Retinal degeneration in the Abyssinian and Somali cat (rdAc): correlation between genotype and phenotype and rdAc allele frequency in two continents

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Abstract

Objective To characterize hereditary retinal degeneration in the Abyssinian cat (rdAc) in a recently established closed colony segregating for the rdAc mutation, and evaluate possible differences in the age of onset and progression of disease phenotype since the initial description of rdAc 25 years ago. The sample size of an earlier study was increased in order to determine the allele frequency in Abyssinian and Somali cats on a worldwide basis.

Animals studied Twenty rdAc affected cats from the closed animal facility, 87 Abyssinian and Somali cats for study of genotype–phenotype concordance, and DNA from 131 Abyssinian and Somali cats from Scandinavia, the UK and Australia for evaluation of the rdAc allele frequency.

Procedures DNA was extracted from blood and buccal swabs using commercially available kits, followed by genotyping. Ophthalmic examinations were performed in the USA and Sweden by two board-certified veterinary ophthalmologists.

Results A greater variation in the age of onset and progression of the disease was observed compared to that previously described. An excellent correlation between genotype and phenotype was observed. A population genetic survey revealed that the rdAc allele is in moderate abundance in the Abyssinian breed in Europe and Australia. Surprisingly, homozygosity for the mutant allele was observed in a Siamese cat with ophthalmoscopic findings similar to those originally described for affected rdAc individuals.

Conclusions Alertness to the potential of rdAc is needed on the part of the veterinary ophthalmology community, not only in Abyssinian and Somali cats but possibly also in other related cat breeds.

Key Words: allele frequency, cat, degeneration, hereditary, photoreceptor, retina

INTRODUCTION

An unusually high incidence of recessively inherited rod-cone degeneration in Abyssinian cats, termed retinal degeneration in Abyssinian cat (rdAc), was first observed in Sweden in 1983.1,2 A high prevalence of affected and carrier animals (45% and 44%, respectively) was identified in the population. It was speculated that the cause for the high prevalence of the disease was the frequent use of close inbreeding among purebred Abyssinian cats, including parent and offspring matings. Within a few years of the report of rdAc, the disease became more widespread and was observed in other Nordic countries, the Netherlands and Germany.

Affected cats with rod-cone degeneration were described to have a normal fundus appearance until they were approximately 1.5–2 years old. Ophthalmoscopically, visible
changes then appeared that were progressive with complete retinal atrophy observed at the end stage. Four specific stages (S1–S4), as observed ophthalmoscopically, have been described in order to correlate funduscopic changes with other clinical and laboratory findings. In stage 1 (S1) (the stage of suspected disease): slight color changes are observed in the central part of the fundus, most often along the visual streak. In stage 2 (S2) (early disease): distinct grayish color changes are observed centrally, with additional and distinct color changes in the peripheral tapetal fundus. In stage 3 (S3): generalized color changes are observed in all of the tapetal area as well as hypo- and hyperreflective areas in the midperipheral and peripheral tapetal fundus. Additionally, vascular attenuation and some minute changes in the non-tapetal fundus, such as mottling and depigmentation are observed. In stage 4 (S4): generalized hyperreflectivity of the tapetal fundus are observed, as well as generalized vascular attenuation, marked depigmentation, some hyperpigmented spots in the non-tapetal fundus and occasionally, hyporeflective areas along the visual streak. The end stage (S4) was usually reached in most affected cats between 3 and 5 years of age.

Electrophysiological studies demonstrated that ERG a-, b- and c-waves were all reduced prior to ophthalmoscopic changes, usually by 8 months of age and, most markedly so, the a-wave. Ultrastructurally, distinct changes in the photoreceptors were observed at 5 months of age, with disorganization mainly of the photoreceptor lamellar discs, followed by degeneration of solitary photoreceptor outer segments and then apoptosis of entire rod cells. These initial changes in photoreceptors occurred while the inner retina was morphologically normal. The disease was always bilateral and slowly progressive with subsequent affection of the cone photoreceptors. The end stage of the disease was characterized by a generalized degeneration of the photoreceptors with secondary changes in the inner retina and in the retinal pigment epithelium. Systemic effects were not observed in affected cats.

Heterozygous cats, identified as described below, were clinically normal but had ERG changes indicative of a reduced number of photoreceptor cells, although no definite ophthalmoscopic changes were observed in these cats. Clinical similarities to human recessive retinitis pigmentosa (RP) were described for the disease.

A pedigree was established to identify the genetic defect causative of rdAc. A single base pair change (single nucleotide polymorphism, SNP) was identified in intron 50 of the centrosomal protein 290 (CEP290) gene (IVS50 + 9T > G), which resulted in alternative splicing of the transcript, with subsequent introduction of a stop codon and truncation of the mature protein. Interestingly, mutations in CEP290 have been reported to be prevalent for some rare, severe, early onset syndromic diseases of humans (the Joubert’s, Senior-Loken, Meckel-Gruber, and Bardet-Biedl syndromes) causing blindness, mental retardation and kidney failure among other severe clinical signs. It has also been reported that 31% of patients with Leber Congenital Amaurosis (LCA) demonstrate mutations in the CEP290 gene. The CEP290 protein, also designated NPNH6, has been shown to be involved with axonemal transport in the photoreceptor connecting cilium and, consequently, renders this disease a primary ciliopathy.

The aim of the present study was to characterize further feline rod cone degeneration in a closed colony of Abyssinian and mixed/breed cats segregating for the rdAc mutation, established nearly 8 years ago at the University of Missouri. We wanted to evaluate whether there are differences in the age of onset and progression in the disease phenotype since its initial description approximately 25 years ago. Furthermore, we wanted to increase the sample size of an earlier study, in order to determine the frequency of the rdAc causative SNP in the Abyssinian cat and its related breed, the Somali, on a worldwide basis.

MATERIALS AND METHODS

Animals and clinical studies

A group of purebred Abyssinian cats affected with hereditary retinal degeneration (rdAc) (n = 10) were moved from Sweden to Columbia, MO, and a new colony was established at the University of Missouri (MU) in 2001. The colony at MU was increased in numbers through outcrossing affected Abyssinian cats with normal European and American Shorthaired cats, and backcrossing the offspring (heterozygous cats) with affected purebred Abyssinians. Thereby, 55 backcrossed cats were produced. Approximately 50% of these cats were homozygous for the rdAc mutation and were used for further matings. Homozygous × homozygous matings were performed resulting in 20 affected offspring, which were used for the present study, with a follow-up time between 1 and 7 years. The rdAc clinical affection status of each cat included in the study was verified by genotyping for the rdAc causative SNP.

Starting at 8 weeks of age, the cats were examined clinically using indirect ophthalmoscopy and slit-lamp biomicroscopy after induction of mydriasis (1% Tropicamide, Bausch and Lomb Inc, Tampa, FL, USA) in each eye. Funduscopic changes were documented using a Nidek NM-100 digital camera (Nidek Co Ltd, Freemont, CA). Simultaneous bilateral scotopic and photopic electroretinography (ERG) was also performed in all cats, every 4–6 months, with results reported in a separate publication.

Information on rdAc clinical affection status in the population

The high frequency of rdAc affected individuals observed in Swedish Abyssinian and Somali populations 25 years ago led to a concerted effort by cat breeders and veterinarians to decrease the incidence of rdAc through the use of selective breeding practices. This ongoing effort included an informative website, the Abyssinian and Somali database (http://www.pawpeds.com/db/?p=aby&date=iso), where cat breeders have listed information on 25 849 individuals providing (i)
date of birth, (ii) multi-generation pedigrees and (iii) annual results of rdAc status, obtained through ophthalmic screenings performed by veterinarians certified to perform evaluations for hereditary eye disease (panelists or diplomats from European countries according to the European College of Veterinary Ophthalmologist’s scheme for prevention of hereditary eye diseases). This informative website was utilized to obtain clinical information of rdAc status and the age of individuals at the time of the examination. Additional cats were evaluated as to their rdAc status through ophthalmic examinations at animal hospitals in Sweden and in the USA by two board-certified veterinary ophthalmologists (KN and DW). A total of 87 Abyssinian and Somali cats were evaluated as to disease status using the informative website or by clinical examination.

**Samples for DNA extraction**

Buccal swab samples from 130 Abyssinian (n = 103) or Somali (n = 27) (Long-haired Abyssinians) cats were collected from cat breeders in Scandinavia (mainly Sweden, Norway and Finland). Sterile Q-tips were used to collect saliva from each cat. The Q-tips were then rolled upon FTA™ paper (Whatman Inc, Florham Park, NJ, USA), allowed to air dry for 30 min, enclosed in a small zip bag and sent to the Laboratory of Genomic Diversity, National Cancer Institute-Frederick, Frederick, MD. Eighty-seven of the individuals had been clinically evaluated for rdAc status, either by the first author or through the informative website maintained by Abyssinian/Somali breeders (see above). Buccal swabs were also obtained, as described above, from 34 Abyssinian or Somali cats from the UK. Finally, DNA was provided from 57 Abyssinian or Somali cats from Australia, screened in a separate study for pyruvate kinase deficiency.25 The British and Australian animals were, however, not evaluated ophthalmoscopically for clinical signs of disease.

In the course of the present study, screening of DNA samples for the rdAc mutation was also performed in a group of purebred Siamese cats. The samples were obtained through a previous study performed at the National Cancer Institute. The cats had not been evaluated ophthalmoscopically as to rdAc status.

**DNA extraction**

DNA was extracted from blood and buccal swab samples using Qiagen QiAmp DNA Blood Midi and Mini Extraction Kits following the manufacturer’s suggested protocols. DNA was quantified using a Hoefer DNA Quant 200 Flurometer (Amersham BioSciences). A proportion of each sample was diluted to a standard concentration of 2.5 ng/μL with sterile distilled water (Quality Biological).

**Genotyping of the rdAc mutation**

Genotyping of the rdAc causative SNP (IVS50 + 9T > G) was performed as previously described by Menotti-Raymond et al.13

**RESULTS**

**Ophthalmic examination**

A great variability in the timing of the occurrence for S1 was observed in the MU pedigree. In the majority of affected cats (75%, n = 20), ophthalmoscopic changes developed between 12 and 18 months, as previously described.3 However, in three affected individuals, fundus changes were observed as early as at 4 months of age. Areas of subtle hyporeflectivity were observed mainly in the central fundus and along the visual streak in the young affected cats indicative of a stage of suspected disease (S1) (Fig. 1). The changes progressed and, within 2–4 months, became more marked especially in the peripheral fundus (S2). Conversely, two other affected cats exhibited a much slower rate of progression, and developed the first ophthalmoscopic signs of disease at age 30 and 36 months (Fig. 2).

The progression of disease was extremely variable. It appeared that if the onset of funduscopic changes was observed early in life, disease progression was more rapid, compared to cats that developed changes at a later time point in life. Thus, the three cases with early onset disease developed clear S2 changes within 4 months, whereas the late onset cases were very slow to progress and S2 was not reached until 1.5 years later.

Ophthalmoscopic changes were always bilateral and mainly at the same stage of disease, as previously reported4 but not always symmetrical in the two eyes. Secondary cataracts were not observed in any of the affected cats at any age.

**Molecular analysis and frequency of disease**

The frequencies of the rdAc allele observed in the Scandinavian, UK and Australian populations were 0.20, 0.21 and 0.11, respectively (Table 1). From a population genetics perspective, the numbers of homozygous affected, unaffected and carrier individuals from all three populations exhibited expectations of a locus in Hardy-Weinberg equilibrium.26 Alleles in Hardy-Weinberg equilibrium associate randomly unless acted upon by selection. The frequency of individuals affected with rdAc, was therefore anticipated to be 0.038, 0.042 and 0.011 in the populations studied in Scandinavia, the UK and Australia, respectively, based upon the present sample set.

Clinical retinal reports of rdAc disease status of 87 individuals were obtained for which DNA samples had been collected. Genotype and phenotypic information for the 87 individuals showed that 82 of 87 samples demonstrated complete concordance between rdAc genotype and phenotype. Five non-concordance cases were observed, some of which were resolved following additional clinical evaluations. These cases are discussed in the following section, which show the subtleties in the clinical diagnosis of feline retinal degeneration.

1. A 1.5-year-old Abyssinian cat homozygous for the mutant allele was found to be normal upon ophthalmoscopic examination. This cat was not obtained for reexamination.
2. An 11-year-old Abyssinian cat, homozygous normal for the rdAc allele, was ophthalmoscopically normal until age 5 years, but had developed advanced retinal degeneration upon a follow-up examination 1 year later.

3. A 7-year-old Abyssinian cat, homozygous normal for the rdAc allele, was diagnosed with advanced retinal degeneration.

4. A 7-year-old cat, ophthalmoscopically normal and, according to the owner, visually normal at 6 years of age and homozygous for the mutant allele, was finally diagnosed at age 7 years with generalized retinal degeneration.

5. A 7-year-old cat, heterozygous for the rdAc mutation, was diagnosed with generalized retinal degeneration at 4 years of age. Re-examination showed distinct hyperreflective changes in the area centralis region on both eyes and less affected areas of both fundi midperipherally and peripherally with mainly normal-sized retinal vasculature.

Interestingly, an 8-year-old Siamese cat from the USA was identified through blood testing to be homozygous for the rdAc mutation. This cat, believed by the owner to be visually normal, was determined to have similar funduscopic changes as those described for an affected Abyssinian cat at stage 4 (Fig. 3).

DISCUSSION

The frequency of the rdAc causative SNP was examined in three populations of Abyssinian and Somali cats from Scandinavia, the UK and Australia. The frequencies observed for the rdAc SNP (at 0.20, 0.21 and 0.11 in the 3 populations, respectively), demonstrate that the allele is in moderate abundance in two continents. A continued source of alertness is therefore needed on the part of the veterinary ophthalmology community to identify rdAc affected individuals.

Through persistent efforts by the Scandinavian Abyssinian and Somali breeders, the prevalence of rdAc has been markedly decreased from a frequency of 45% to below 4% of the population (Unpublished results, Laboklin GmbH & Co. KG, Bad Kissingen, Germany, 2008). However, with a commercially available diagnostic test for the rdAc mutation available, it will now be possible for informed cat breeders to decrease the frequency of the rdAc allele even further in the population.

The diagnosis of retinal degeneration due to the rdAc mutation is not always simple. The present study shows that there is a great variation as to age of onset of the first ophthalmoscopic signs of the disease. A few cats were observed through clinical examination to be affected at the

Table 1. Frequencies observed for rdAc genotypes

<table>
<thead>
<tr>
<th>Population</th>
<th>Number of individuals</th>
<th>CEP290 +/+ (normal)</th>
<th>CEP290 +/– (carrier)</th>
<th>CEP290 −/– (affected)</th>
<th>Frequency of CEP290 allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scandinavia*</td>
<td>130</td>
<td>85</td>
<td>39</td>
<td>6</td>
<td>0.196</td>
</tr>
<tr>
<td>UK</td>
<td>34</td>
<td>22</td>
<td>10</td>
<td>2</td>
<td>0.206</td>
</tr>
<tr>
<td>Australian</td>
<td>57</td>
<td>46</td>
<td>10</td>
<td>1</td>
<td>0.105</td>
</tr>
<tr>
<td>Total</td>
<td>221</td>
<td>153</td>
<td>59</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

*Individuals registered with Swedish, Norwegian or Finnish cat registries.
ERGs. It has been shown that in cats homozygous for the mutant allele, ERGs are diagnostic for the disease at approximately 8 months of age, with affected individuals exhibiting a reduction mainly of a-wave amplitudes.\(^5\)\(^6\)\(^7\)\(^8\) Increased b : a wave ratios were also described before significant reductions in b-wave amplitudes were observed, using scotopic high intensity light stimulation.\(^24\)

Other diseases may cause retinal degeneration in cats such as feline central retinal degeneration (FCRD),\(^29\)\(^30\) and toxic\(^31\) or inflammatory retinopathies.\(^32\) Initial changes in FCRD can be described as distinct hyperreflective lesions in the area centralis region sometimes with extension horizontally along the visual streak. The end-stage of FCRD is, however, generalized retinal degeneration, which is indistinguishable from advanced retinal degeneration (S4) caused by the \(rdAc\) defect. In toxic retinopathies, early changes may be subtle as well and sometimes patchy, but the end stage is often observed as generalized retinal degeneration. For retinal disease caused by inflammatory reactions, the clinical signs are more variable in the active stage depending on disease etiology with fundus discoloration, hypo- and hyperreflective areas, sometimes granulomas, and possibly retinal hemorrhage, with generalized retinal atrophy as the end-stage, although most often with significant retinal scarring.

The five non-concordancy examples we observed reflect some of these subtleties in diagnosing \(rdAc\). Case #1 was likely non-concordant due to the young age of the cat at the time of examination. Thus, it is probable that the retinal degeneration was not yet far enough advanced to show ophthalmoscopic changes. For cases #2 and #3, it is probable that these cats were affected by a non-hereditary type of retinal degeneration, such as toxic retinopathy or advanced FCRD. In case #4 the \(rdAc\) disease was ultimately diagnosed in accordance with homozygosity for the \(rdAc\) mutation, but progression was slower than previously reported. In case #5, the diagnosis was most certainly FCRD. There were typical signs of hyperreflectivity of the area centralis region and degenerative changes spreading peripherally from the central fundus (not the other way around as in most cases of the moderately advanced hereditary rod cone degenerations of dogs and cats\(^33\)). Furthermore, retinal vasculature would be more attenuated at this stage of disease if the degeneration was due to the \(rdAc\) mutation.

As an alert to the veterinary community, we have observed \(rdAc\) disease in a breed other than the Abyssinian or the Abyssinian-related cat breed. An earlier mini-screen of cat breeds for the \(rdAc\) allele,\(^13\) in which two individuals were genotyped from each of 21 breeds, demonstrated that the \(rdAc\) SNP was confined to Abyssinian and Somali populations and a related breed: a single \(rdAc\) allele was identified in an Ocicat, a relatively new breed generated with genetic input from Abyssinian, American Shorthair and Siamese breeds (http://www.cfainc.org/breeds/profiles/ocicat.html).

Then, in an on-going expanded breed survey (Menotti-Raymond \textit{et al.}, in preparation) the presence of two \(rdAc\)
alleles in an 8-year-old Siamese cat was identified. This cat was evaluated and found to be clinically affected with typical funduscopic changes of advanced stage of retinal degeneration (S4) previously described only for Abyssinian and Somali cats. This shows that there is a potential for the rdAc mutation to be present in breeds other than Abyssinian or Somali cats.

It is exciting to note that through the concerted efforts by breeders mainly in Scandinavia, remarkable results have been obtained in reducing the prevalence of the disease in the Abyssinian and Somali cat breeds. The high concordancy between rdAc genotype and phenotype status demonstrates that the rdAc genotype is highly predictive of the rdAc clinical affection status \( P = 3.24E-8 \). With the ready availability of a commercial mutation detection test for the rdAc mutation it will be possible to reduce the disease frequency even more among the Abyssinian and Somali populations world-wide. Furthermore, cats of other breeds possibly carrying the rdAc mutation can be tested and if found positive, not used for breeding purposes in order to prevent the spreading of the disease causing mutation further in the feline population.

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