Novel retinopathy in related Gordon setters: a clinical, behavioral, electrophysiological, and genetic investigation

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Abstract

Purpose To conduct ophthalmic, behavioral, electrophysiological, and genetic testing on two related Gordon setters presented for day blindness and compare findings with those of nine related and unrelated Gordon setters.

Methods All dogs underwent comprehensive ophthalmic examination. Maze testing was conducted under different light intensities. Rod and cone function was assessed electroretinographically. DNA samples were screened for five canine retinal disease gene mutations.

Results Ophthalmic examination was unremarkable in all dogs. There was no notable difference between day blind dogs and the reference population in scotopic and mesopic maze tests. Day blind dogs performed worse in the photopic maze with slower course completion time and more obstacle collisions. Electroretinography revealed extinguished cone function in day blind dogs and depressed rod responses in all but two reference dogs. One reference population dog presented with day blindness 1 year after initial examination. Mutations that cause achromatopsia (in *CNGB3*) and cone-rod dystrophies (in *ADAM9* and *IQCB1*) were not detected in any dog tested, although five reference dogs were carriers of the mutation in *C2orf71* that causes rod–cone degeneration 4 (*rcd4*) in Gordon setters and in polski owczarek nizinny dogs.

Conclusions This report describes a novel retinopathy in related Gordon setters that has clinical signs and vision testing results consistent with achromatopsia but electroretinographic results suggestive of cone-rod dystrophy. The majority of Gordon setters in this study had low rod responses on electroretinography but it is unclear whether this was indicative of rod dysfunction or normal for the breed. Longer-term observation of affected individuals is warranted.

Key Words: achromatopsia, cone-rod dystrophy, day blindness, dog, electroretinography, night blindness

INTRODUCTION

Progressive retinal atrophy (PRA) is the collective name for a group of inherited retinal disorders characterized by progressive retinal degeneration culminating in complete vision loss.[1] It is the leading hereditary cause of blindness in dogs.[2–4] PRA has been reported in more than 100 dog breeds.[5] Most forms of PRA have a rod-led degeneration followed by a secondary degeneration of cones.[4] As such, affected dogs initially experience night blindness but daytime vision loss, and hence complete blindness, is soon to follow. Classic ophthalmoscopic changes include tapetal hyper-reflectivity and retinal vascular attenuation, with optic nerve head atrophy present in the later stages of the disease. Hyper- and hypopigmentation in the nontapetal region of the fundus can also be seen.[1,6]

Although rod–cone degenerations and dysplasias are more prevalent, cone degeneration and cone-rod dystrophies have also been documented in dogs.[7–15] Cone degeneration (*cd*), also referred to as achromatopsia, is a

retinal degenerative disorder characterized by specific functional and structural abnormalities limited to the cones.[9,16–19] Affected dogs are therefore visually impaired in bright light (photopic) conditions. Rod structure and function remain unaltered in dogs with achromatopsia, and hence, affected dogs have normal vision under dim light (scotopic) conditions.[17,18] Achromatopsia is an autosomal recessive disorder in Alaskan malamutes, German shorthaired pointers, and miniature Australian shepherds and may also occur in Siberian huskies, Alaskan sled dogs, German shepherd dogs, and other breeds.[7,8,10,20] Case reports describing day blindness in a Chihuahua, Australian cattle dog, and Rhodesian ridgeback cross have also been described.[9] Cone-rod dystrophy (*crd*) is a diverse group of inherited retinal degenerative disorders characterized by the simultaneous degeneration of both cones and rods with cones being most severely affected initially.[11,12,21] Known breeds with *crd* include the miniature longhaired dachshund, Glen of Imaal terrier, American pit bull terrier, and the standard wirehaired dachshund (SWHD).[13–15,22] Fundus examination of *crd* dogs reveals signs of retinal degeneration including tapetal hyperreflectivity, vascular attenuation, and optic nerve head pallor, while the fundus of *cd* dogs typically appears normal.[8,9,11,23]

The first detailed report of inherited canine retinal degeneration was in the Gordon setter breed in Sweden and was provided by Magnusson in 1911.[24] Clinically, PRA reported in the Gordon setter is characterized by initial night blindness progressing to complete vision loss.[24,25] The typical age of diagnosis is around ten years, leading many to refer to it as a late-onset PRA.[25] Recently, a novel PRA locus termed rod–cone degeneration 4 (*rcd4*) and containing a frameshift mutation in the *C2orf71* gene was discovered in Gordon Setters and polski owczarek nizinny dogs.[25,26] While *rcd4* is the most common form of PRA among Gordon setters, at least one more form of PRA appears to segregate in the breed.[25] The purpose of this study was to conduct ophthalmic, behavioral, electrophysiological, and genetic testing on two related Gordon setters presented for day blindness and compare these findings with those of nine additional related and unrelated Gordon setters. To our knowledge, this is the first report of suspected cone dysfunction in this breed.

MATERIALS AND METHODS

Animals

The probands of this study were two Gordon setters (dogs 1 and 2) who were presented to separate but geographically close veterinary ophthalmologists due to owner suspicion of visual impairment in photopic conditions since approximately 1 year of age. These dogs were then referred to the Ophthalmology Service of the University of California, Davis Veterinary Medical Teaching Hospital (UCDVMTH), for further characterization of their visual impairment and comprehensive electroretinographic assessment. Nine additional Gordon setters (dogs 3–11) were recruited to form a reference population against which data from dogs 1 and 2 could be compared. These dogs were chosen based on their breed, age, gender, owner-reported relationship to Dog 1 or 2, and owner belief that they were normally sighted. Attempts to recruit dogs from the east coast of the USA in order to genetically distance the reference population from the two probands were unsuccessful. All dogs were included in this study only after obtaining permission from the UCDVMTH Clinical Trials Review Board and informed owner consent. Their clinical history and pedigrees were obtained from the owners.

Behavioral testing

A standardized maze with plastic obstacles was used to evaluate the dogs' ability to navigate under different light intensities. The obstacles were yellow and pink caution cones and floor signs that were of two different sizes, 9 inches (base width) × 24 inches (height) and 12 inches (base width) × 36 inches (height). Maze testing was conducted prior to clinical and electroretinographic evaluation in order to avoid the effects of pupillary dilation or recent bright illumination. Obstacle placement was varied for each trial to avoid dogs memorizing the course. For indoor maze testing, the subject was restrained by an assistant in one corner of a large (370 sq ft.) equine examination room. The dog's owner stood at the opposite end of the room (19.7 ft.). The owner was instructed to call the dog's name once, the assistant would let go of the dog, and the dog was allowed to navigate through the maze. Each dog was tested indoors in four mesopic and scotopic light intensities in the following order: 60 lux (indoors with doors open to outside and no overhead lights), 10 lux (indoors with doors closed and white overhead lighting), 2 lux (indoors with doors closed and red overhead lighting), and 1 lux (indoors with closed doors and no overhead lighting). Luminance was assessed using a photometer (Sekonic Studio Deluxe II model L-398M photometer; Sekonic Co. LTD, Tokyo, Japan), and each dog was allowed to adapt to the new lighting for 2 min prior to test commencement. After the four indoor trials, a photopic maze test was conducted outdoors on a bright, sunny day during which the measured light intensity was 3229 lux. When navigating the outdoor maze, the subjects were started inside a building in a dimly lit corridor and then walked outside to mimic the scenario in which owners had reported that their vision seemed most affected. For all five trials, the time from release by the assistant to arrival at the owner's side, as well as the number of times the dog bumped into an obstacle within the maze, was recorded by an observer masked to the identity of each dog. Dogs completed the maze three times for each lighting condition, and results were averaged. In addition, four

dogs (dogs 1–4) were video-taped while navigating the maze.

Dogs 1, 3–5, and 8–10 were assessed again by maze testing under three lighting conditions in an examination room (fluorescent lights on, red lights on, or all lights off) 1.5 years after the initial evaluation. On this occasion, maze testing could not be conducted at the original location and was in a smaller room so it was not timed. Dogs 2 and 11 were unavailable for retesting due to owner refusal and euthanasia due to metastatic osteosarcoma, respectively. Dogs 6 and 7 were not invited for retesting.

Clinical examination

Following maze testing, all eleven dogs underwent ophthalmic and general physical examinations. Ophthalmic examination included (in the following order) evaluation of the menace response under three lighting conditions in an examination room (fluorescent lights on, red lights on, or all lights off) and in bright outdoor light, direct and indirect pupillary light reflexes (PLRs) using a Finnoff transilluminator (Welch Allyn Inc., Skaneateles Falls, NY) in an examination room with fluorescent lights on, applanation tonometry (Tono-Pen Vet™; Reichert, Inc. Depew, NY), pupil dilation with 1% tropicamide (Falcon Pharmaceuticals, Ltd. Fort Worth, TX), slit-lamp biomicroscopy, indirect ophthalmoscopy, and fundus photography (RC-2 and GenesisD fundus cameras; Kowa Co Ltd Tokyo Japan). Ophthalmic examination was repeated 2–3 months after the initial evaluation in dogs 1–4 and 1.5 years after the initial evaluation in dogs 1, 3–5, and 8–10. In six dogs (dogs 1, 3, 4, 8–10), horizontal pupil diameter was measured at their second examination using a Jameson caliper in an examination room with fluorescent lights on and prior to pharmacologic pupil dilation. Fundus photographs of each dog were evaluated at the conclusion of the study to assess for evidence of retinal degeneration. All ophthalmic examinations were performed by the same ophthalmologist (KLG) who was not masked regarding other study data.

Electroretinography

Electroretinography (ERG) was performed in all dogs by one of the authors (RO) masked as to each dog's visual status. Recordings were conducted at least one hour following ophthalmic examination and fundus photography to avoid confounding effects of previous light exposure.[27] After maximal pupillary dilation was confirmed, each dog was premedicated with intramuscularly administered butorphanol 0.2 mg/kg (Fort Dodge Animal Health, Fort Dodge, IA) and glycopyrrolate 0.01 mg/kg (Baxter Healthcare Corp., Deerfield, IL). General anesthesia was induced with intravenously administered propofol (approximately 4 mg/kg (PropoFlo, Abbott Laboratories, N. Chicago, IL)) and maintained in a stable anesthetic plane with 1.2–2% isoflurane (Vet One, MWI, Meridian, ID) in 100% oxygen. Systolic blood pressure (Ultrasonic Doppler Flow Detector Model 811-B; Parks Medical Electronics, Inc. Aloha, OR), saturation of hemoglobin with oxygen, body temperature, frequency and character of respiration, and pulse rate and quality were continuously monitored by an experienced veterinary technician.

Electroretinography was performed using a handheld multispecies ERG (HMsERG) unit that includes a built-in mini Ganzfield stimulator which was used to deliver both the background illumination and the flash stimuli (HMsERG Model: 1000; OcuScience, Henderson, NV). The left eye only of each dog was evaluated. Once anesthetized, dogs were placed in right lateral recumbency and eyelids retracted using a Barraquer wire eyelid speculum. After topical application of 0.5% proparacaine hydrochloride (Falcon® Pharmaceuticals, Ltd. Fort Worth, TX), a dorsal episcleral stay suture was placed using 6-0 silk and the globe fixed in a central forward gaze. A contact lens electrode (ERG-jet®, Fabrinal SA, Switzerland) was applied to the cornea and electrical conduction enhanced by topical application of hydroxypropyl methylcellulose gel (Gonak™; Akorn, Inc. Lake Forest, IL). Subdermal reference and ground electrodes (F-E2; OcuScience, Henderson, NV) were placed 5 cm from the temporal canthus and at the base of the left pinna, respectively. Electrode impedance was maintained at <5 kΩ, and bandpass was 0.3–300 Hz. Animals were prepared in routine fluorescent lighting.

Rod and cone function was recorded using the HMsERG's preprogrammed 'Dog Diagnostic Protocol'. This included a 20-min dark adaptation period, during which rod function was tested every 4 min using a dim stimulus (average of 10 flashes, 0.5 Hz, 10 mcd*s/m^2). Subsequently, the mixed rod–cone response to a standard intensity (average of four flashes, 0.1 Hz, 3 cd*s/m^2) and high-intensity (average of four flashes, 0.05 Hz, 10 cd*s/m^2) stimulus was recorded. Cone function was recorded following 10 min of light adaptation (30 cd/m^2) using a standard intensity flash (average of 32 flashes, 2 Hz, 3 cd*s/m^2) and the cone flicker test (128 flashes, 31 Hz, 3 cd*s/m^2). Initially, all 11 dogs were tested. Electroretinographic testing of dogs 1–4 was repeated 2–3 months after the initial recording, and Dog 4 underwent a third electroretinographic test a year and a half after initial testing.

Mutation analysis

Whole blood was collected in tubes with EDTA from all dogs, and genomic DNA was extracted using the QIAmp DNA Blood Mini Kit (Qiagen; Valencia, CA) according to the manufacturer's protocol. The DNA samples were submitted to commercial testing laboratories to determine the presence or absence of specific mutations known to cause *cd* or *crd* in other breeds and for *rcd4* in Gordon setters (Table 1).

Table 1. Genetic mutations evaluated

Gene	Mutation	Disease	Breeds affected	References
CNGB3	140-kb deletion (removal of all exons)[a]	Achromatopsia (cone degeneration)	Alaskan malamute, miniature Australian shepherd, Siberian husky	Sidjanin *et al.*[8]; Yeh *et al.* 2011
CNGB3	D262N missense mutation[a]	Achromatopsia (cone degeneration)	German shorthaired pointer	Sidjanin *et al.*[8]
C2orf71	Frameshift[b]	Rod-cone degeneration 4 (*rcd4*)	Gordon and Irish setters, polski owczarek nizinny	Downs *et al.* 2013; Svensson *et al.*[26]
IQCB1 (NPHP5)	Cytosine insertion in exon 10[a]	Cone-rod dystrophy 2 (*crd2*)	American pit bull terrier	Goldstein *et al.* 2013
ADAM9	23-kb deletion (removal of exons 15–16)[a]	Cone-rod dystrophy 3 (*crd3*)	Glen of Imaal terrier	Goldstein *et al.*[14]; Kropatsch *et al.* 2010

Testing performed by [a]Optigen, Ithaca, NY, USA or [b]Animal Health Trust, Kentford, Newmarket, Suffolk, UK.

Table 2. Signalment, age at examination, testing performed, and *rcd4* status of all dogs evaluated

Dog no.	Gender	Known relation to probands	Age at exam(s) (years)	*rcd4* mutation status
1	FS	Proband	5.3[a,b,c] 5.5[a,f] 7[a,d,e]	Normal
2	MC	Proband	4.3[a,b,c] 4.6[f]	Normal
3	FS	Mother of both probands	9[a,b,c] 9.2[a,f] 10.7[a,d,e]	Carrier
4	M	Littermate of Dog 1	5.3[a,b,c] 5.5[a,f] 7[a,d,e,g]	Carrier
5	M	Grandsire of Dog 2	10.5[a,b,c] 11.8[a]	Carrier
6	F	Distant cousin of Dog 1	8[a,b,c]	Normal
7	M	Distant cousin of Dog 1	3[a,b,c]	Normal
8	F	NR	3.5[a,b,c] 4.8[a,d,e]	Normal
9	M	NR	3.5[a,b,c] 4.8[a,d,e]	Carrier
10	F	NR	6[a,b,c] 7.4[a,d,e]	Carrier
11	M	NR	9[a,b,c]	Normal

NR = not related. Testing performed: [a]complete ophthalmic examination, [b]ERG, [c]timed maze testing, [d]untimed maze testing 1 1/2 years later, [e]pupillary diameter measured [f]repeat ERG 2–3 months later, [g]repeat ERG 1 1/2 years later.

RESULTS

Animals, clinical history, and pedigree analysis

All study dogs were pure-bred, American Kennel Club registered Gordon setters residing in Northern California. The two probands were companion dogs dual bred for both field and show; Dog 1 was a 5.5-year-old spayed female and Dog 2 was a 4.5-year-old neutered male. Their owners reported that at approximately 1 year of age, dogs 1 and 2 did not track thrown balls well while outside during the day; however, they did track birds and bats flying overhead at dusk without apparent difficulty. By 4 years of age, Dog 1 became very hesitant whenever outside and could not navigate outdoor stairs or curbs. Dog 2 was reported to run into trees and other objects outdoors by 2 years of age. Both owners reported that daytime visual impairment was most notable immediately after entering a brightly lit outdoor area from a dimly lit indoor area.

The reference population included nine Gordon setters. Although some were related to the probands, none of the owners reported any visual deficits in their dogs. Median (range) age of dogs in the reference population was 6 (3–10.5) years. There was one spayed and three intact females and five intact males (Table 2). Three dogs were show dogs. Six dogs were dual bred for field and show.

Based on pedigree data provided by the owners, an extensive pedigree for related Gordon setters was established (Fig. 1). The owners reported that dogs 1 and 2 were from different litters but born to the same dam. In all, five dogs within the reference population (dogs 3–7) were reported to be related to the 2 days blind dogs. Of those five, the two most closely related were Dog 3 who was the mother of the probands and Dog 4 who was a littermate of Dog 1. In addition, there were two closely related and two distantly related dogs to the probands that were reported to be day blind but never evaluated by a veterinary ophthalmologist for confirmation (Fig. 1). One of those reportedly day blind relatives was a common ancestor to both Dog 1 and Dog 2 that traced back five generations.

Behavioral testing

There were no obvious differences between the probands and the reference population in the indoor maze testing under any of the four indoor scotopic and mesopic conditions. Only dogs 1 and 2 bumped into obstacles, and they both did so only while navigating the photopic outdoor maze. For the three timed photopic outdoor maze runs, each had a total of four bumps. These dogs also had the longest passage times through the photopic outdoor maze

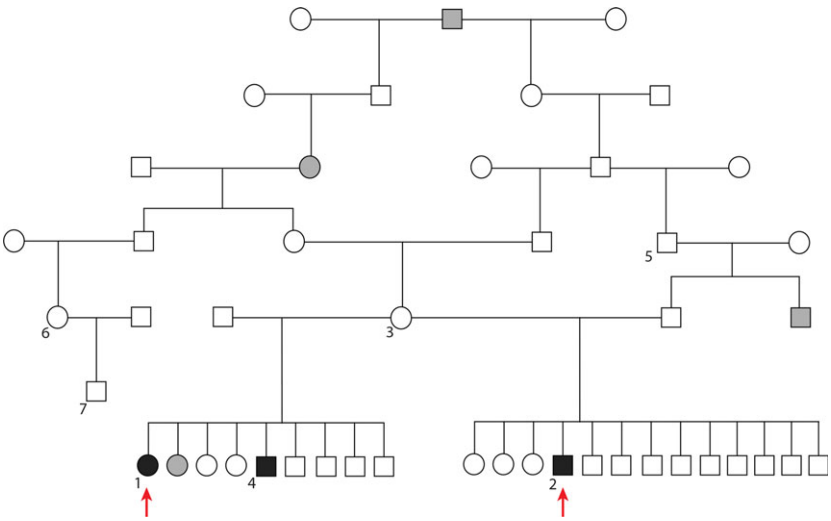

Figure 1. Pedigree of related Gordon setters with or without evidence of photopic visual impairment. Numbers correspond to the dogs included in this study (Table 2). Circles represent females, squares represent males, open symbols represent animals that are phenotypically normal (as reported by owners or determined during this study), solid black symbols represent day blind animals studied by the authors, and solid gray symbols represent dogs reported to be day blind but never examined by a veterinary ophthalmologist. Red arrows denote probands.

(Fig. 2). In addition, individual behavioral characteristics became apparent as the day blind dogs attempted the photopic outdoor maze. Dog 1, whom the owner described as a cautious dog, refused to move and displayed a very high-stepping gait when she did finally ambulate (Video S1). Dog 2, whom the owner described as fearless, ran through the course without any hesitation despite bumping into obstacles (Video S2). Following return indoors, both study dogs required several minutes to adapt before they moved in a way that suggested they were visual again. This behavior was not noted in any dog in the reference population.

Clinical examination

Menace response was normal in all dogs in all indoor scotopic and mesopic conditions. Dogs 3–11 also demonstrated a photopic menace response outdoors; however, dogs 1 and 2 had no demonstrable photopic menace response when tested outdoors. PLRs were normal in all dogs. Slit-lamp biomicroscopy and ophthalmoscopy revealed a variety of lesions in the probands and reference dogs, but none was considered sufficient to explain visual disturbance. Slit-lamp biomicroscopy revealed persistent pupillary membrane remnants on the anterior lens capsule in Dog 2 and incipient anterior cortical cataract in three dogs (dogs 6 and 10, unilateral; Dog 11, bilateral). Ophthalmoscopy revealed small, bilateral RPE colobomas in the inferotemporal region of Dog 5. Dog 7 had a small zone of vitreous hemorrhage for which an underlying cause was not found and which had resolved by follow-up examination 2 weeks later. Results of a complete blood count, serum chemistry panel, serology for *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Dirofilaria immitis*, and systolic blood pressure measurement performed on Dog 7 at the time when the vitreous hemorrhage was noted were all normal. Additional work up for the hemorrhage was declined by the owner. Intraocular pressure measurements were normal in both eyes of all dogs.[28] General physical examination findings and results of pre-anesthetic assessment of blood glucose, PCV, TP, and blood urea nitrogen were normal in all dogs at all times.

In the seven dogs that underwent serial evaluations, owner observations and ophthalmic examination findings did not change in any dog except Dog 4. According to the owner, Dog 4 developed uncharacteristically aggressive behavior toward people when outdoors approximately 1 year following initial evaluation. Soon thereafter, the owner noticed that when the dog went from a shaded patio into bright sunlight he became very hesitant and unwilling to walk. The dog had two separate episodes of falling into a pool on sunny afternoons and preferentially walked in shaded areas when outdoors. He was re-examined at 7 years of age (20 months after initial evaluation). Results of ophthalmic examination remained unremarkable. On an untimed maze test, he was obviously hesitant when maneuvering around obstacles outdoors, but had no difficulties negotiating a maze under fluorescent lighting indoors or when the room lights were turned off. Menace response was present in all indoor lighting conditions but was absent when tested outdoors. He was mydriatic (pupillary diameter 11.5 mm OU) and had delayed and incomplete PLRs bilaterally. Median (range) pupillary diameter in all other reference dogs assessed (dogs 3, 8, 9, and 10) was 8 (8–8.5) mm. Horizontal pupillary diameter of the one proband assessed (Dog 1) was 9.5 mm.

Electroretinography

Representative parameters of dark-adapted, mixed rod–cone and photopic flicker responses recorded at the first ERG examination of all eleven dogs are presented in Fig. 3, and sample traces are shown in Fig. 4. Dogs 1 and 2 had nonrecordable photopic single-flash (not shown) and flicker responses (Fig. 3). It is noteworthy that Dog 4, which went on to develop signs of disease

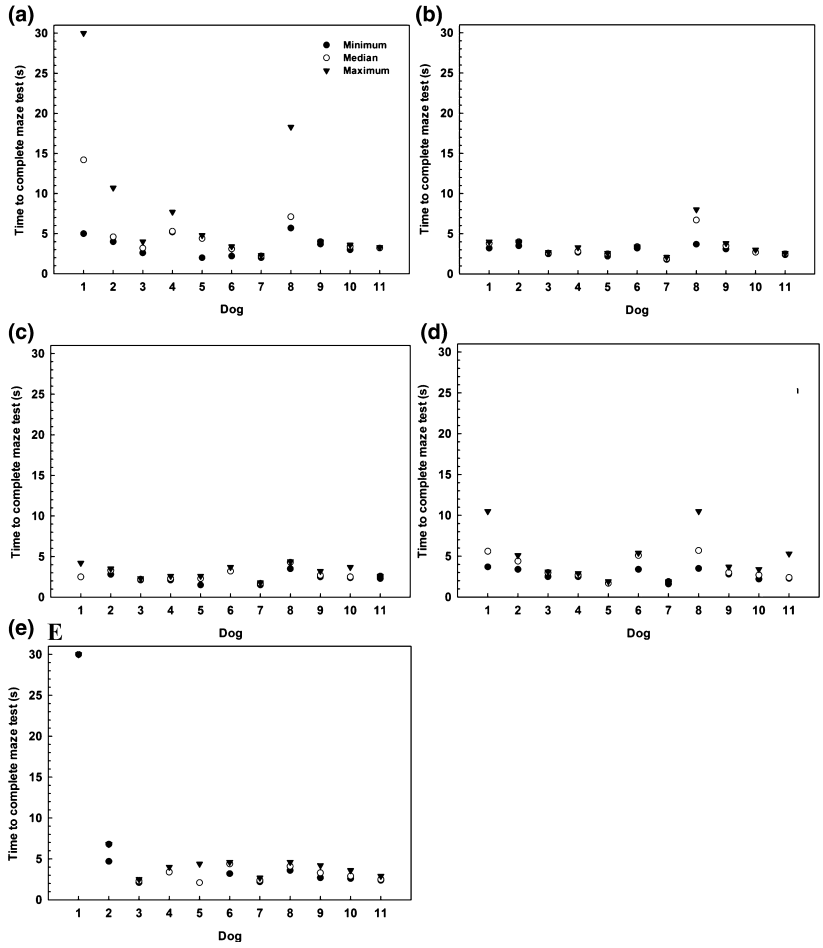

Figure 2. Scatter plot representing the maze completion times in seconds (minimum, maximum, and median) of all 11 study dogs at five different light intensities. a = 1 Lux, b = 2 Lux, c = 10 Lux, d = 60 Lux, e = 3229 Lux. Three runs were performed per light intensity. Cut-off time to complete the test was 30 s. The legend is depicted in (a) only but pertains to all plots.

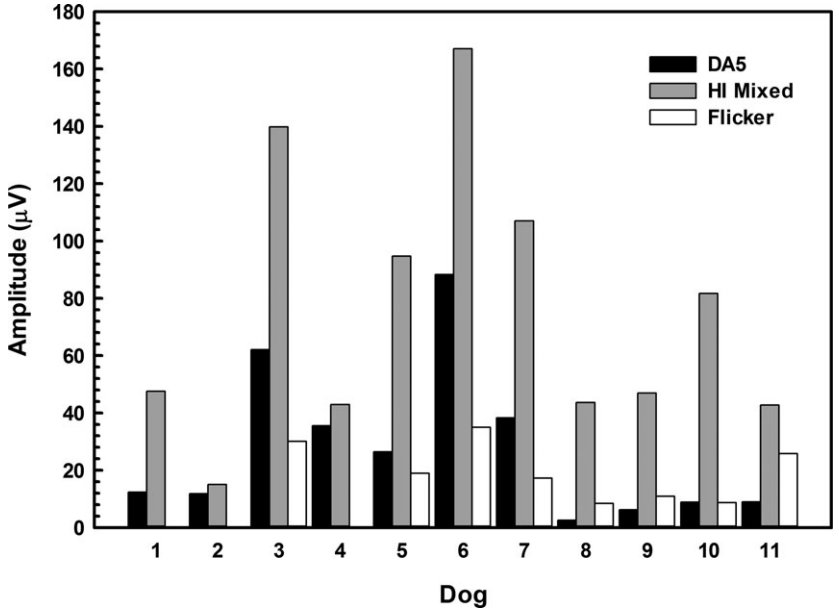

Figure 3. Bar graph presenting, for each dog, the b-wave amplitude of the 5th dark-adapted response (DA5, recorded 20 min after onset of dark adaptation), the b-wave amplitude of the dark-adapted, high-intensity, mixed rod–cone response (HI Mixed) and the amplitude of the flicker response recorded following 10 min of light adaptation (Flicker). Note the low DA5 amplitudes of proband dogs 1 and 2 as well as that of the majority of the reference population. Also notice the absence of a flicker response in both probands and sibling Dog 4.

1 year later, was the only other dog to have nonrecordable photopic flicker responses at this first recording. Furthermore, his photopic single-flash b-wave amplitude was only half of his mother's, Dog 3 (data not shown). Repeat ERG recordings were conducted in dogs 1–4 three months after their initial evaluation. Results were similar to those presented in Fig. 3. Dog 4 was recorded a third time a year and a half after initial testing. At this time, he had nonrecordable rod and cone function (Fig. 5).

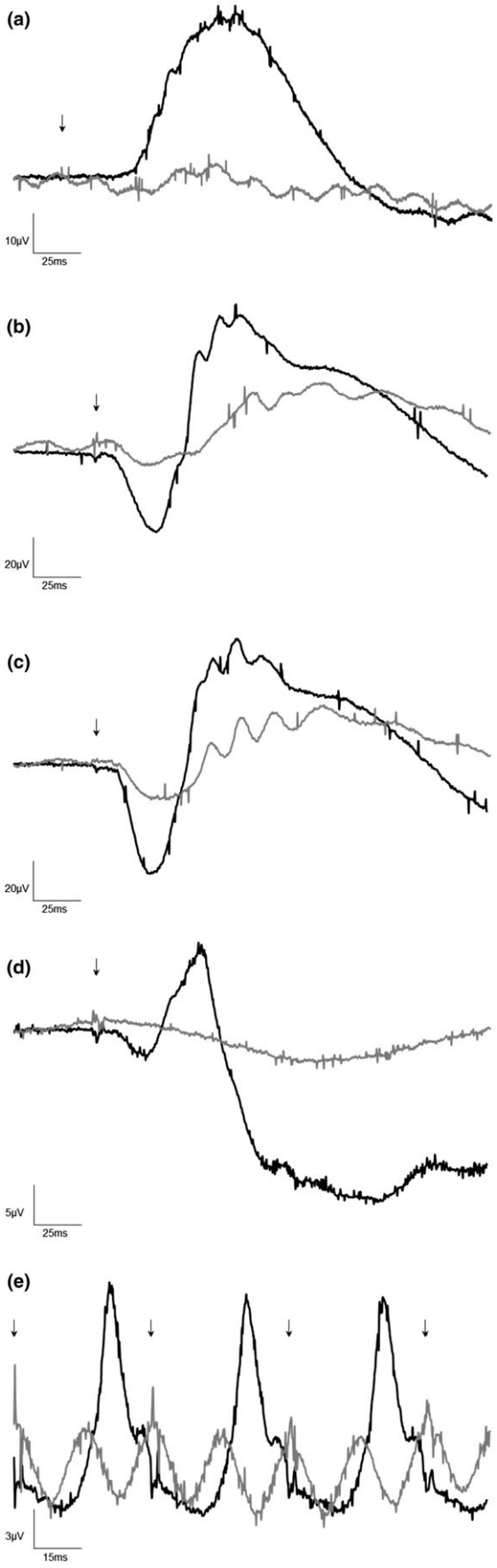

Figure 4. Representative ERG traces from an unaffected (black traces, Dog 7) and an affected (gray traces, Dog 1) Gordon setter. (a) scotopic responses recorded after 20 min of dark adaptation. (b, c) mixed rod–cone responses to standard and high-intensity stimuli, respectively. (d) Photopic single-flash responses following 10 min of light adaptation. (e) Photopic flicker responses. Looking at the onset of flash stimulus (arrows), it can be appreciated that the flicker 'signals' recorded from Dog 1 (panel e) are in fact 60 Hz noise and that this dog had no flicker responses. Additional low amplitude, very brief 'spikes' seen in (a–d) are also artifactual, most likely equipment-generated. See text for stimulus parameters. Horizontal scale bar-10 ms. Vertical bar: (a) 10 μV, (b, c) 20 μV, (d) 5 μV, (e) 2 μV.

Mutation analysis

Specific mutations known to cause *cd* or *crd* in other breeds (Table 1) were not detected in any dog tested. However, five of the eleven dogs (dogs 3, 4, 5,9, and 10) were identified as carriers of the mutation responsible for *rcd* 4 in Gordon setters (Table 2).

DISCUSSION

Achromatopsia in dogs was first reported in 1960 in the Alaskan malamute.[16,23,29] Based on these studies, dogs with achromatopsia have normal funduscopic examination and have no difficulty avoiding obstacles indoors or outside under dimly lit or dark conditions, but they cannot navigate obstacles in brightly lit conditions.[23,29] Electroretinography reveals absence of the photopic b-wave as well as absence of the cone branch of the flicker fusion response curve but a normal scotopic ERG remains.[17,30,31] The three affected dogs in our study were unable to successfully navigate in outdoor photopic conditions but navigated well when indoors even with the lights on and the doors open to the outdoors. ERG recordings were diagnostic and demonstrated complete lack of cone function in agreement with these previous studies of canine and ovine achromatopsia.[17,32] It can be appreciated that in Dog 4 (Fig. 5), an a-wave is present in the earlier recording of mixed rod–cone responses (black traces) and that this negative peak becomes more prominent and of longer duration in the subsequent recording (gray traces). This progressive 'unmasking' of the a-wave is likely due to attenuation of the b-wave which has been described in other studies[17,30,31] and suggests that functional progression of the disease may be due to early bipolar cell dysfunction. These behavioral and electrophysiological findings in dogs with normal-looking fundi made achromatopsia our leading differential diagnosis. While none of our dogs carry the known cyclic nucleotide-gated channel beta-3 (*CNGB3*) mutations that have been shown to lead to achromatopsia in Alaskan malamutes and German shorthaired pointers, it is possible that they suffer from a different mutation in *CNGB3* or other genes.[8,33] Indeed, a mutation in *CNGA3* has recently been shown to cause achromatopsia in a German shepherd dog as well as in sheep.[20,34]

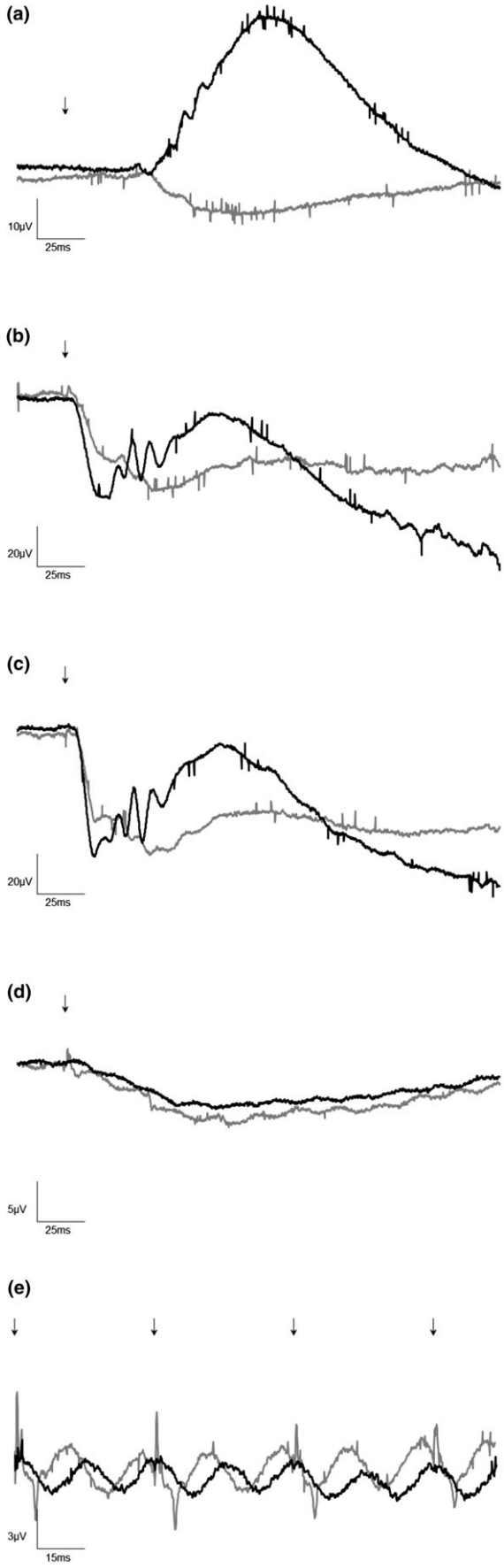

Figure 5. ERG traces recorded from Dog 4 at initial presentation (black traces), when it was behaviorally unremarkable, and 18 months later (gray traces), when it developed behavioral day blindness. (a) scotopic responses recorded after 20 min of dark adaptation. (b, c) mixed rod–cone responses to standard and high-intensity stimuli, respectively. (d) Photopic single-flash responses following 10 min of light adaptation. (e) Photopic flicker responses. The flicker 'signals' recorded on both occasions (panel e) are in fact 60 Hz noise (their frequency is twice as high as the stimulus artifact), and in fact at both recordings, the dog had no flicker responses. Arrows denote onset of flash stimulus. Additional low amplitude, very brief 'spikes' seen in (a–c) are also artifactual, most likely equipment-generated. See text for stimulus parameters.

Another differential diagnosis we considered is *crd*, a disease in which ERG recordings reveal significant reduction in both rod and cone responses.[21,35] In fact, our proband ERG results are more supportive of *crd* than achromatopsia (Figs 3,4). However, SWHDs with *crd* present with funduscopic and pupillary abnormalities not seen in our affected dogs during their initial examination or 1.5 years later.[11]Behavioral testing in *crd* SWHDs shows no differences between photopic and scotopic performances of affected dogs, nor between affected and control dogs, while behaviorally our affected dogs had no scotopic deficits but did have obvious photopic deficits compared to the reference population (Fig. 2; Videos S1, S2).[11] Dog 1 (proband) had a prolonged maze completion time at the lowest scotopic light intensity but she, along with Dog 2 (proband) at all luminance levels, confidently navigated the indoor maze at all other scotopic and mesopic light intensities. Furthermore, all of our dogs were negative for two known mutations causing canine *crd* (Table 1). It is important to point out that the behavioral testing in the SWHD study was only performed indoors and our study, as well as others, has shown that standard indoor testing is insufficient for diagnosing cone dysfunction and that outdoor testing is necessary to obtain reliable behavioral results. [9,11]

We were rather surprised by the extremely low amplitude and abnormal waveform of the scotopic ERG responses of our probands (Figs 3,4), which were inconsistent with their normal fundi and lack of scotopic and mesopic visual deficits (Fig. 2). The unexpectedly low values and abnormal waveforms led us to repeat the recordings in dogs 1–4 three months later and were reconfirmed in these repeat recordings. These decreased rod responses and abnormal waveforms were not unique to the probands, however. As Fig. 3 illustrates, most of the reference population of dogs also had depressed values and abnormal waveforms, and in four dogs, amplitudes were <10 μV after 20 min of dark adaptation. Ideally, the ERGs should have been repeated for the reference population of dogs to know whether these low amplitudes were repeatable and hence more supportive of normal rod values in Gordon setters, as breed variation in normal ERG values and

waveforms is recognized (personal experience, and Simon Petersen-Jones and Gus Aguirre, personal communications). Alternatively, it may be that these attenuated rod responses, and their abnormal waveforms, are due to the presence of another unidentified mutation in our dogs. In some cases (e.g., dogs 9 and 10), these depressed responses could potentially be explained by the dogs' *rcd4* carrier status (Table 2). However, this explanation is discredited by the fact that another *rcd4* carrier, Dog 3, had the second highest scotopic amplitudes of all dogs (Table 2; Fig. 3). Furthermore, the scotopic responses of reference dogs that were not *rcd4* carriers were also characterized by similar low amplitudes (Table 2; Fig. 3) and abnormal waveforms (see control Dog 7 in Fig. 4). Evaluating genetically distanced Gordon setters from a different geographic location of the country would help determine whether these depressed rod ERG amplitudes and abnormal waveforms were unique to these Northern Californian Gordon setters or a breed variation. Despite the low amplitudes and abnormal waveforms of the scotopic ERG responses, all reference and affected dogs (with the exception of Dog 1 at the lowest intensity) navigated the indoor maze with certainty and the owners, including those of both probands, did not report signs of night blindness. It is of interest to note, however, that Dog 8, who was not related to the probands, had the lowest DA5 b-wave amplitude and also had the slowest transit times under the lowest lighting condition through the obstacle course of all reference population dogs. Long-term monitoring with repeat ERG and vision testing in this dog is warranted.

One of the most interesting facets of this study is the results of Dog 4 who went on to develop day blindness 1.5 years after his initial examination. At the time of the initial testing, he was clinically and behaviorally normal yet his photopic single-flash b-wave amplitude was only half of his mother's, Dog 3. In addition, his cone flicker responses, which are of particular interest in diagnosing *cd*, were undetectable similar to the two probands (Fig. 3).[17,32] In view of his normal clinical and behavioral examinations, his depressed photopic ERG responses were puzzling. The client was advised of our concerns that based on the ERG findings, this dog may indeed be either a carrier or an affected individual, concerns that were justified 18 months later (Fig. 5). Electroretinography was therefore able to diagnose cone dysfunction in this dog 18 months prior to behavioral manifestations of day blindness. It is noteworthy that Dog 4 had a delayed onset of day blindness and a seemingly more rapid progression once behavioral signs were first noticed, in contrast to the disease onset and progression noted in his day blind littermate (Dog 1) in which behavioral signs of the disease were noticed at 1 year of age. His rod function also continued to decline and was nonrecordable on ERG 1.5 years after the initial examination. It has become increasingly observed that more than one form of PRA segregates in many breeds, and hence, this phenomenon could explain the phenotypic differences between affected Gordon setters.[5]

This study raises several interesting and important clinical implications for the veterinary practitioner. When evaluating dogs for possible cone photoreceptor dysfunction, the ability to adequately navigate under lighted indoor (mesopic) conditions can be misleading. In this study as well as others, standard indoor ambient light proved inadequate for making the presumptive diagnosis of *cd*.[9,11] Outdoor maze testing on a bright sunny day is needed for diagnostic confirmation although an indoor obstacle-avoidance course with ambient light intensities ≥25 lux has been constructed to successfully identify dogs with achromatopsia.[36] Also of note is that the most dramatic evidence of visual impairment in *cd* dogs is when going from inside a dimly lit building into the outdoors and they commonly have a lag in vision recovery when transitioning from the bright outdoors to the dimly lit building.[9,23] Both of the aforementioned traits were seen in our probands. Individual dog personalities may also either help or hinder the efforts to pinpoint whether a dog is afflicted with *cd*. It is important to note that when assessing vision impairment, dogs that are inherently shy and fearful (see Dog 1 in Video S1) may display extreme caution or simply refuse to navigate in different light conditions that alter their vision, whereas dogs that are innately fearless and carefree may maintain their confidence despite their repeated collisions with obstacles (see Dog 2 in Video S2).

Given the client-owned nature of the dogs in this study, histological evaluation of the globes of our affected dogs was not performed. One of the control dogs (Dog 11) was euthanized for a hind limb osteosarcoma 1 year after the study commenced. This individual did have depressed rod function on ERG but unfortunately the authors were not made aware of his illness and euthanasia, and hence, the globes were not collected for histopathological analysis. *In vivo* optical coherence tomography imaging and histological evaluation of the retina in the probands and phenotypically normal Gordon setters may help delineate whether the electroretinographically depressed scotopic responses are a variation of normal for this breed or a manifestation of PRA.

In summary, this report describes a novel retinopathy in related Gordon setters that has clinical signs and vision testing results consistent with achromatopsia, but electroretinographic results suggestive of *crd*. Longer-term observation of affected individuals and studies in additional dogs are warranted to further classify and diagnose this suspected inherited retinopathy in the Gordon setter.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Video S1. Dog 1 performing the photopic outdoor maze test.

Video S2. Dog 2 performing the photopic outdoor maze test.