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Widespread retinal degenerative disease mutation (*rdAc*) discovered among a large number of popular cat breeds

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ABSTRACT

The recent discovery of a mutational variant in the *CEP290* gene (*CEP290*: IVS50 + 9T>G), conferring recessive retinal degeneration in Abyssinian and Somali (long-haired Abyssinian) cats (*rdAc*) prompted a survey among 41 cat breeds (846 individuals) to assess the incidence, frequency and clinical consequence of *rdAc*. The *rdAc* allele displayed widespread distribution, observed in 16/43 (37%) breeds, exhibiting a high allele frequency (~33%) in North American and European Siamese populations. Clinical evaluations demonstrated high concordance between *rdAc* pathology and the *CEP290* (IVS50 + 9T>G) homozygous genotype ($P = 1.1E{-}6$), with clinical disease similar to affected Abyssinians/Somalis. This retinal degeneration has not been reported in breeds other than the Abyssinian/Somali and poses a significant health risk particularly in the Siamese breed group. Alertness of the veterinary community and the present availability of commercial diagnostic testing could synergistically enable breeders to reduce the incidence of *rdAc* blindness in pure-bred cat populations.

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Introduction

Cat breeds exhibit a high incidence of hereditary disease pathologies (Pontius et al., 2007), as has been seen in other domesticated animal breed populations (Higgins and Nicholas, 2008; Taberlet et al., 2008). Small effective population sizes, the use of popular sires, line breeding and founder effects increase the likelihood of expression of rare pathogenic mutations. Over 280 pathologies with a hereditary component have been reported in the domestic cat.[1] Many conditions are not seen outside of the breed or breed group in which the causative mutation occurred, such as gangliosidosis in the Korat (Baker et al., 2001; Martin et al., 2004; Muldoon et al., 1994) and Type IV glycogen storage enzyme disease in the Norwegian Forest cat (Fyfe and Kurzhals, 1998), possibly due to the constraints that registration rules may place on gene transfer between breeds. Others such as polycystic kidney disease have most likely spread into newer breeds from contributing parental breeds (Persian) (Barthez et al., 2003).

Whilst there have been reports of sporadic retinal pathologies identified in cats (Bistner et al., 1976; Glaze, 2005; Gould and Sargan, 2002; Narfström, 1999, 1983; Rah et al., 2005), hereditary retinal blindness has not generally been considered a significant health factor in pure-bred cats. With the discovery of an unusually high prevalence (45%) of hereditary rod cone degeneration in the Abyssinian cat approximately 25 years ago in Sweden, it became clear that there was increased risk for hereditary retinal dystrophies causing blindness within some cat breeds (Narfström, 1983, 1985a; Narfström and Nilsson, 1983). Hereditary blindness was observed in a group of American short-haired cats (West-Hyde and Buyukmihci, 1982) and another Abyssinian cat model was subsequently described for retinal blinding disease in the United Kingdom (Barnett and Curtis, 1985; Curtis et al., 1987). More recently, an autosomal recessive form of early onset progressive retinal atrophy has been described in Persian cats (Rah et al., 2005).

The development of critical genetic mapping resources in the cat, including comprehensive genetic maps (Davis et al., 2009; Menotti-Raymond et al., 1999, 2003, 2009; Murphy et al., 2007), the recent 1.9X whole genome sequence of the cat (Pontius et al., 2007; Pontius and O'Brien, 2007) and the generation of a pedigree segregating for *rdAc* (Narfström et al., in press) has enabled the identification of the causal mutation for *rdAc* (Menotti-Raymond et al., 2007b). A single base pair substitution in an intron of the centrosomal protein 290 gene (*CEP290*) (IVS50 + 9T>G) (previously referred to as the *rdAc* allele) results in alternative splicing of the *CEP290* transcript, with subsequent introduction of a premature

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[1] See: http://omia.angis.org.au.

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stop codon and truncation of the mature protein (Menotti-Raymond et al., 2007b).

Mutations in the homologous human *CEP290* gene are a common cause of human blindness, including approximately 30% of patients with Leber's congenital amaurosis (Hollander et al., 2006). Additionally, mutations in *CEP290* are causative of several rare, severe, early onset syndromic diseases in humans including Joubert's, Senior-Loken, Meckel-Gruber, and Bardet-Biedl syndromes, which cause blindness, mental retardation and kidney failure among other severe clinical signs (Baala et al., 2007; Leitch et al., 2008; Sayer et al., 2006; Valente et al., 2006). None of these additional clinical manifestations are observed in *rdAc* affected cats (Narfström et al., in press).

A recent survey of 21 cat breeds in the USA (n = 2/breed) (Menotti-Raymond et al., 2007b) suggested that the *rdAc* allele was confined to the Abyssinian/Somali (Somali cats are long-haired Abyssinians) and Abyssinian related breeds, including a single *rdAc* allele identified in an Ocicat. This recently generated hybrid breed has had input from both Abyssinian and Siamese cats (Helgren, 1997). Furthermore, a recent genetic survey of Abyssinian and Somali cat populations from the USA, UK, Australia and Scandinavia, identified *rdAc* allele frequencies of 0.07, 0.21, 0.11, 0.20, respectively (Menotti-Raymond et al., 2007b; Narfström et al., in press). In this extended survey the *rdAc* allele genotype was in complete concordance with the presence or absence of retinal atrophy (P = 3.2E−8), demonstrating that the *rdAc* genotype is highly predictive of *rdAc* disease progression (n = 846).

As the *rdAc* allele has a world-wide distribution in Abyssinian and Somali populations with a significant clinical impact on homozygous affected cats, it became imperative to determine whether the *rdAc* allele is present in additional related breeds. In this report 41 further breeds (n = 846 individuals) and 92 outbred (random bred) cats were genotyped at the *rdAc* locus, and 27 individual cats screened for evidence of retinal degeneration.

Surprisingly, the *rdAc* allele was detected in 34% of the cat breeds examined with relatively high frequencies in Siamese and Siamese-related breeds in both North America and Europe. Based on a recent publication on the genetic relatedness of cat breeds (Menotti-Raymond et al., 2007a), we were unable to identify genetic distinctiveness between the Siamese, Colorpoint Shorthair, Oriental Shorthair, Balinese and Javanese breeds, which we will refer to in this study as 'Siamese related breeds' or the 'Siamese breed group'. In addition, the *rdAc* genotype was predictive of the disease phenotype, where all homozygous *rdAc* pure-bred cats that were examined in the study had evidence of retinal degeneration, similar to that observed in Abyssinian and Somali cats. Clearly the *rdAc* mutation has a global distribution in multiple breeds of cats, and this form of heritable retinal degeneration has been previously unrecognised in several popular breeds of cats.

Materials and methods

DNA and tissue samples

DNA samples representing 43 cat breeds and one outbred population were used in the study (Table 1). DNA samples of individuals representing 36 breeds were used from a previous collection (Menotti-Raymond et al., 2005). DNA from five additional breeds was obtained from a population genetic survey (Thai) (see below), the Laboratory of Genomic Diversity DNA resources (Tennessee Rex, Munchkin, Angora) and from a commercial DNA testing laboratory (see below) which included the Peterbald, a breed recently developed with Siamese influence (Fogle, 2001).

DNA from 92 random bred, feral cats was extracted from discarded tissues provided by veterinary hospitals and spay clinics in Frederick and Howard County, Maryland. Additionally, DNA samples (n = 54) were obtained from a commercial testing laboratory in Europe LABOKLIN (Bad Kissingen, Germany), including 28 Siamese cats, 1 Balinese, 8 Peterbald, 3 Ocicat, 12 Oriental Shorthair, and 2 Bengal, under the condition of anonymity of the individuals and their owners.

Animals

For cats of recognised breeds, buccal swab samples were obtained, under an approved Animal Care and Use Protocol, from Siamese (n = 107), Oriental Shorthair (n = 40), Javanese (n = 4), Balinese (n = 11), and Colorpoint Shorthair (n = 3) cats from cat breeders in Maryland, Massachusetts, Pennsylvania, Virginia, Texas and Ohio. Breeders' names were withheld under request for anonymity.

DNA extraction and genotyping of the rdAc allele

DNA was extracted from buccal or tissue 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 Fluorometer (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* causative single nucleotide polymorphism (SNP) (IVS50 + 9T>G) was performed as described by Menotti-Raymond et al. (2007b).

Clinical examinations

Clinical study of *rdAc* has been extensively characterized in the Abyssinian cat (Narfström, 1983, 1985a,b; Narfström et al., 1989, 2001, 1988; Narfström and Nilsson, 1986, 1989). To determine if the *rdAc* genotype correlated with the development of retinal degeneration pathology as observed in the Abyssinian, a subset of individuals (n = 27) from 8 months to 18 years of age, representing six specific breeds in the Siamese breed group (Table 2), were clinically evaluated after genotyping. Ophthalmologic examinations were performed in a masked fashion, in that the veterinary ophthalmologist (KN) examined cats without knowledge of their *rdAc* genotype. Pupils were dilated using short acting mydriatics (Tropicamide 1%, Alcon), and fundic examinations were performed through indirect ophthalmoscopy (Welch Allyn Distributors) in all cats included in the study.

Full-field flash electroretinography (ERG) was performed in a 1-year old Siamese with suspected disease (stage 1) according to the ophthalmoscopic examination and normal age-matched cat, both under medetomidine, 150 μg/kg, equivalent to 0.15 mL/kg (Domitor, Pfizer) and ketamine anaesthesia (5 mg/kg IM). A portable ERG unit was used (HMsERG, RetVetCorp.) with an automated protocol for evaluation of rod and cone function (Narfström et al., 2002; Katz et al., 2008).

Results

In a genetic survey of 846 pure-bred and 92 outbred cats, the *rdAc* single nucleotide polymorphism (*CEP290*: IVS50 + 9T>G) previously characterized in the Abyssinian and Somali breeds was detected in 14/41 breeds (34%) (Table 1). Frequencies for the *rdAc* allele in the cat breeds from the US ranged from 0.02 (Cornish Rex) to 0.36 (Balinese) (Table 1), although sample sizes were small for some of the breeds and may not accurately reflect the true frequencies. While the *rdAc* allele was also detected in two carriers out of 92 outbred cats sampled in Maryland, the frequency was extremely low (0.01). Whereas, individuals in the Siamese and Siamese-related breeds (Colorpoint Shorthair, Oriental Shorthair, Balinese, Javanese), which will be referred to in this study as the 'Siamese breed group', exhibited the highest allele frequencies for the *rdAc* mutation ranging from 0.27 to 0.36 (Table 1).

A significant part of our study focused on the Siamese breed group, as initial estimates in 36 individuals had demonstrated elevated *rdAc* allele frequencies (data not shown). To confirm these initial estimates within the Siamese and Siamese-related breeds, additional samples were collected from several independent US breed registries (n = 187) (Table 3) and European countries (n = 52) (Table 1). Samples obtained from 52 cats from six breeds (Balinese, Bengal, Ocicat, Oriental Shorthair, Peterbald, Siamese) from geographically separated countries, demonstrated presence of the *rdAc* allele in 5/6 breeds with elevated frequencies in the Siamese breed group (Table 1).

Within the US additional samples were obtained from populations of Siamese cats maintained in separate registries (CFA, TICA, TCA) (Table 3). Within the TCA registry, breeders have developed two populations of Siamese cats with different conformational standards. Siamese cats which exhibit a rounder head shape and more robust body (reminiscent of the old-style Siamese conformation) are referred to as classic or 'appleheads', and are shown and