An Early-Onset Retinal Dystrophy With Dominant Inheritance in the Abyssinian Cat

Clinical and Pathological Findings
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The clinical and pathological features of an early-onset autosomal dominant photoreceptor degeneration in the Abyssinian cat are described. Ophthalmoscopic evidence of retinal disease at 8–12 weeks of age was always preceded by marked dilatation of the pupils, impairment of the pupillary light reflex, and nystagmus. The electroretinogram was unrecordable in all but one of the affected individuals examined. Abnormal photoreceptor development was observed by both light and electron microscopy in retinas of a 22-day-old kitten; in this individual, no outer segment material was detected, and inner segments showed impaired development which was more severe towards the posterior pole. In a 40-day-old kitten, the inner segments were relatively well-formed, whereas the outer segments, though present, showed marked disorganization and degenerative change. The retinas of older individuals showed more advanced photoreceptor degeneration, with thinning of the neural retina. This early-onset retinopathy, which may be classified as a rod-cone dysplasia, is distinct from the hereditary retinal dystrophy (progressive retinal atrophy) previously described in this breed. The gene symbol Rdy has been adopted. Invest Ophthalmol Vis Sci 28:131–139, 1987

Inherited retinal degenerations have been studied extensively in several species, including man, dog, rat, and mouse. Instances of bilateral generalized retinal degeneration have been described in the cat, but only in some has inheritance been established. A form of progressive retinal atrophy (PRA) was diagnosed in the Abyssinian cat in Sweden in 1977, and, subsequently, elsewhere in Europe. In this autosomal recessive disease, the first clinical signs become apparent at approximately 1½–2 yr of age. Feline retinal dystrophies of much earlier onset have been reported in the United States in the Persian breed and in three generations of domestic cats of mixed breeding. In the latter condition, the appearance of clinical signs and virtual extinction of the electroretinogram occurred as early as 4 weeks of age. More recently, in England, a condition of similarly early onset has been described in the Abyssinian cat, and breeding studies involving 14 litters have confirmed an autosomal dominant mode of inheritance. Clinical, pathological, and ultrastructural features of this condition are reported in the present paper.

Materials and Methods

Animals

All affected and unaffected cats studied were descended from a single affected male Abyssinian cat with bilateral advanced generalized retinal atrophy and a history of defective vision first noticed by the owners at 4 months of age. Fourteen litters comprising 57 individuals were produced: 3 from matings in which both parents were affected, and 11 from affected male × unaffected female matings; the unaffected females were either unrelated cats or unaffected sibs of affected individuals. These litters have been described elsewhere. The distribution of unaffected and affected individuals within these litters was consistent with an autosomal dominant mode of inheritance. The investigations described in this paper were carried out in accordance with the ARVO Resolution on the Use of Animals in Research.

Clinical Observations

All individuals were examined at approximately weekly intervals until 4 months of age, and, thereafter, at least monthly. Some affected animals have been observed to beyond 4 yr of age. Direct or indirect ophthalmoscopy of each fundus was performed following mydriasis with 1% tropicamide (Mydriacyl, Alcon Laboratories, Watford, England) and retinal photographs were taken with a Kowa RC2 fundus camera.
Electroretinography

Electroretinography was carried out under general anaesthesia on 12 affected and 12 unaffected individuals, at ages ranging from 17 days to 17 weeks and 17 days to 19 weeks, respectively. Prior to anaesthesia, pupils were dilated with 10% phenylephrine and 1% tropicamide (Mydriacyl, Alcon). Anaesthesia was induced with alphaxalone/alphadalone acetate (Saffan, Glaxo-ovet Ltd., Harefield, Middlesex, England), administered intravenously, and maintained with halothane (Fluothane, ICI, Macclesfield, Cheshire, England). A clear plastic contact lens electrode was placed on the cornea after application of a mixture of physiological saline and 1% hypromellose. Monopolar insulated stainless steel needles were inserted subcutaneously adjacent to the lateral canthus and at the base of the ear to serve as reference and ground electrodes, respectively. Flash stimuli of varying intensity were provided by a Grass Photic Stimulator PS22 (Grass Medical Instruments, Quincy, MA) used in conjunction with a series of neutral density filters. Potentials were recorded using a Medelec (Medelec Ltd., Old Woking, Surrey, England) mainframe incorporating AC amplifiers and a signal averager. Responses to single flashes, or averaged responses to multiple flashes, of white light were obtained after a period of 30 min of dark adaptation.

Light and Electron Microscopy

During the course of this investigation, 11 affected and 8 unaffected cats ranging in age from 22 days to 27 months were killed with pentobarbitone sodium (Euthesate, Willows Francis Veterinary, Horsham, Sussex, England) administered intravenously. The eyes were extirpated within 3 min of death. One eye was fixed in Davidson’s fluid;* 3 μm sagittal sections, prepared from paraffin-embedded material and stained with haematoxylin and eosin, were examined by light microscopy. The other eye was used for electron microscopy; tissue fragments including retina, choroid, and sclera were taken from the mid-tapetal region and from an equivalent site inferior to the optic disc. More extensive samples, including peripheral and posterior polar retina, were taken from the eyes of two kittens aged 22 and 40 days, the youngest affected individuals to be examined. Samples were fixed in 2.5% glutaraldehyde in phosphate buffer pH 7.2, dehydrated in a series of ethanol, and embedded in Araldite. Silver sections cut on an ultramicrotome and stained with uranyl acetate and lead citrate were examined with a Philips 410 LS electron microscope.

Results

Clinical Observations

The first sign of visual abnormality in affected kittens was an increase in pupil size, the disparity between affected and unaffected littermates becoming evident in moderate daylight between 2 and 3 weeks of age. The pupillary light reflex was present, but sluggish and incomplete. There followed, at 4–5 weeks of age, the development of an intermittent nystagmus varying in character between individuals. The direction was usually either vertical or horizontal, but occasionally rotary; the excursion was short, and it was frequently impossible to distinguish fast and slow components. Accompanying head movements (nodding) were apparent in young kittens with severe nystagmus. The nystagmus later became less obvious with advancing retinal degeneration.

The first ophthalmoscopic sign of retinal degeneration, visible at about 8 weeks of age, was dullness and slight loss of detail of the tapetal fundus. Subsequently, the tapetal area became hyper-reflective, the normal yellow-green color becoming less intense and more silvery and mirror-like in appearance. The early changes were more severe in the area centralis, but were always diffuse and never demarcated. These signs were accompanied by progressive attenuation of the retinal blood vessels, both venous and arterial (Fig. 1A–C). By approximately 1 yr of age, the fundus appeared avascular, apart from isolated ghost vessels visible in the tapetal region. In some individuals, patchy tapetal degeneration, identified by the absence of tapetal structure and the exposure of choroidal pigment and sometimes vessels, was observed in advanced cases (Fig. 1D). The non-tapetal fundus showed little change until late in the course of the disease; by 18 months of age, pallor of the region was evident, and, by 2 yr, distinct, rounded, pale patches were present with darker pigment between. In advanced cases, the optic disc was pale and atrophied, with a slightly crenated edge.

Lens changes were not observed in affected cats, the oldest of which exceeded 4 yr of age. Prior to the appearance of nystagmus, it was not possible to distinguish affected kittens using simple behavioural tests based on the ability to fixate. However, there was no evidence that potentially affected kittens ever had significant vision. From about 5 weeks of age, it was obvious that affected individuals had a visual deficit, being less inclined to play and more precise and careful in their movements.

Although marked dilatation of the pupil was a constant finding from a very early age, some retention of the pupillary light reflex was notable. It was usually possible to elicit a residual pupillary light reflex, even

* 4% formaldehyde, 10% acetic acid, and 28.5% ethyl alcohol in aqueous solution.
Fig. 1. Fundus photographs. A, Normal 12-week-old kitten. B, Affected kitten aged 13 weeks showing blood vessel attenuation. C, Affected 16-month-old cat showing advanced retinal degeneration; a single “ghost” vessel is visible in the tapetal area. D, Affected 2-yr-old cat with advanced retinal atrophy and tapetal degeneration.
in advanced cases aged 4 yr or older with ophthalmoscopically avascular and hyper-reflective fundi.

**Electroretinography**

All unaffected individuals had electroretinograms (ERGs) which, in terms of waveform morphology, a- and b-wave amplitude, and form of the b-wave amplitude/log relative intensity curve, resembled those of unrelated normal cats tested by similar means in this laboratory. In contrast it was impossible to elicit an ERG from any of the affected individuals, with the sole exception of one 17-day-old kitten (Fig. 2); in this animal, a small electropositive response was recordable, but only with white light stimuli of high intensity. However, it proved to be impossible to elicit a response when this individual was retested 5 days later. Seven other kittens were tested at an early age: three affected individuals aged 17, 18, and 24 days had no recordable ERG, while four unaffected kittens aged 17, 24 (2), and 26 days had normal ERGs.

**Retinal Pathology**

With light microscopy, it was possible to distinguish the affected and unaffected eyes of kittens aged 22 and 24 days, respectively; the main difference being the greatly reduced thickness of the layer of photoreceptor inner and outer segments in the affected eye (Fig. 3A, B). There was no significant difference in the number of nuclei in the outer nuclear layer: in equivalent areas of both tapetal and non-tapetal fundus, there were approximately 11 rows. However, the nuclei of the outer nuclear layer in the affected eye were oval, and did not have the normal elongated form of those in the unaffected eye. Examination of the affected retina revealed various stages of photoreceptor degeneration in different areas. Inner segments were scarce, small, and stubby near the posterior pole, whereas, in more peripheral areas of retina, they were present in greater numbers and appeared more elongated.

In electron micrographs of the unaffected retina of the 24-day-old kitten, rod and cone inner and outer segments were relatively well-developed, with rod outer segments containing regular stacks of discs extending to the pigment epithelium. In contrast, photoreceptor inner segments of the affected eye of the 22-day-old kitten were represented by rudimentary club-like protrusions through the external limiting membrane (Fig. 4). Many of these were seen to contain both a basal body and a centriole. Although well-developed cilia were seen, there was no evidence of outer segment material or the elaboration of disc lamellae, even in the more peripheral areas where the inner segments were slightly longer. Pigment epithelial cells were characterized by numerous prominent microvilli, which were frequently in contact with the rudimentary inner segments. The remaining layers of the retina of the affected eye appeared normal.

In the eye of an affected kitten aged 40 days, the layer of photoreceptor inner and outer segments was of almost normal thickness, and the other retinal layers appeared largely normal (Fig. 3C). However, under the electron microscope, there were marked degenerative changes in the inner segments and severe degeneration and disorganization of the outer segments, the presence of which contrasted with their absence in the 22-day affected retina (Fig. 5A, B). The inner segments, although shortened, were still readily identifiable, and the photoreceptor external receptor fibers were clearly distinguishable at the level of the external limiting membrane. Vesicular dilatation of the smooth-surfaced
Fig. 3. Light micrographs of retinas of affected and unaffected Abyssinian kittens at various ages. A, Affected 22 days. The layer of photoreceptor inner and outer segments, especially near the posterior pole, is undeveloped at this stage, but the remaining layers appear normal. B, Unaffected 24 days. There are distinct photoreceptor inner and outer segment layers. C, Affected 40 days. Both the photoreceptor inner and outer segment and outer nuclear layers are developed. D, Affected 12 weeks. The photoreceptor inner and outer segment layer is becoming thinner, although the inner segments are still discernible. E, Affected 19 weeks. Degeneration and thinning of the photoreceptor inner and outer segment and outer plexiform layers are now obvious, and the nuclei of the outer nuclear layer are becoming pyknotic and spatially separated. F, Affected 27 months. The layer of photoreceptor inner and outer segments is absent, and the outer nuclear layer is reduced to one layer of nuclei. The retinal pigment epithelium, with which it is in contact, shows marked loss of cells. (H and E original magnification ×170).

Endoplasmic reticulum was present in the myoid, in which many free ribosomes and neurotubules were still present. The mitochondria of the ellipsoid appeared distended and were few in number. However, endoplasmic vesiculation and distended mitochondria were also seen in the unaffected 24-day-old kitten, and were probably artefactual. Connecting cilia and their accompanying basal bodies were readily identifiable, and root filaments were prominent in the inner segments, sometimes lying in close proximity to the external limiting membrane. Cilia cut in cross-section showed the normal microtubular arrangement of nine doublets. In a few sections, connections could be established between inner and outer segments; however, the normal arrangement of disc lamellae was almost totally lost, with disc material being represented by irregular sheets of lamellae with mainly axial orientation. Towards the pigment epithelium, it became difficult to distinguish individual outer segments, and bizarre arrays of disc material in the form of sheets or whorls, often with severe vesiculation, were evident. In this, as in the 22-day affected eye, great difficulty was experienced in distinguishing between rod and cone photoreceptors, both of which were clearly severely affected.

The pigment epithelium in this eye was intact, and the cells contained many mitochondria and normal endoplasmic reticulum. Numerous microvilli were seen to surround the masses of degenerative outer segment material (Fig. 5C), but phagosomes, although present, were very infrequent. Often, the cytoplasmic processes extended deeply into the photoreceptor outer segment material, and pigment granules were prominent in non-tapetal regions of the retina. At this stage, the developing tapetum appeared to have normal structure.

By 10 weeks of age, there was obvious thinning of the layer of photoreceptor inner and outer segments, and greater disorganization of the outer segment material. The RPE cells were hypertrophied with promi-
Fig. 4. A, Electron micrograph of the outer retina (mid-tapetal region) of a 22-day-old affected kitten. The photoreceptor inner and outer segment layer is virtually absent. The inner segments are represented by club-like protrusions (arrows) through the external limiting membrane; basal bodies and cilia are present, but outer segment material is lacking (original magnification ×11,670). B, Electron micrograph of the outer retina of a 24-day-old unaffected kitten showing developing photoreceptor inner and outer segments. (The vacuolation evident in this section is artefactual) (original magnification ×5,600). ONL = outer nuclear layer, RPE = retinal pigment epithelium, IS = photoreceptor inner segment layer, OS = photoreceptor outer segment layer.

Discussion

In the present study, the first clinical sign, dilatation of the pupils, was usually present by 3 weeks of age. This, together with the appearance of secondary nystagmus at 4–5 weeks of age, suggests severe visual deprivation during the early stages of photoreceptor differentiation. These clinical signs are in accordance with the histological and ultrastructural findings present in the retina of the earliest affected kitten at 22 days of age, and the virtual extinction of the ERG in the same animal at 17 days and in other affected individuals. In contrast, an unaffected eye at 24 days showed advanced photoreceptor inner and outer segment development consistent with the observation of Donovan that, in

Slight disruption of the normal arrangement of nuclei in the outer nuclear layer was discernible. In an eye examined at 12 weeks of age, occasional adventitious cells, presumably macrophages, were present within the photoreceptor inner and outer segment layer. However, the inner segments were still discernible in this eye (Fig. 3D).

In the retinas of affected cats aged 17 weeks and older, there was marked thinning of the photoreceptor inner and outer segment, outer nuclear and outer plexiform layers, and of the retina as a whole. Nuclei of the outer nuclear layer became markedly pyknotic and spatially separated (Fig. 3E). Subsequently, both inner and outer nuclear layers continued to thin and, at 8 months of age, were virtually fused, with loss of the outer plexiform layer. In an affected eye examined at 27 months of age (Fig. 3F), the total thickness of the retina was about 40% of that of the retina of an unaffected and histologically normal littermate; the outer nuclear layer was reduced to a single discontinuous row of nuclei. At this late stage, the retinal pigment epithelium showed a reduction in the number of cells.

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the cat, outer segments are clearly recognisable 8–10 days after birth. In affected eyes examined before the normal age of maturation of the retina, the inner segments were diminutive, and the outer segments were either absent or showed severe lamellar disorganisation and vesiculation. These degenerative changes, the in-

Fig. 5. Electron micrographs of the photoreceptor inner and outer segment layer and retinal pigment epithelium of a 40-day-old affected kitten. A, The layer of photoreceptor inner and outer segments is of almost normal thickness; degenerate photoreceptor outer segments are represented by bizarre arrangements of disc lamellae, often vesiculated, in clumps, sheets and whorls (original magnification ×4,915). B, Inner segment containing a ciliary rootlet and part of a connecting cilium, together with outer segment lamellar material. Some stacking of rod disc lamellae is evident, but, elsewhere, outer segment lamellae show disorganisation and vesiculation (original magnification ×20,395). C, The photoreceptor/retinal pigment epithelium interface showing a RPE cytoplasmic process with several pigment granules extending into the degenerate inner and outer segment layer (original magnification ×11,045).
ability to distinguish between degenerating rod and cone photoreceptors in EM sections, and the absence of both rod and cone components of the ERG in most affected individuals permits the condition to be classified as a rod-cone dysplasia. The retention of integrity of the retinal pigment epithelium, except in the later stages of retinal degeneration, and the initial normal development of the inner retinal layers confirm the primary nature of the photoreceptor disease. The degenerative tapetal changes which permitted visualization of the choroidal vasculature on ophthalmoscopic examination were probably secondary. The gene symbol Rdy has been adopted for this condition.

The apparently greater severity of EM changes seen towards the posterior pole of the eye of the 22-day-old kitten may reflect the more advanced development of the central retina in this species. Regional variations were not studied in detail in other eyes, although this aspect is currently under investigation. It is of interest that, clinically, the first fundus changes were seen in the region of the area centralis, and appeared to spread peripherally.

Electron microscopy showed that, in the 22-day retina, the photoreceptor inner segments were rudimentary and the outer segments were absent, whereas, in the 40-day retina, both had developed, although the outer segments were very degenerate. Assuming that these animals were of similar genotype, it would seem that photoreceptors exhibiting retarded development (as at 22 days) are capable of further differentiation to produce outer segments, including significant amounts of lamellar material. However, it remains possible that these individuals were of different genotype, and the disease was thus of different severity. Whereas most of the affected animals in the study could not, by virtue of their breeding, have been homozygous, the 22-day-old kitten, both parents of which were affected, might well have been. It may be of relevance, however, that, in the small number of litters which might have included both homozygotes and heterozygotes, there were no apparent differences in clinical severity among affected individuals. Studies are in progress to investigate possible differences between the heterozygous and homozygous states.

The clinical features of the disease, including the early onset of tapetal hyper-reflectivity and retinal vascular attenuation and the extinction of the electroretinogram before maturation of the retina, are all consistent with the condition described by West-Hyde and Buyukmihci. However, these authors make no reference to nystagmus, and their published light and micrographs suggest earlier thinning of the outer retinal layers. The published breeding data are similarly consistent with a dominant mode of inheritance. Comparison with the condition described by Rubin and Lipton in two successive litters of Persian kittens born to unaffected parents is more difficult. Visual impairment, evident by 12–15 weeks of age, seemed to develop later, but the histological picture was similar to that of affected Abyssinian cats of similar age.

Classification of the condition as a photoreceptor dysplasia invites comparison with hereditary photoreceptor dysplasias in the Irish Setter and Collie breeds of dog and the rd mouse, all of which are due to autosomal recessive genes. There is in these conditions a defect in cyclic nucleotide metabolism in which impaired phosphodiesterase activity leads to the accumulation of retinal cyclic GMP, although the mechanism may differ between diseases. However, preliminary studies on cyclic GMP in affected Abyssinian kittens has not suggested an abnormality in this respect (M. J. Voaden, personal communication). It is of interest that, in the rds mouse, a morphologically early-onset photoreceptor dysplasia with autosomal recessive inheritance does not exhibit high retinal cyclic GMP levels.

Because, in this study, affected kittens could not be positively identified earlier than the age of onset of clinical signs at about 2–3 weeks, early pathology is lacking, and the age of onset in morphological terms is not known. Attempts will be made to identify, by test mating, homozygous individuals which can be used to produce known homozygous, and therefore potentially affected, progeny. More detailed comparisons with the recessively inherited retinal photoreceptor dysplasias in dogs and mice should then be possible.

Key words: retinal dystrophy, photoreceptor dysplasia, electroretinography, electron microscopy, autosomal dominant inheritance, Abyssinian cat

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