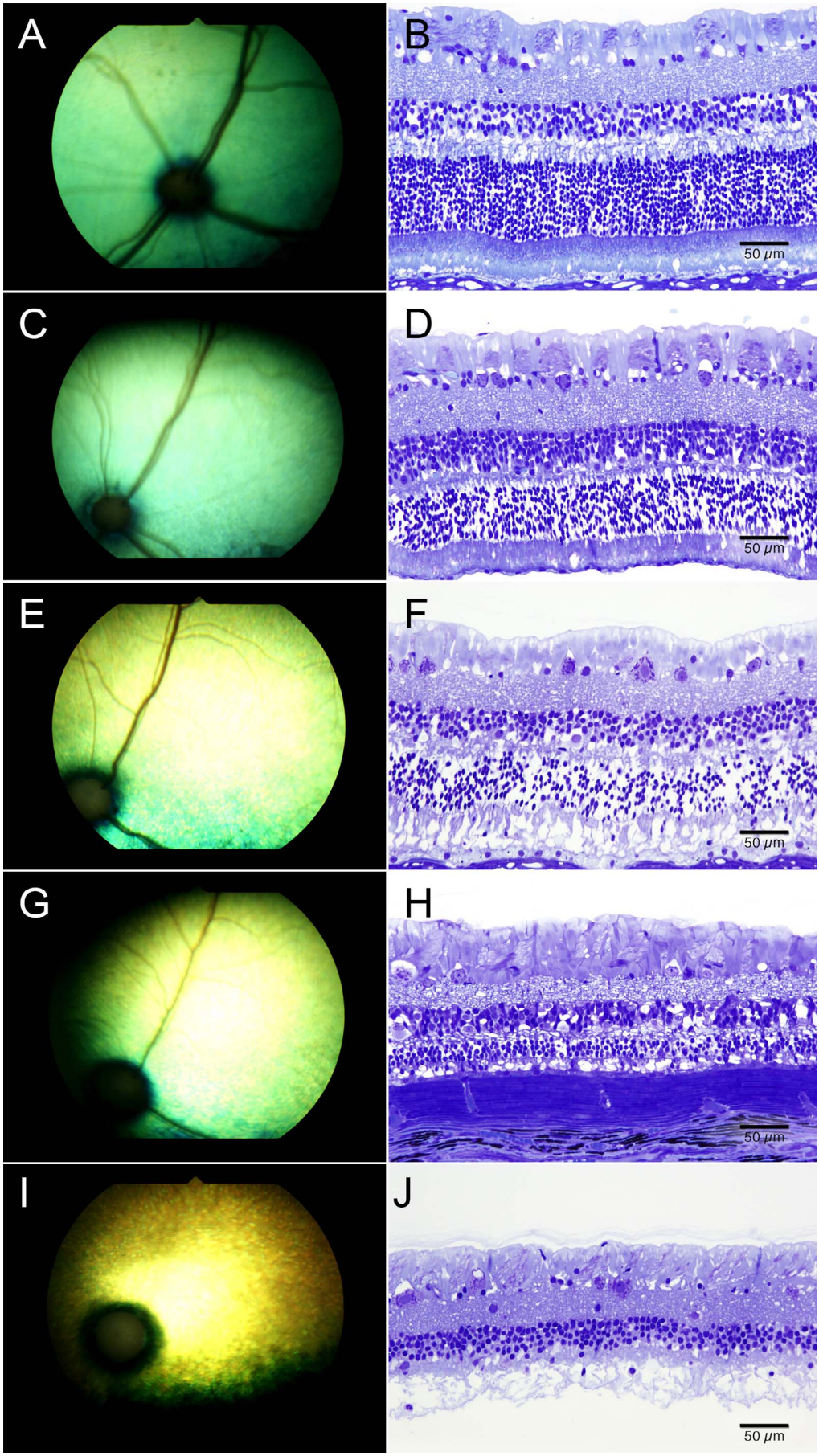

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Figure 2. Color fundus photographs of cats with Bengal PRA. Color fundus photographs (**A**, **C**, **E**, **G**, **I**) and photomicrographs of the area centralis region (**B**, **D**, **F**, **H**, **J**) from an obligate carrier Bengal kitten aged 8 weeks (**A**, **B**) and PRA-affected Bengal kittens (**C**–**J**) aged 8 weeks (**C**, **D**), 25 weeks (**E**, **F**), 41 weeks (**G**, **H**), or 62 weeks (**I**, **J**). Photomicrograph magnification ×400; toluidine blue stain.

by 20 to 25 weeks tapetal hyperreflectivity remained generalized but was moderate to marked, with vascular attenuation evident as reduced secondary branching. The most advanced clinical changes occurred between approximately 40 and 60 weeks of age by which time complete and pan retinal degeneration was readily apparent as marked tapetal hyperreflectivity and absence of discernible retinal arterioles or venules. Ophthalmic signs were always symmetrical.

No obvious reduction in extent or speed of direct or consensual PLRs, or loss of dazzle reflex, was noted until the terminal stages of the degeneration (one cat at 62 and one at 143 weeks). Horizontal pupil diameter was compared between cats subsequently assigned as affected and those determined to be normal using 20 weeks of age as a cut-point. Considering only cats 20 weeks of age or younger, (median; range) pupil diameter was not significantly between affected (4.0; 4–5 mm) and control (4.8; 3–8 mm) cats ($P = 0.47$; Mann-Whitney rank sum test). By contrast, for animals older than 20 weeks (median; range) pupil diameter of affected cats (7; 5–8 mm) was significantly greater than that of control cats (4.8; 3–8 mm; $P = 0.003$; Mann-Whitney rank sum test). Vision assessment typically revealed detectable photopic deficits only in the terminal stages of the disease; commonly from approximately 60 weeks of age onward, though in one cat scotopic visual deficits were noted at 44 weeks.

Based only on results of the final funduscopic examination for all 33 cats, 12 were diagnosed as affected, and 18 as unaffected. The ophthalmoscopic diagnosis remained uncertain at the final exam in three cats aged 12, 14, and 20 weeks, respectively. These cats were adopted and their new owners were suspicious that they exhibited signs consistent with compromised vision, although vision status was never confirmed by a veterinarian. Median (range) age at clinical diagnosis for all 12 affected cats was 20 (9–44) weeks; however, three cats had marked retinal degeneration at the time of their first examination at 29, 44, and 44 weeks of age, respectively. Affected cats were of diverse coat colors,

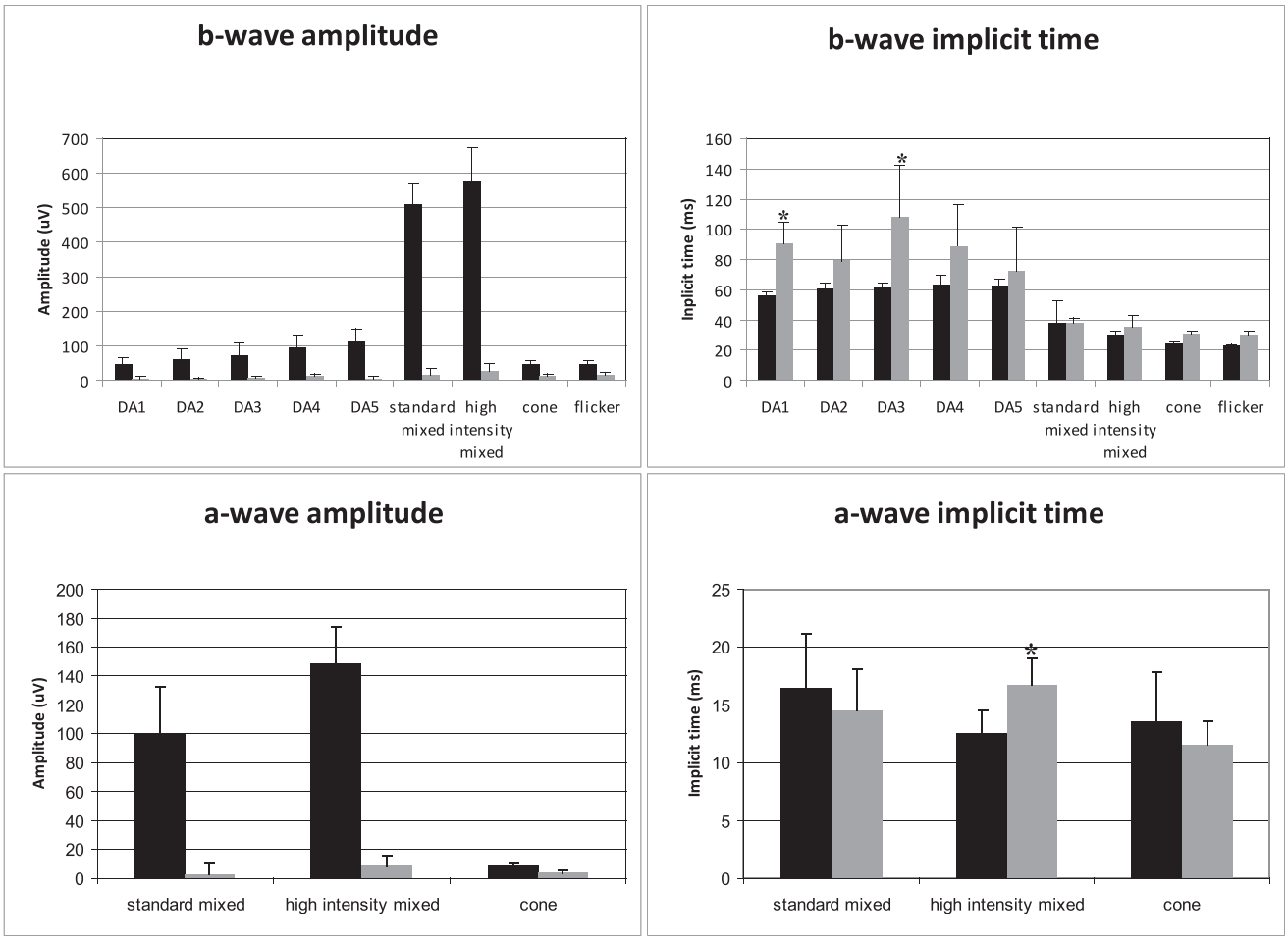

Figure 3. Retinal function in PRA-affected and control cats. Mean ± SD amplitudes and implicit times of a- and b-waves of ERG responses in control (*black bars*, $n = 6$) and PRA-affected (*gray bars*, $n = 5$) Bengal cats. Mean ± SD (median) ages of affected and unaffected cats were 25.0 ± 2.8 (25) and 26.3 ± 3.6 (28) weeks, respectively. Responses of rods (recorded 4, 8, 12, 16, and 20 minutes after the start of dark adaptation and labeled DA1–DA5, respectively), mixed rod–cone and cones are presented. All a- and b-wave amplitudes of normal cats were significantly higher than those of the affected cats. Implicit times of several responses were significantly prolonged in affected cats, and are denoted by an *asterisk*.

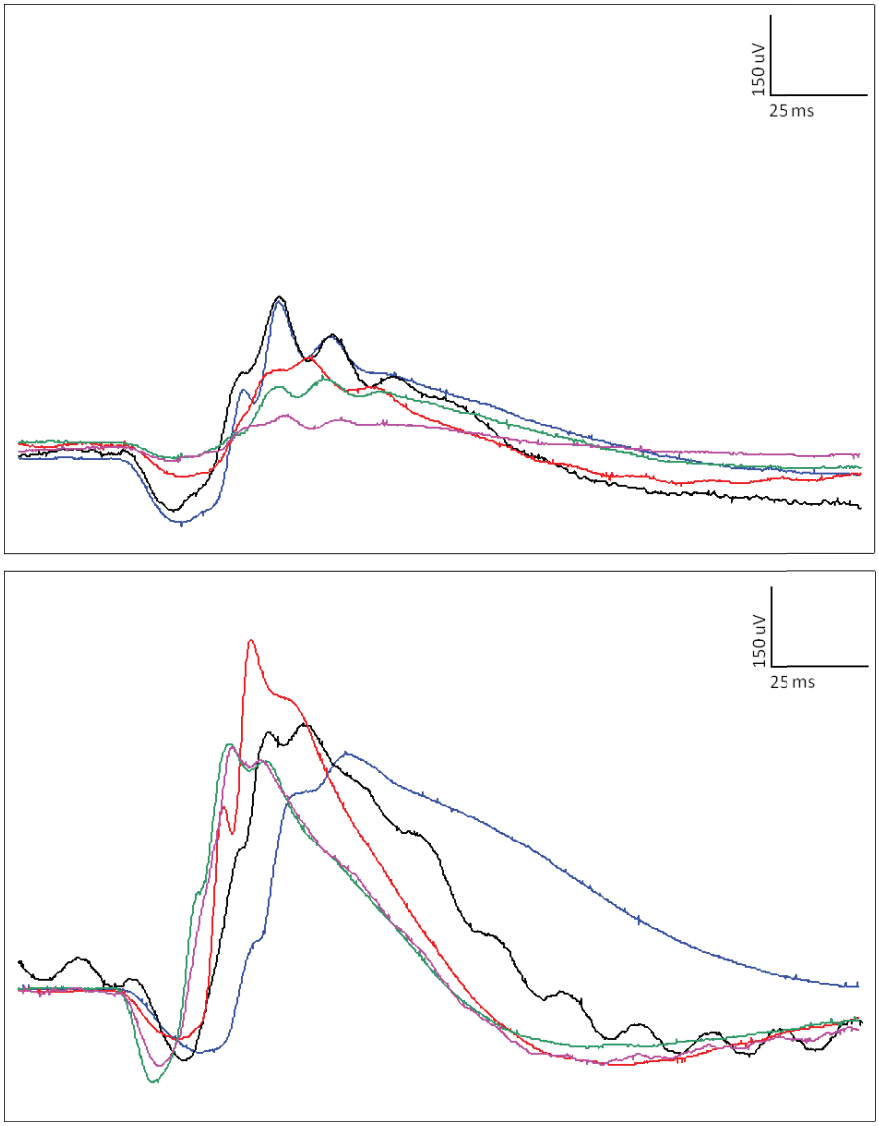

FIGURE 4. Mixed rod–cone responses in a control and PRA-affected cat. High intensity mixed rod–cone responses in a PRA-affected Bengal cat (*top*) and an age-matched normal, control (*bottom*). Sequential recordings were conducted at 5 (*blue*), 7 (*black*), 9 (*red*), 11 (*green*), and 14 (*pink*) weeks of age. Note the progressive decline of retinal function in the affected cat, while amplitudes in the control cat are much higher, and mostly unchanged.

including some with the dilute coat color and subalbinotic choroid/RPE typical of Siamese and Burmese breeds.[29,30]

Electroretinography

Recordings in affected adult cats showed significant ($P < 0.05$) attenuation of a- and b-wave amplitudes of all rod, mixed rod–cone and cone responses, as well as some prolonged implicit times (Fig. 3). Sequential recordings in two affected cats showed early signs of attenuated retinal function starting at 7 weeks of age, which continued to decline in subsequent recordings (Fig. 4). Initial signs of attenuated rod function were seen at age 7 weeks, and early signs of cone dysfunction, as well as a further decrease in of rod function, were seen at the next recording at age 9 weeks (not shown). Differences between affected and normal cats, with severely attenuated rod responses (Figs. 5A, 5B) and reduced mixed rod–cone (Fig. 5C) and cone (Figs. 5D, 5E) responses were obvious at age 11 weeks (Fig. 5). By age 14 weeks, rod responses were regarded as extinguished, and by age 26 weeks only minimal cone responses were recordable (Fig. 3).

Histologic Examination

Most examined retinas were mildly altered by artifacts such as retinal separation, vacuoles, and folds; however, all retinal layers could be evaluated and were comparable across ages and phenotype/genotype. As changes were generally proportional across regions of the retina, lesions from the superior retina (near the area centralis) are described and depicted in Figure 2. In an unaffected, obligate carrier cat at 8 weeks of age (Fig. 2B), photoreceptor inner and outer segments (PIS and POS, respectively) were distinctly visible, and outer nuclear layer (ONL) thickness was 12 to 15 cells. The outer plexiform layer (OPL) was distinct, and the inner nuclear layer (INL) was composed of 4 to 6 layers. At the same age (Fig. 2D), POS of the affected cat was mildly thinned and less distinct. The OPL was also thinned by approximately 50% relative to the unaffected

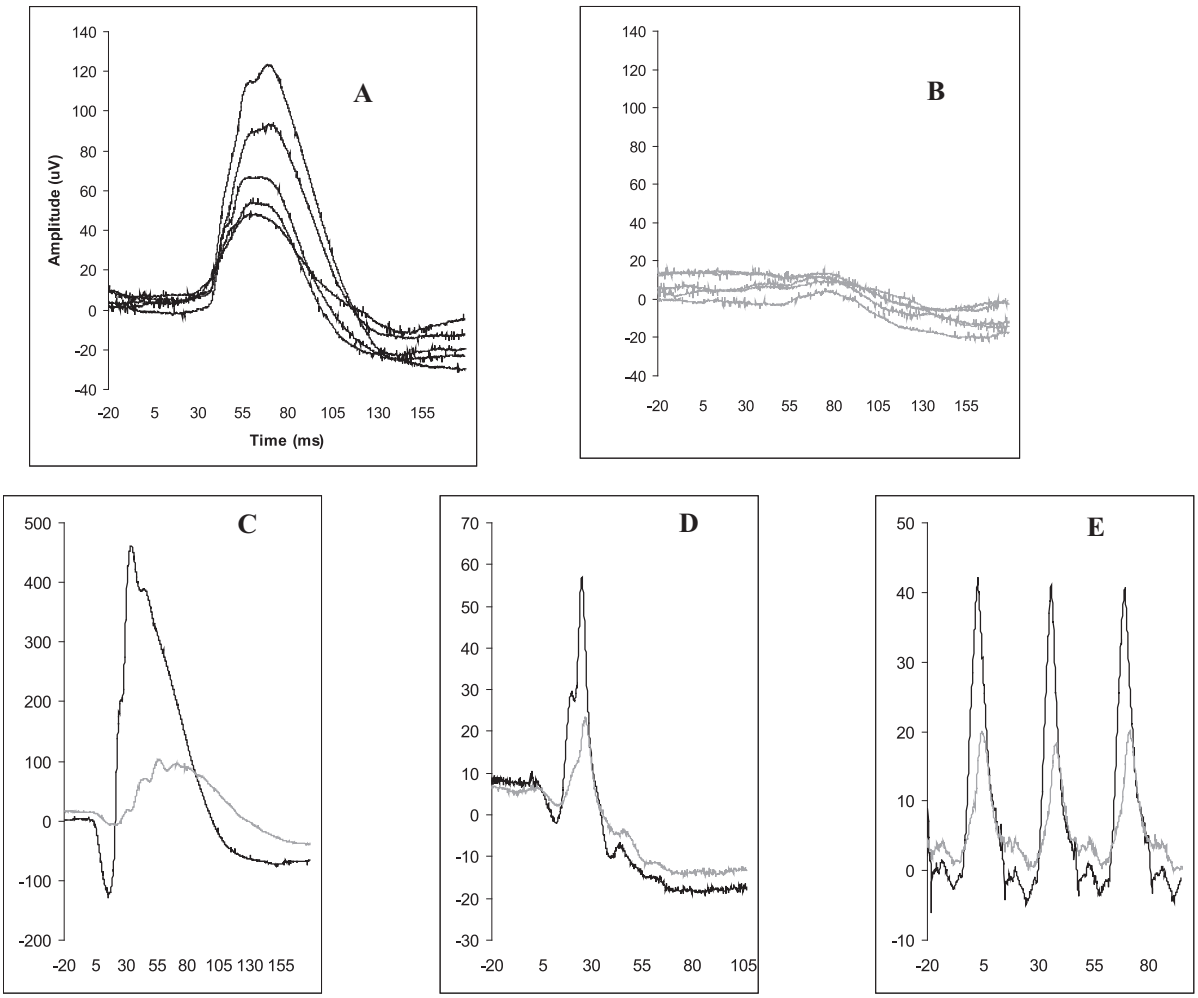

FIGURE 5. Electroretinogram responses of a control and a PRA-affected cat. The dark adaptation process in 11-week-old normal control (**A**) and PRA-affected (**B**) Bengal cats. Responses to scotopic stimuli were recorded every 4 minutes. A progressive increase in responsiveness as the animal spends more time in the dark is evident in the normal cat (**A**), but not in the affected cat (**B**). Amplitude differences between the responses of the two animals are also obvious. Following dark adaptation, responses to standard mixed rod–cone (**C**), photopic (**D**), and cone flicker (**E**) stimuli were recorded in the same two cats. Differences between traces obtained from the normal (*black traces*) and affected (*gray traces*) cats are obvious.

obligate carrier. By 25 weeks (Fig. 2F), photoreceptor outer segments (POS) of two affected cats were completely disorganized or absent, and photoreceptor inner segments (PIS) were markedly distorted and swollen. There were scattered nuclei, presumed to be from the ONL, sclerad of the outer limiting membrane (OLM). The ONL was reduced to 6 to 8 layers, the OPL disorganization and thinning had continued, and the INL was reduced to 2 to 4 layers. Retinal pigment epithelial cells were slightly swollen, relative to unaffected carriers. By 41 weeks of age (Fig. 2H), inner and outer segments of both rods and cones were almost completely absent in the affected cat, and there were increased numbers of aberrant nuclei outside the OLM; RPE changes were similar to those noted at age 25 weeks. The ONL was reduced to 2 to 4 layers. The INL was reduced to two to three layers. At 62 weeks (Fig. 2J), in the oldest affected cat examined, no cellular detail remained in the POS, PIS, or ONL; there were scattered aberrant nuclei outside the OLM. The RPE was unchanged from previous examinations (not shown). The INL was reduced to one to three layers of nuclei. No significant lesions were noted in any affected cat's inner plexiform, ganglion cell or nerve fiber layers.

Genetic and Breeding Studies

Based on examination by board-certified veterinary ophthalmologists and pedigree data provided by breeders, an extensive pedigree was established (Fig. 1). Pedigree analysis of all cats presented to the VMTH and identified in the breeding community revealed that all affected cats had one common ancestor, which appeared at least six generations previously (data not shown). According to breeder reports of behavior, all affected cats had apparently normally-sighted parents.

Several breedings were performed to confirm the mode of inheritance and determine if the form of PRA in Bengal cats was allelic to the form of PRA in Persian cats. Results of test and complementation breedings are presented in the Table. The matings that produced cats with ophthalmic examinations are presented in Figure 1; all performed breedings used to determine segregation are presented in Supplementary Figure S1. Of 38 litters producing 124 offspring, 20 kittens were stillborn or died prior to ophthalmic examinations. Complementation breedings of the affected males with six affected female Persian cats produced 24 kittens (8 males, 16 females). At clinical examinations as late as 1.5 years of age, all offspring from these breedings retained apparently normal vision, normal neuro-ophthalmic reflexes and responses, and normal

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Table. Complementation and Test Breedings to Determine the Mode of Inheritance of PRA in Bengal Cats

			Offspring										
			Affected			Normal				Total			
Sire ♂	Queen ♀	Litters	♂	♀	All	♂	♀	All	Died Prior Diagnosis‡	♂	♀	All	Expected Affected%
Bengal PRA*	Wild-type	4	0	0	0	5	5	10	1	5	5	10	0
Bengal PRA§	Persian PRA	6	0	0	0	8	16	24	0	8	16	24	0
Bengal PRA	Persian Carrier	2	0	0	0	1	3	4	1	1	3	4	0
Bengal Carrier	Persian PRA	1	0	0	0	4	2	6	0	4	2	6	0
Persian PRA	Bengal Carrier	1	0	0	0	2	3	5	0	2	3	5	0
BPRA, PPRA†	Persian PRA	2	0	0	0	4	4	8	0	4	4	8	0
Persian PRA	Bengal PRA	2	0	0	0	1	1	2	0	1	1	2	0
Bengal PRA	Bengal PRA	3	6	2	8	0	0	0	1	6	2	8	100
Bengal PRA	Bengal Carrier	6	5	1	6	5	5	10	5	10	6	16	50
Bengal PRA	BPRA&PPRA†	1	1	1	2	1	0	1	0	2	1	3	50
Bengal Carrier	Bengal PRA	3	3	3	6	1	2	3	1	4	5	9	50
BPRA&PPRA†	Bengal PRA	3	1	0	1	2	1	3	4	3	1	4	50
	Sub-total 50%	13	10	5	15	9	8	17	10	19	13	32	15/32 = 47
Bengal Carrier	Bengal Carrier	1	0	2	2	0	0	0	2	0	2	2	25
BPRA&PPRA†	BPRA&PPRA†	3	0	2	2	1	0	1	5	1	2	3	25
	Total Offspring	38	16	11	27	30	37	67	19	46	46	94	

* Proband bred with normally sighted cats of different breeds (wild-type); offspring not subjected to clinical examinations except for one kitten that had ERG recorded at 21 weeks. These cats were not included in any data interpretations.

† BPRA and PPRA implies obligate carriers for both Persian and Bengal PRA. For breedings of the affected proband to wild-type cats, only cats examined are presented in Figure 1.

‡ Stillborn or died of unrelated disease prior to clinical characterization; sex also unknown.

§ Complementation test breedings of the affected male Bengal proband with Persian cats that were confirmed by clinical examination and pedigree analysis as affected with the early-onset PRA identified in this breed.[15]

funduscopic appearance. Additionally, several F1 offspring were confirmed to have normal retinal function as assessed using ERG, suggesting that the forms of PRA seen in Bengal and Persian cats are nonallelic and unlikely autosomal dominant. The affected female was also bred twice to male Persians with PRA, no offspring were affected. Thus, 26 normal kittens from eight breedings of the affected Bengals to non-Bengal sighted or Persian PRA affected cats suggest that this form of PRA in Bengal cats is not autosomal dominant. Subsequent breedings between F1 and affected cats were performed so that offspring could be reliably predicted to be homozygous affected or heterozygous carriers. Thirteen matings of affected cats with presumed obligate carriers (clinically normal animals with one affected parent) produced 32 kittens and 10 additional stillborn or kittens that died prior to examination. For seven kittens, clinical examination identified them as affected, and five cats had unremarkable funduscopic and neuro-ophthalmic examinations, supporting a recessive mode of inheritance. Overall, 15 of 32 (47%) cats that were evaluated clinically or by owners supported 50% affected from affected by obligate carrier cat breedings. Pedigree analysis also revealed that normal males produced affected females, excluding an X-linked mode of inheritance. The distribution of affected and normal males and females from all test and complementation breedings did not support an X-linked mode of inheritance. These data suggest an autosomal recessive mode of inheritance for the Bengal PRA.

Direct Sanger sequencing was used to genotype the cytosine deletion (n.546delC) in *CRX* and the intronic SNP (IVS50 + 9T > G) in *CEP290*. Both affected Bengal founders, the one presumed obligate carrier Bengal founder and all normal non-Bengal outcross cats had wild-type, normal variants at *CEP290,* homozygous for thymine and *CRX*, no cytosine deletion present; sites known to cause Abyssinian *rdAc* and *rdy* PRA, respectively.[26,27]

Discussion

Spontaneously occurring retinal diseases causing blindness are perhaps the most frequently studied inherited diseases of animals at the molecular level.[31] One reason for this is most inherited retinal diseases of dogs and cats are monogenic and comparatively easy targets for gene mapping, thereby allowing for elimination of specific diseases through DNA-based testing. Another reason is the similarity of various inherited canine and feline retinal diseases, such as various forms of retinal degenerations, dysplasias, and dystrophies, to human retinal diseases including RP and LCA. Well known is the successful gene therapy performed in *RPE65* mutant Briard dogs[32] that paved the way to clinical trials in LCA patients.[33–38] Thus, well-characterized animal models play an important part in basic research and, as in the aforementioned case, in the development of new treatment strategies.

This study identifies and characterizes a novel, relatively early-onset, autosomal recessive retinal degeneration in Bengal cats. The following observations support our hypothesis that this disease has a simple autosomal recessive mode of inheritance: (1) affected kittens were produced from visually normal cats, (2) the proband and the other founder cats for the colony had evidence of consanguinity, (3) affected kittens of both sexes were observed in similar proportions, (4) affected-to-affected breedings produced 100% affected offspring, (5) affected-to-unrelated Persian cats with PRA produced only normal offspring, and (6) affected to obligate carrier breedings produced approximately 50% affected offspring.

Clinical, electrophysiological, and morphologic evidence confirms the retinopathy described here is distinct from the previously described hereditary retinal disease of Persian cats.[15] Firstly, clinical and histologic evidence confirmed that the disease in Persian cats occurs earlier than the disease described here, with complete retinal degeneration reported at

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16 weeks in Persians.[15] Further, complementation breedings of affected Persian cats with an affected Bengal showed that the Bengal and the Persian retinal diseases are nonallelic as all offspring had normal vision.

The Bengal has an obvious risk for the *CEP290* and *CRX* variants since Abyssinians were used in the foundation of this relatively recently-developed breed. Although the *CEP290* variant, locus designated as *rdAc*, was demonstrated to have a low to moderate frequency in Abyssinian and Somali cat populations and in several other breeds worldwide,[39] the *CEP290* mutation was excluded as causative for the retinal degeneration reported herein for Bengal cats. The exclusion of *rdAc* was not surprising since the phenotype of the *CEP290* mutation is different from that of affected Bengal cats; the funduscopic appearance of cats homozygous for the *CEP290* variant typically is normal until approximately 1.5 to 2 years of age.[40,41]

Morphologically, the disease in *rdAc* and in Bengal cats is also different, with more severe degenerative changes in the midperipheral and peripheral retina in early-stage *rdAc*, while the central retina is relatively spared until late in the disease process. In Bengal cats, however, the outer retinal degenerative changes occur both centrally and, to a lesser extent, peripherally in early stages of disease, with generalized and complete retinal atrophy observed only in a 62- week-old cat. Although causal variants for *CRX* and *CEP290* have been excluded other variants in these genes could be present.

The *CRX* Abyssinian mutation was also not identified in the Bengal cats. This, too, is supported by clinical data that revealed the Bengal retinal degeneration was distinct from the early-onset dominant retinal dysplasia of Abyssinian cats, designated *rdy*.[13,42] In *rdy*, cats show nystagmus and abnormal PLRs as early as 2 weeks of age with early ophthalmoscopic changes observed in the area centralis region at 7 weeks. Electroretinographic recordings in cats with *rdy* are usually nonrecordable at 7 to 11 weeks of age and, histologically, generalized retinal atrophy is observed at 12 weeks. Therefore, the temporal characteristics of the Bengal disease are intermediate between those seen in *rdAc* (*CEP290*) and the *rdy* (*CRX*) cats, with the Bengal retinopathy described here having a later onset and slower progression than *rdy*, but earlier onset and more rapid progression than *rdAc*.

The gene variant causing the Bengal disease is likely to be different than those previously described in cats and may be derived from domestic or wild felid progenitors. Fundus examinations can detect generalized increase in granularity and subsequently reflectivity of the tapetum lucidum, along with mild retinal vascular attenuation as early as 8 weeks of age, however, the disease has a varied course of progression. Some cats seem sighted based on behavior over 1 year of age. Electroretinogram studies supported the histologically findings of a photoreceptor degeneration, suggesting a rod–cone degeneration. Bengal cats affected by this comparatively early-onset disease (PRA–Bengal) leading to blindness within only 1 year have the potential to become a valuable animal model for early-onset and rapidly progressing human retinal diseases, and this cat breed may be especially useful in studying treatment strategies for hereditary retinal blindness.

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References

1. APPMA. *National Pet Owner's Survey*. Greenwich: American Pet Product Manufacturing Association; 2008.
2. AVMA. *US Pet Ownership and Demographics Sourcebook*. Schaumburg: American Veterinary Medical Association; 2007.
3. Lyons LA. Genetic testing in domestic cats. *Mol Cell Probes*. 2012;26:224–230.
4. Nicholas FW, Harper PAW. Inherited disorders: the comparative picture. *Aust Vet J*. 1996;73:64–66.
5. O'Brien SJ, Wienberg J, Lyons LA. Comparative genomics: lessons from cats. *Trends Genet*. 1997;13:393–399.
6. Vigne JD. The origins of animal domestication and husbandry: a major change in the history of humanity and the biosphere. *C R Biol*. 2011;334:171–181.
7. Vigne JD, Briois F, Zazzo A, et al. First wave of cultivators spread to Cyprus at least 10,600 y ago. *Proc Natl Acad Sci U S A*. 2012;109:8445–8449.
8. Vigne JD, Guilaine J, Debue K, Haye L, Gerard P. Early taming of the cat in Cyprus. *Science*. 2004;304:259.
9. CFA. *The Cat Fanciers' Association Complete Cat Book*. 1st ed. New York: Harper Collins Publishers; 2004.
10. Morris D. *Cat Breeds of the World*. New York: Penguin Books; 1999.
11. Nicholas FW, Brown SC, Le Tissier PR. *Online Mendelian Inheritance in Animals, OMIA*. Sydney, University of Sydney; 1998.
12. Narfstrom K, Holland Deckman K, Menotti-Raymond M. The domestic cat as a large animal model for characterization of disease and therapeutic intervention in hereditary retinal blindness. *J Ophthalmol*. 2011;2011:906943.
13. Curtis R, Barnett KC, Leon A. An early-onset retinal dystrophy with dominant inheritance in the Abyssinian cat. Clinical and pathological findings. *Invest Ophthalmol Vis Sci*. 1987;28:131–139.
14. Narfström K. Hereditary progressive retinal atrophy in the Abyssinian cat. *J Hered*. 1983;74:273–276.
15. Rah H, Maggs DJ, Blankenship TN, Narfstrom K, Lyons LA. Early-onset, autosomal recessive, progressive retinal atrophy in Persian cats. *Invest Ophthalmol Vis Sci*. 2005;46:1742–1747.
16. Narfstrom K, Deckman KH, Menotti-Raymond M. Cats: a gold mine for ophthalmology. *Annu Rev Anim Biosci*. 2013;1:157–177.
17. Narfstrom K. Hereditary and congenital ocular disease in the cat. *J Feline Med Surg*. 1999;1:135–141.

18. Johnson G. *The Bengal Cat*. Greenwell Springs: Gogees Cattery; 1991.
19. Kurushima JD, Lipinski MJ, Gandolfi B, et al. Variation of cats under domestication: genetic assignment of domestic cats to breeds and worldwide random-bred populations. *Anim Genet*. 2012;44:311–324.
20. Tuntivanich N, Mentzer AL, Eifler DM, et al. Assessment of the dark-adaptation time required for recovery of electroretinographic responses in dogs after fundus photography and indirect ophthalmoscopy. *Am J Vet Res*. 2005;66:1798–1804.
21. Narfstrom K. Electroretinography in veterinary medicine–easy or accurate? *Vet Ophthalmol*. 2002;5:249–251.
22. Ekesten B, Komáromy AM, Ofri R, Petersen-Jones SM, Narfström K. Guidelines for clinical electroretinography in the dog – 2012 update. *Doc Ophthalmol*. 2013;127:79–87.
23. Jacobson SG, Ikeda H, Ruddock K. Cone-mediated retinal function in cats during development. *Doc Ophthalmol*. 1987; 65:7–14.
24. Hamasaki DI, Maguire GW. Physiological development of the kitten's retina: an ERG study. *Vision Res*. 1985;25:1537–1543.
25. Lipinski MJ, Froenicke L, Baysac KC, et al. The ascent of cat breeds: genetic evaluations of breeds and worldwide random-bred populations. *Genomics*. 2008;91:12–21.
26. Menotti-Raymond M, Deckman KH, David V, Myrkalo J, O'Brien SJ, Narfstrom K. Mutation discovered in a feline model of human congenital retinal blinding disease. *Invest Ophthalmol Vis Sci*. 2010;51:2852–2859.
27. Menotti-Raymond M, David VA, Schaffer AA, et al. Mutation in *CEP290* discovered for cat model of human retinal degeneration. *J Hered*. 2007;98:211–220.
28. Gandolfi B, Alhaddad H, Joslin SE, et al. A splice variant in *KRT71* is associated with curly coat phenotype of Selkirk Rex cats. *Scientific Reports*. 2013;3.
29. Ishida Y, David VA, Eizirik E, et al. A homozygous single-base deletion in *MLPH* causes the dilute coat color phenotype in the domestic cat. *Genomics*. 2006;88:698–705.
30. Lyons LA, Foe IT, Rah H, Grahn RA. Chocolate coated cat: *TYRP1* mutations for brown color in domestic cats. *Mamm Genome*. 2005;16:356–366.
31. Miyadera K, Acland GM, Aguirre GD. Genetic and phenotypic variations of inherited retinal diseases in dogs: the power of within- and across-breed studies. *Mamm Genome*. 2012;23: 40–61.
32. Acland GM, Aguirre GD, Ray J, et al. Gene therapy restores vision in a canine model of childhood blindness. *Nat Genet*. 2001;28:92–95.
33. Maguire AM, Simonelli F, Pierce EA, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med*. 2008;358:2240–2248.
34. Bainbridge JW, Ali RR. Keeping an eye on clinical trials in 2008. *Gene Ther*. 2008;15:633–634.
35. Bainbridge JW, Ali RR. Success in sight: the eyes have it! Ocular gene therapy trials for LCA look promising. *Gene Ther*. 2008; 15:1191–1192.
36. Bainbridge JW, Smith AJ, Barker SS, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. *N Engl J Med*. 2008;358:2231–2239.
37. Banin E, Bandah-Rozenfeld D, Obolensky A, et al. Molecular anthropology meets genetic medicine to treat blindness in the North African Jewish population: human gene therapy initiated in Israel. *Hum Gene Ther*. 2010;21:1749–1757.
38. Hauswirth WW, Aleman TS, Kaushal S, et al. Treatment of Leber congenital amaurosis due to *RPE65* mutations by ocular subretinal injection of adeno-associated virus gene vector: short-term results of a phase I trial. *Hum Gene Ther*. 2008;19: 979–990.
39. Menotti-Raymond M, David VA, Pflueger S, et al. Widespread retinal degenerative disease mutation (rdAc) discovered among a large number of popular cat breeds. *Vet J*. 2010; 186:32–38.
40. Narfstrom K, Nilsson SE. Hereditary retinal degeneration in the Abyssinian cat: correlation of ophthalmoscopic and electroretinographic findings. *Doc Ophthalmol*. 1985;60:183–187.
41. Eizirik E, David VA, Buckley-Beason V, et al. Defining and mapping mammalian coat pattern genes: multiple genomic regions implicated in domestic cat stripes and spots. *Genetics*. 2010;184:267–275.
42. Barnett KC, Curtis R. Autosomal dominant progressive retinal atrophy in Abyssinian cats. *J Hered*. 1985;76:168–170.

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