Assessment of a Novel Pigmentary Chorioretinopathy in the Chinese Crested Dog

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Abstract

One hundred and twenty seven privately owned Chinese Crested Dogs (CCDs) were screened for Presumed Inherited Eye Disease (PIED). Thirty five cases of a recently discovered presumed inherited pigmentary chorioretinopathy were diagnosed and used for preliminary characterization of the disease using clinical and laboratory methods. Electroretinography (ERG) in ten affected and in nine normal CCDs was performed and morphology was obtained in one normal and in three affected 6-8 year-old dogs. A bilateral and mainly symmetrical retinal dystrophy with circumscribed pigmented lesions was observed, the earliest changes observed in 3-year-old dogs. The lesions showed a lighter center (pimple-like) and areas between lesions were often grayish and hypo reflective. Changes were mainly prevalent in the peripheral fundus initially. In more advanced cases the circumscribed pigmented changes were also observed centrally. In some cases there was diffuse hyper reflectivity in the area of the pigmented changes and slight vascular attenuation, indicating more generalized retinal degeneration. ERGs were not diagnostic in the early disease stage. Morphology showed changes at the level of the Retinal Pigment Epithelium (RPE), with focal areas of increased layering of RPE cells and detachment of RPE cells with subretinal migration. There were also areas of severe RPE and photoreceptor degeneration or atrophy with outer nuclear layer remnants in direct contact with the choroidal tissue. The circumscribed pigmented lesions observed ophthalmoscopically corresponded to these areas of geographic atrophy. The disease appears to be slowly progressive and in some cases, but not all, the disorder results in visual impairment or blindness.

ABBREVIATIONS

CCD: Chinese Crested Dog; PIED: Presumed Inherited Eye Disease; ERG: Electretinography; RPE: Retinal Pigment Epithelium; prcd: Progressive Rod Cone Degeneration; PRA: Progressive Retinal Atrophy; DNA: Deoxynucleic Acid; PCR: Polymerase Chain Reaction; HMsERG: Hand-held Multi-Species Electroretinograph; FA: Fluorescein Angiography; LM: Light Microscopy; EM: Electron Microscopy; IHC: Immunohistochemistry; KCS: Keratoconjunctivitis Sica; Hz: Herz; ONL: Outer Nuclear Layer; INL: Inner Nuclear Layer; ID: Identification; AMD: Age-related Macular Degeneration; cmr: Canine Multifocal Retinopathy; RPED: Retinal Pigment Epithelial Dystrophy; CNV: Choroidal Neovascular Membrane; cd.s/m2: Candela Seconds Per Square Meter; Psd: Photopic Standard light intensity recordings; Sh: Scotopic High Light intensity recordings; Pf: Photopic Flicker Recordings; POS: Photoreceptor Outer Segments; PIS: Photoreceptor Inner Segments; INL: Inner Nuclear Layer; GCL: Ganglion Cell Layer; Chor: Choroid; DIC: Differential Interference Contrast; Tap: Tapetum lucidum

INTRODUCTION

The Chinese Crested Dog (CCD) is considered one of the oldest of purebred dog breeds, and for centuries the popularity of hairless dogs appears to have been significant. Already the Aztecs, approximately 3700 years ago, considered the Mexican hairless dog as a sacred and ancient literature describes the Chinese hairless dog as having one external use: for people afflicted with rheumatism as bed warmers, and one internal: to assuage
the pangs of hunger (in: Chinese Crested Dog, 1899, unknown author). The past years the CCD has become the 10th most popular breed in Sweden (Swedish Kennel Club, 2010).

In Europe there appears to have been a dramatic reduction of the number of CCDs available for breeding after World War II. Further, during the 1990s in Scandinavia, frequent breeding between close relatives occurred as well as the use of specific sires and dams repeatedly in the same pedigree. It is thus likely that the breed has lost much genetic variation in the post-war era. It is affected by several systemic diseases [1-3] as well as severe ophthalmic diseases, such as progressive rod cone degeneration (prcd) type of progressive retinal atrophy (PRA) [4] (www.OptiGen.com) and lens luxation [5], both potentially blinding disorders.

The aim of the present study was to describe a recently discovered pigmentary chorioretinopathy in the CCD breed and to document the typical clinical and morphological findings in an outer retinal disease that appears different from classical canine hereditary retinal degeneration/Progressive Retinal Atrophy (PRA) [4].

MATERIALS AND METHODS

Animals and clinical examinations

A total of 127 CCD dogs (age range 1-10 years) were included in the study in which 35 cases (19 females and 16 males, age range 3-10 years) with bilateral retinal degenerative changes were found and used for a preliminary characterization of disease (see Table 1 for dogs examined by the first author). Nine 4-8-year-old CCDs of both sexes with normal fundus appearances were initially evaluated as part of a separate study to obtain normal canine ERG parameters, including those for CCDs [6]. The dogs from the previous and the current studies were all privately owned pets. Owners or breeders had requested eye examinations, according to eye scheme routines (www.ecvo.org), since their dogs or their close relatives were used for breeding. Pups were diluted with short acting mydriatics, 20 minutes before examination of the internal structures, using 1-2 drops of 1% tropicamide in each eye (Mydriacyl, Bausch and Lomb Inc., Tampa, FL) [7]. Standard ophthalmic examination was then performed using indirect ophthalmoscopy (Welch Allyn Distributors, Medical Device Depot, Inc., MD, USA) and slitlamp biomicroscopy (SL14, KOWA Co Ltd, Tokyo, Japan). Fundus appearance was documented in some of the normal and affected dogs with a digital fundus camera (Nidek NM-100, Nidek Co Ltd, Freemont, CA, USA, or the Smartscope V3-1 Digital Camera, Acrivet, Henningsdorf, Germany). Informed consent was received from dog owners and the study was approved by the regional ethical committee.

DNA samples

Blood samples from 29 CCD dogs were collected in conjunction with eye examinations performed. The age of 6 affected dogs (Table 1) ranged from 5-8 years and 23 normal CCDs were between 4-11 years of age. The blood was collected into EDTA tubes and genomic DNA was extracted manually from peripheral blood leukocytes using QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) or automatically on a QIAmp Genotyping Master Mix (Qiagen, Hilden, Germany) or automatically on a QIAmp DNA Blood Midi Kit (Qiagen, Hilden, Germany) or automatically on a QIAmp DNA Blood Midi Kit (Qiagen, Hilden, Germany). The 29 dogs were tested for the mutation causing prcd-PRA [8] using a real-time PCR assay (Custom TaqMan® Assay, Applied Biosystems™, Foster City, CA, USA) with a forward (5'-CTTTCCTCGCGAGACTCTG-3') and a reverse (5'-CAAGAGTGGTGAAGAAGAG-3') amplification primer and two allele-specific fluorescent labeled probes (VIC-5'-TGAGCCATGTCCACCAC-3' and FAM-5'-TGAGCCATGTCCACCAC-3'). Following the manufacturer’s instructions, about 100 ng genomic DNA was mixed with 1 μl Custom TaqMan® Assay, 10 μl TaqMan Genotyping Master Mix (Applied Biosystems™) and water to a final reaction volume of 20 μl. The PCR was performed on a StepOnePlus™ Real-Time PCR system (Applied Biosystems™, Foster City, CA, USA) using the thermal program: 62°C for 30 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 s, and 62°C for 1 minute.

A pedigree of examined and not examined dogs was constructed with 3 cases of the pigmentary chorioretinopathy. Information about the ophthamal status of each dog and relationships between individuals of the pedigree were obtained from the Swedish Kennel Club.

Electroretinography (ERG)

Dogs were chosen for retinal functional evaluation in accordance with the owner's consent and availability. For the present study Electroretinographic (ERG) evaluations were performed in 10 dogs (age range 4-9.5 years). A follow-up ERG was performed in one of the dogs 1.5 years after the first examination (Table 1, dog ID 104).

A portable ERG unit was used (HMSERG model 1000, RetVet Corp., Columbia, MO), with a mini-Ganzfeld dome. The procedures using this equipment and a standardized protocol for evaluation of rod and cone function, recommended by the European College of Veterinary Ophthalmologists, have been previously reported [7,8]. For light intensities used for stimulation of the retina and background light used during the procedure, see Figures 4 and 5.

In short dogs were deeply sedated by using intravenous administration of medetomidine (Domitor, Novartis, Pfizer Animal Health, Exton, PA), up to 150 micrograms/kg, equivalent to 0.15 ml/kg, and prepared for the ERG session in ordinary room light. Heart and respiratory rates were closely monitored before and throughout the procedure and the dogs were temperature controlled. The dog’s head was positioned on a cushion for stabilization. Maximal pupillary dilation was provided for by the use of short-acting mydriatics (Tropicamide, Mydriacyl, Bausch and Lomb Inc., Tampa, FL) and the eye was topically anesthetized using 0.5% proparacaine hydrochloride (Alcaine, Alcon, Fort Worth, TX). A lid speculum was inserted to ensure that the nictitating membrane and the upper and lower eyelids did not interfere with light exposure to the maximally dilated pupils. Platinum subdural needle electrodes (model E2, Grass Instrument Division, Astro-Med, Inc., West Warwick, RI) were used for the ground electrode, positioned on the occipital crest, and for the reference electrode, positioned 2 cm from the lateral canthus, close to the base of the ipsilateral ear. An active contact lens electrode (ERG-Jet, Universo Plastique, LKC Technologies Inc., Gaithersburg, Md) was placed on the cornea after instillation of one drop of 2% methylcellulose (Methocel, Ciba Vision, Munich, Germany). The electrodes were connected to a preamplifier and
the signals were amplified with a band pass filter between 0.3 and 300 Hz.

After termination of the ERG session an injection of atipamazole hydrochloride (Antisedan, Pfizer Inc. St Louis, MO) was administered intramuscularly to reverse the deep sedation (at a dosage 5 times higher than that given of the medetomidine; similar volumes were injected).

**Angiography**

Fluorescein Angiography (FA) was performed in one CCD dog, 7-years-old, with typical bilateral funduscopic findings corresponding to the chorioretinopathy currently described, and in one 5-year-old dog with a normal fundus appearance. Both dogs were diagnosed in France and not included in Table 1. An intravenous sodium fluorescein injection was performed (0.1 mL/kg of a 1g/5 mL solution, Sterop-Pharmacobel, Brussels, Belgium) without sedation, into the cephalic vein. The FA was documented using a KOWA RC2 fundus camera with a recycling microscope.

**LM** was performed using a JEOL 1200 EX transmission electron microscope.

**EM** was performed using a Zeiss Axiophot microscope and conventional transmission electron microscopy (Olympus FluoView 1000 confocal, Center Valley, PA).

**RESULTS AND DISCUSSION**

**Clinical findings**

A total of 35 cases of a bilateral, mainly symmetrical chorioretinopathy were observed in the 127 examined CCDs. The earliest cases were diagnosed in 3-year-old dogs (Table 1). Affected individuals of both genders and with both the coated (powder-puff) and hairless coat types were found.

There were a number of normal variations as to fundus appearance and coloration in the examined CCDs (Figure 1A). Some dogs showed thin layering of tapetal cells, resulting in an even, slight grayish coloration in the periphery of the tapetal area of the fundus. Subalbinotic fundus appearances were observed in both normal and affected dogs (for normal fundus variations in dogs, see reference 9).

Fundus changes in dogs affected by the disease consisted of circular, darkly pigmented areas with a lighter center or an irregular dark region with a centrally located lighter ring, sometimes with a dark pigmentation in the center (doughnut or pimple-like appearance) (Figure 1B,C). The changes were most often located in the tapetum near the junctional area between the peripheral tapetal fundus and the non-tapetal fundus. The largest lesions were approximately ¼ disc diameter, but most of the changes were smaller. An area with tapetal hyporeflectivity was usually observed between and in the vicinity of these lesions. Retinal vasculature appeared normal in young affected dogs (Figure 1B). In the older dogs (6-8-year-old), the circular lesions were more prevalent and some appeared larger in diameter (Figure 1C). In the older animals the lesions were usually observed also in the central part of the fundus. At this time there was a slight generalized vascular attenuation and there were areas of tapetal hyper- and/or hyper reflectivity interspersing the pigmented lesions. Also, at this stage of disease hyperpigmented circular lesions, with small centrally located depigmented areas, were observed in the non-tapetal fundus. The oldest dog found during routine screening with this specific chorioretinopathy was 10 years old. This dog had no visual problems according to the owners, however, ophthalmoscopy showed the circular pigmented lesions, some with pale or dark spots, spread all over
Figure 1 Composite of fundus photographs from a normal 4-year-old CCD (A). Fundus appearances of two affected dogs, age 4 and 6 years, respectively, with typical changes observed in the disease designated as a pigmentary chorioretinopathy (B,C). In B, note the grayish discoloration in the peripheral tapetal fundus with distinct circular pigmented areas (arrow), some with a darker center or small light colored spots. In C, the lesions are more advanced with generalized hyporeflectivity in most of the tapetal fundus. There are circular and pigmented lesions also in the central part of the fundus. None of the lesions appear elevated.

Figure 2A 6-year-old CCD affected with pigmentary chorioretinopathy (A) (not included in Table 1). Follow-up 2 years later show mild progression with more circular lesions, some of which have become more extended (B). A slight generalized vascular attenuation is observed in (B).

the tapetal and non-tapetal fundus (not shown). These were more prevalent in the midperiphery/periphery than centrally. In four affected dogs, age 3-6 years (Table 1, case ID: 57, 105, 106, 107), a more generalized change in tapetal reflectivity was also observed, with hyporeflectivity especially in the visual streak region. In these dogs the tapetal reflectivity changes were observed in addition to the circular pigmented lesions with centrally located spots in the midperipheral and peripheral fundus. In another three CCDs, 5-9 years old (Table 1, case ID: 1, 10, 91), a bilateral generalized retinal atrophy was observed without the doughnut-like pigmented spots or lesions described above.

During the study period 4 affected dogs were obtained for ophthalmic evaluation a second time, 6-24 months apart, and in one case also with ERG (see below). It was found that the progression of the fundus lesions was highly variable. In two affected dogs there was only slight progression of the retinopathy. Figure 2 shows this slow progression of the fundus lesions in a case with a 24 month-follow-up. In the other two cases, however, the pigmented lesions progressed to become more widespread within 6 months.

Only two cases of cataracts were observed among the affected dogs in the study (Table 1, case ID: 114 and 122). More prevalent were cases of vitreal degeneration, some of which also had vitreal strands in the anterior chamber. Among the examined CCDs were also 2 cases with bilateral lens subluxation, one of which was diagnosed also with unilateral Keratoconjunctivitis Sicca (KCS), and the other dog with bilateral KCS. The latter 4 dogs all had normal fundus appearances. There appeared to be no relationship between the pigmentary chorioretinopathy and the other disease entities presently described in the breed.

Genetics

Segregation analysis for the disease in a family of CCDs is shown in Figure 3. The occurrence of an affected member in every generation of the family indicates that there is a familial disposition for the disorder. The pigmentary chorioretinopathy was found to affect both male and females. Molecular genetic studies of blood obtained from affected animals showed that none of the cases with or without the more generalized retinal atrophic changes were homo- or heterozygote for the prcd mutation.
Table 1: Details in regards to 34 Chinese crested dogs diagnosed by the first author (KN) to be affected by retinal degenerative disease.

<table>
<thead>
<tr>
<th>Dog ID</th>
<th>Gender</th>
<th>Age</th>
<th>Examinations performed</th>
<th>Diagnosis and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>5</td>
<td>PIED screen</td>
<td>Generalized retinal degeneration Blind</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>3</td>
<td>PIED screen</td>
<td>PC</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>5</td>
<td>PIED screen, ERG</td>
<td>Generalized retinal degeneration Blind</td>
</tr>
<tr>
<td>32</td>
<td>F</td>
<td>6</td>
<td>PIED screen</td>
<td>PC</td>
</tr>
<tr>
<td>39</td>
<td>M</td>
<td>6</td>
<td>PIED screen</td>
<td>PC</td>
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<td>6</td>
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<td>PC</td>
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<td>PC</td>
</tr>
<tr>
<td>54</td>
<td>F</td>
<td>5</td>
<td>PIED screen, ERG</td>
<td>PC</td>
</tr>
<tr>
<td>55</td>
<td>F</td>
<td>6</td>
<td>PIED screen</td>
<td>PC</td>
</tr>
<tr>
<td>57</td>
<td>M</td>
<td>3</td>
<td>PIED screen</td>
<td>PC, hyporeflective fundus</td>
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<td>63</td>
<td>F</td>
<td>7</td>
<td>PIED screen, ERG, MG</td>
<td>PC</td>
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<td>73</td>
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<tr>
<td>77</td>
<td>M</td>
<td>7.5</td>
<td>PIED screen, ERG, MG</td>
<td>PC, Vitreous degeneration Vitreous prolapse Moderate visual impairment</td>
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<tr>
<td>78</td>
<td>F</td>
<td>6</td>
<td>PIED screen</td>
<td>PC</td>
</tr>
<tr>
<td>79</td>
<td>M</td>
<td>3</td>
<td>PIED screen</td>
<td>PC</td>
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<td>PC</td>
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<td>3</td>
<td>PIED screen</td>
<td>PC</td>
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<tr>
<td>89</td>
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<td>7</td>
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<td>PC</td>
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<tr>
<td>90</td>
<td>F</td>
<td>6.5</td>
<td>PIED screen</td>
<td>PC, Moderate visual impairment</td>
</tr>
<tr>
<td>91</td>
<td>M</td>
<td>9</td>
<td>PIED screen</td>
<td>PC, Severe visual impairment Euthanized</td>
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<td>104</td>
<td>M</td>
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<td>PIED screen, ERG, MG</td>
<td>PC, Vitreous degeneration</td>
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<tr>
<td>105</td>
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<tr>
<td>106</td>
<td>F</td>
<td>6</td>
<td>PIED screen, ERG, MG</td>
<td>PC, Generalized retinal degeneration Severe visual impairment</td>
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<tr>
<td>107</td>
<td>F</td>
<td>5</td>
<td>PIED screen, ERG</td>
<td>PC, Generalized retinal degeneration</td>
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<tr>
<td>108</td>
<td>F</td>
<td>4</td>
<td>PIED screen, ERG</td>
<td>PC</td>
</tr>
<tr>
<td>109</td>
<td>F</td>
<td>5</td>
<td>PIED screen</td>
<td>PC</td>
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<td>3</td>
<td>PIED screen</td>
<td>PC</td>
</tr>
<tr>
<td>114</td>
<td>F</td>
<td>6</td>
<td>PIED screen</td>
<td>PC, Cataracts (ant. suture line)</td>
</tr>
<tr>
<td>116</td>
<td>M</td>
<td>4</td>
<td>PIED screen</td>
<td>PC</td>
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<td>117</td>
<td>F</td>
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<td>PC</td>
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<tr>
<td>122</td>
<td>M</td>
<td>10</td>
<td>PIED screen</td>
<td>PC, Cataract (minor post cortical)</td>
</tr>
</tbody>
</table>

All dogs screened for Presumed Inherited Eye Disease (PIED), using indirect ophthalmoscopy and slitlamp biomicroscopy. Visual behavior, when spontaneously reported by the owner, is shown in italics.

M: Male intact dog; F: Female intact dog; age given in years; ERG: Electoretinography; MG: Blood obtained for molecular genetics; LM: Light Microscopy; EM= Electron Microscopy; IHC: Immunohistochemistry; PC: Pigmentary Chorioretinopathy
Electroretinography

Results of ERG recordings from 4-8 year-old normal CCDs and three of the affected dogs of comparable ages are shown in Table 2. ERGs from some of the affected dogs did not deviate from parameters obtained from previously examined normal CCDs in regards to a- and b-wave amplitudes and implicit times (Table 1 and 2, dog ID 108, 104 and Figure 4 and 5). Thus, ERG was not diagnostic for the retinopathy shown for two of the affected dogs at 4 and 8 years of age, respectively. However, in some of the other affected dogs with more advanced disease (Table 2, dog ID 106, also used for morphology, Figure 9 and 10), markedly reduced ERG amplitudes could be observed (Table 2). The process of dark adaptation was abnormal, starting off with barely recordable b-wave amplitudes. The scotopic rod cone responses were reduced as well as the photopic 30 Hz responses. ERGs were non-recordable in a case (dog ID:10, Table 1) with generalized retinal degeneration (flat line, not shown).

Only one dog (Table 1, dog ID 104) was obtained for follow-up ERG. Surprisingly ERG parameters were still within the normal range for CCDs (not shown). Fundus changes had not progressed markedly and the dog was still visual.

Fluorescein angiography

FA of the normal dog was unremarkable (Figure 6 A-D). In the affected dog (Figure 7), however, in the arterial phase hyperfluorescent lesions, which appeared to be in the Bruch’s membrane/retinal pigment epithelium complex, were observed (Figure 7A). These defects, categorized as window defects, became more obvious in the arterio-venous phase (Figure 7B). A spotted diffusion (staining) was observed during the arterio-venous phase (Figure 7C). During the venous and throughout the late phase, there was a slow but gradual increase in fluorescence (Figure 7D).

Morphology

In the affected dogs obtained for morphology there was a marked difference in changes depending on the retinal region examined. In the central fundus the retina appeared mainly normal with symmetrical, long, slender and normally extended photoreceptor outer segments (Figure 8A, dog ID 77) and a normal appearing RPE cell layer was observed (not shown). In the midperipheral parts, however, there were significant changes: RPE cells were thick and bulging with organelles, mainly pigment granules (Figure 8B). Some RPE cells appeared to be detaching from the one cell-layered structure. In these abnormal regions, cells including pigment granules and phagosomes were seen in the subretinal space (Figure 8C,D). In another case (Table 1, dog ID 106) areas of geographic atrophy were observed in parts of the midperipheral and peripheral retina. In these regions there was a loss of RPE, photoreceptor outer and inner segments and most of the outer nuclear and plexiform layers (Figure 9 A,B). In focal areas there was direct contact between remnants of outer nuclear layer and the choroidal layer (Figure 9B,C). In other parts, RPE cells were spherical and formed a multi-cellular layer (Figure 9D), whereas some regions were much less affected and showed normal cell layering with rod and cone nuclei in the ONL and the RPE as a single-cell structure (Figure 9E: shown is the transition zone between affected and non-affected retina). Figure 10 shows an example of the midperiphery of the superior retina of the same case with solitary RPE cells (white stars), conglomerates of RPE cells and possibly macrophages, observed subretinally. There were also RPE cells that appeared to be detaching (white stars) from the (normally) one cell-layered structure. Rods (white...
Table 2: ERG results from 9 normal 4-8-year-old CCDs and from 3 affected CCDs, dog ID: 106, 108, 104 (Table 1), the latter dogs at age 6, 4 and 8 years, respectively.

<table>
<thead>
<tr>
<th>Response</th>
<th>Normal</th>
<th>Case 106</th>
<th>Case 108</th>
<th>Case 104</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave</td>
<td>Median</td>
<td>5th</td>
<td>95th</td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>b</td>
<td>28</td>
<td>14</td>
<td>60</td>
</tr>
<tr>
<td>S2</td>
<td>b</td>
<td>50</td>
<td>23</td>
<td>79</td>
</tr>
<tr>
<td>S3</td>
<td>b</td>
<td>72</td>
<td>51</td>
<td>95</td>
</tr>
<tr>
<td>S4</td>
<td>b</td>
<td>84</td>
<td>67</td>
<td>115</td>
</tr>
<tr>
<td>S5</td>
<td>b</td>
<td>90</td>
<td>67</td>
<td>125</td>
</tr>
<tr>
<td>Ssd</td>
<td>a</td>
<td>111</td>
<td>69</td>
<td>169</td>
</tr>
<tr>
<td>b</td>
<td>192</td>
<td>158</td>
<td>232</td>
<td>118</td>
</tr>
<tr>
<td>Sh</td>
<td>a</td>
<td>152</td>
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<td>b</td>
<td>227</td>
<td>180</td>
<td>264</td>
<td>155</td>
</tr>
<tr>
<td>Psd</td>
<td>a</td>
<td>11</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>b</td>
<td>38</td>
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<td>27</td>
</tr>
<tr>
<td>PfI</td>
<td>b</td>
<td>15</td>
<td>12</td>
<td>19</td>
</tr>
</tbody>
</table>

The dark adapted recordings consisted of scotopic low intensity responses (S: 0.01 cd•s/m²), which were elicited after 4, 8, 12, 16 and 20 minutes of dark adaptation, S1-S5, and scotopic standard intensity responses, Ssd: 3 cd•s/m² and scotopic high intensity responses, Sh 3 cd•s/m². Photopic ERGs consisted of photopic single flash responses: Psd, and 30 Hz flicker responses: PfI, using 3 cd•s/m² for both responses, after at least 10 minutes of light adaptation using 30 cd/m² of background light.

arrows) and cones (orange arrows) were partially misaligned and some had lost their outer segments. The choriocapillaris in this area appeared enlarged (red star) with many erythrocytes detectable in the subretinal space (green arrows). In affected dog with ID 90 (Table 1), LM and EM findings (not shown) were comparable to those of dog with ID 77 (Table 1).

The retinas from affected dog with ID 90 (Table 1) were examined using RPE65 immunohistochemistry in order to further evaluate the RPE cell layer (Figure 11). Multiple focal areas were identified within the tapetal and non-tapetal areas that contained more than a single layer of RPE cells, indicating RPE layer duplication (Figure 11A), in comparison with interspersing regions within the same retina that contained normal appearing single-layered RPE (Figure 11B).

DISCUSSION

The retinopathy of CCDs appears to be a very specific disease entity. The disease has been observed in a large number of CCDs mainly in Scandinavia, but sporadic cases have also been observed in Germany and France during the past three years (Personal communications, Dr. Corinna Eule and Dr. Gilles Chaudieu, 2011). The specific ophthalmoscopic characteristics of the disease, its bilateral nature with similar lesions in both eyes, and a clear familial disposition for the disorder suggest that hereditary factors are involved in the disease process. Both the coated, “powder puff” type and the hairless, mutant type of CCD are affected by the retinopathy. Thus, the normal or mutant types of CCDs and coat color do not appear to play a role in development of the defect. Although the disease appears to be familial, with affected dogs observed in three consecutive generations (Figure 3), the specific mode of inheritance has been difficult to establish because of the multiple inbreeding loops in the natural CCD pedigrees that have been available for investigation. Since no significant sex bias in affected dogs has been observed, it is unlikely that pigmentary chorioretinopathy in the CCD is an x-linked disease.

The disease appears to progress in both eyes simultaneously. The progression, however, was shown to be highly variable. For instance, in two of the affected cases evaluated twice during a 6-month period, with both dogs obtained for morphologic studies (Table 1, dog ID 77 and 90), there had been a deterioration of vision observed by the owner. In another two dogs there did not appear to be progression or there was a very slow progression (Table 1, dog ID 104 and 122). The reason for the variable progression is not understood at the present time.
Figure 4 Dark and light-adapted ERG recordings of a normal CCD at age 7 years. The former consisted of scotopic low intensity responses (S: 0.01 cd•s/ m²), which were elicited after 4, 8, 12, 16 and 20 minutes of dark adaptation (responses in A show the increasing b-wave amplitude with increasing dark adaptation time) and scotopic standard intensity responses, Ssd: 3 cd•s/ m² (C). Photopic ERGs consisted of photopic single flash responses: Psd (B), and 30 Hz flicker responses: Pfl (D), using 3 cd•s/ m² for both, after at least 10 minutes of light adaptation using 30 cd/ m² of background light.

Figure 5 ERG recordings of an affected CCD at age 4 years, which are within normal limits for the breed (Table 1, dog ID 108, numerical results shown in Table 3). The dark adapted recordings consisted of scotopic low intensity responses (S: 0.01 cd•s/ m²), which were elicited after 4, 8, 12, 16 and 20 minutes of dark adaptation (responses in A show the increasing b-wave amplitudes with increasing dark adaptation time) and scotopic standard intensity responses, Ssd: 3 cd•s/ m² (C). Photopic ERGs consisted of photopic single flash responses: Psd (B), and 30 Hz flicker responses: Pfl (D), using 3 cd•s/ m² for both, after at least 10 minutes of light adaptation using 30 cd/ m² of background light.
Figure 6 Fluorescein angiograms of a 5 year-old ophthalmoscopically normal CCD. The retinal arterial phase (A), arterio-venous phase (B), venous phase (C) and late phase (D) are all normal.

Figure 7 Fluorescein angiograms in an affected 7-year-old CCD. The arterial phase (A): the choroidal fluorescence at the level of the tapetum lucidum is still visible, the arteries are normally filled with fluorescent dye, while the veins are not filled during this phase. At the level of the venous bow, it is possible to distinguish two hyperfluorescent lines (arrows) which appear as window defects in the choriocapillaris/Bruch's membrane/retinal pigment epithelium complex. Arterio-venous phase (B): the fluorescein still slightly fills the retinal arteries but also the capillary network and the marginal part of the largest veins. The fluorescence of the two lines (small arrows) is more visible and the beginning of a spotted diffusion (staining) becomes visible (large arrows). Venous phase (C): the temporal retinal vein is still completely fluorescent and the leakage of fluorescein becomes more extended in the abnormal RPE. Late phase (D): the venous fluorescence diminishes but remains normal. There is a gradual increase in fluorescence, however, due to leakage at the level of the RPE.
Figure 8 Ultrastructure of affected retina, dog ID 77 (Table 1). (A) Outer layers of the central retina with normal appearing photoreceptor outer and inner segments. (B) Outer layers of the peripheral retina showing loss of inner and outer segments with RPE cells filled with organelles bulging into the subretinal space. (C) RPE and chorio-capillaris region in midperipheral retina: note the rounded RPE cell that appears to be detaching (arrows). (D) Parts of a detached RPE cell (star), including phagosomes and pigment granules, invading the subretinal space. Scale bars, as indicated.

POS: Photoreceptor Outer Segments; PIS: Photoreceptor Inner Segments; RPE: Retinal Pigment Epithelium; ONL: Outer Nuclear Layer

Figure 9 (A) Morphology of the midperipheral region of the inferior retina of affected dog with ID 106 (Table 1). An area with geographic atrophy is shown, corresponding to a circumscribed pigmented lesions observed by ophthalmoscopy. (B-E) show enlargements of the boxed areas shown in (A).

RPE: Retinal Pigment Epithelium; ONL: Outer Nuclear Layer; INL: Inner Nuclear Layer; GCL: Ganglion Cell Layer Scale bars, as indicated.
Mechanistically this is an interesting form of retinopathy. The RPE monolayer sustains the outer blood-retina barrier [10,11]. If patches of these cells degenerate this barrier is disrupted. It is also known that defects in Bruch’s membrane or, for example, when there are changes of this structure, such as in human Age-Related Macular Degeneration (AMD), the attachment of the RPE cells becomes abnormal [12]. This may inhibit normal RPE function and lead to RPE cell death. The symbiotic relationship of the RPE and the photoreceptor cells is well understood, especially in regards to the process of phototransduction and the visual cycle [13]. As long as the majority of RPE cells function normally the visual cycle may be uninterrupted. However, in regions with wide-spread degenerative changes, such as with degenerated or abnormal layers of RPE cells, possibly with a focal lack of outer retinal barrier, a severe and detrimental insult upon photoreceptor cell structure and, consequently, cell function [14] occurs.

The circumferential pigmented and demarcated lesions observed by ophthalmoscopy corresponded to the areas of geographic atrophy observed by morphology. Focal degenerative changes of RPE cells were observed by FA as window-defects in one case diagnosed as a pigmentedary chorioretinopathy. There was also leakage of dye in the late phase of the FA, indicating vascular abnormalities in the disease process. A hallmark feature of AMD is chorioretinal neovascularization [15]. This is not a feature identified in the affected CCDs in our study.

In the present study 3 CCDs were found with bilateral generalized retinal degeneration. In another 4 cases there were bilateral generalized changes in tapetal reflectivity, one with a distinct grayish discoloration along the visual streak area, but simultaneously with signs of the CCD retinopathy. The clinical signs of the first 3 cases were similar in appearance to “end stage” Progressive Retinal Atrophy (PRA). This is a hereditary primary photoreceptor disorder leading to bilateral blindness, with the prcd type PRA [4] known to affect the CCD (www.OptiGen.com). For this reason dogs included in the study were screened for the prcd mutation. None of the dogs showed an affected genotype. The generalized retinal degenerative changes observed in the 7 dogs could be secondary to the CCD chorioretinopathy or, possibly a separate disease entity.

Presumed inherited retinal diseases of dogs in which the RPE is primarily affected include canine multifocal retinopathy (cmr). This is a multifocal bullous retinopathy first described in the Great Pyrenees and the Mastiff dog breeds [16], and later in the Coton de Tulear, the Lapponian herder and the Australian shepherd dog breeds [17-19]. The disease appears to be stationary, e.g. lesions do not increase in size with time, rather they are stationary or become less marked with time. Serous lesions and focal detachments are detected with multifocal, gray to tan funduscopic patches that vary in size from barely visible to lesions larger than the optic disc [17], usually developing at 11 weeks of age. Changes are observed bilaterally in the peripheral tapetal fundus and most frequently around the optic disc. It was suggested that the retinopathy could be due to focal dysplasia of specific RPE cells, however, a generalized defect in the outer blood ocular barrier was not shown. Three different mutations in the VMD2 gene in affected dogs [18] have been elucidated. The disease is comparable with vitelliform macular dystrophy type 2, or Best’s disease found in humans. In contrast to the clinical findings in cmr, changes are observed in affected CCDs primarily in the peripheral fundus, and not until in adult dogs. Further,
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Figure 11 Confocal microscopy of RPE65 immunohistochemistry of the retina of affected dog with ID 90 (Table 1). Multiple areas were identified that contained more than a single layer of RPE cells (A), in comparison with regions that contained single-layered RPE (B). An image showing incubation with secondary antibody and the primary antibody omitted is shown (C).

Chor: Choroid; RPE: Retinal Pigment Epithelium; tap: Tapetum; IS/OS: Inner and Outer Segments of Photoreceptors; ONL: Outer Nuclear Layer; INL: Inner Nuclear Layer; DAPI: stain for nuclei in the neuro-retina. In each image the left panel shows the fluorescent signal. The right panel shows the fluorescent image superimposed of the Differential Interference Contrast (DIC) image to further highlight the retina layering.

in affected CCDs the changes become more prevalent and are observed also centrally with time. Ultrastructurally, geographic retinal atrophy is discerned in affected CCDs with more advanced disease, a finding that has not been described in cases due to cmr.

Another inherited pathology of the RPE cell layer, is the retinal dystrophy described in Briards [20]. The disease is due to a mutation in the RPE65 gene [21], similar to the defect found in a subtype of human hereditary blindness, Leber congenital amaurosis [22]. The disorder causes congenital night blindness and partial day blindness in dogs. Although there is a normal fundus appearance dark-adapted ERG recordings are non-recordable in young affected Briards [23]. Histologically, inclusions are observed in the RPE cells also with degenerative changes in photoreceptor cells [24]. The disease is slowly progressive and funduscopic changes were observed in one strain of affected dogs by 3-5 years of age [20]. In affected CCDs the disease is quite different since the ERG in affected CCDs appears normal initially, at a time when the chorioretinopathy is observed ophthalmoscopically. Further, the fundus changes are larger and more distinctly pigmented in affected CCDs, when compared to the small grayish to white discrete spots found in the central fundus of Briard dogs with the RPE65 mutation [20]. In the present study immunohistochemical stainings were performed using RPE65 antibodies in order to evaluate the RPE cell layer. Abnormal areas with multiple layers of RPE cells were discerned in affected CCDs. Morphology (LM) showed areas with atrophic patches with a lack of RPE cells. In the same affected retinas there
were also areas in which the RPE cell layer appeared structurally normal. These studies were aimed at evaluating the RPE cell layer structure and to investigate if the RPE65 protein was prevalent in the RPE of affected CCDs, which was not the case in Briard dogs with RPED. Ophthalmoscopically, changes in the disease were characterized by accumulation of irregular foci or light-brown pigment spots, initially always in the central tapetal fundus but slowly spreading over the entire fundus with time. Morphology indicated that the primary defect was in the RPE. The cells accumulated a light-brown granular material in the cytoplasm, and with progression of disease, hypertrophic RPE cells appeared to form multicellular nests, with secondary focal degeneration of photoreceptors [27]. Similar funduscopy lesions have been produced in dogs fed diets deficient in vitamin E, an antioxidant that retards the intracellular accumulation of lipofuscin pigment [29]. Naturally acquired retinopathy resulting from vitamin E deficiency has also been described in dogs [30-32]. There is a similarity between the retinopathy of CCDs and RPED, in that ERGs are not diagnostic for either disease. A difference is that the chorioretinopathy of CCDs is observed mainly in the peripheral tapetal fundus and initially not in the central parts of the fundus as in RPED affected animals. Another difference is that in the former, areas of geographic atrophy are found with involvement of the outer retina and choriocapillaris, an aberration not observed in RPED.

Other breed-related retinopathies or chorioretinopathies have been reported in the Borzoi [33], the Czechoslovakian Wolfdog [34], and in the German Shepherd dog [35]. The pathophysiology of these diseases is still mainly unclear, however, infectious processes, cardiovascular events and excessive physical strain, have been mentioned as possible causative factors. In the Border Collie [36] a high prevalence of focal or generalized chorioretinal changes were described, and the disease designated a “working dog retinopathy”. Studies indicated a high prevalence for the disease with large number of dogs in enclosed environments, and with simultaneous heavy exposure to parasites, especially Toxocara canis species. Hereditary factors could also be involved in the expression of the working dog retinopathy [37].

The presently described chorioretinopathy of CCDs, with marked differences in regards to the specific ocular disorders in other canine breeds described above was designated as a pigmentary chorioretinopathy [38]. So far, a precise counterpart to this disease has not been found in other dog breeds, however, a geographic atrophy is described in conjunction with human AMD [11], with the clinical appearance of sharply demarcated islands and continents of RPE atrophy in the macula, typical for the advanced stage of the dry form of AMD [39].

Studies are in progress in order to further define the pathophysiology of the disease at the ultrastructural and immunohistochemical levels. Also, imaging studies of affected CCDs using optical coherence tomography, fluorescein and indocyanine green angiographies with scanning laser ophthalmoscopy are warranted as well as further molecular genetic studies.

CONCLUSION

Pigmentary chorioretinopathy is a presumed inherited ophthalmic disease of the Chinese crested dog, affecting mainly the retinal pigment epithelium, the choriocapillaris and the photoreceptor layer. Clinical findings include bilateral, demarcated, circumferential, pigmented lesions (less than 1/4 of disc diameter, approximately) with a light- or dark-colored center, usually first observed in the peripheral tapetal fundus, with hyperreflective areas between the lesions. Electoretinography is not diagnostic in mild cases. The disease appears slowly progressive in that lesions become more prevalent with time and changes are observed also in the central parts of the fundus. ERGs then become abnormal. Vision in affected animals is reduced to variable degrees from no observable visual problems to blindness. Morphology shows abnormalities in the retinal pigment epithelium with areas of duplication of the cell layer, degeneration and/or migration of cells and areas of geographic atrophy. The clinical and morphological findings in the disease have no counterpart in other disease entities of canines, however, geographic atrophy is described in conjunction with human age related macular degeneration.

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