Cosseggregation of Codon 807 Mutation of the Canine Rod cGMP Phosphodiesterase β Gene and rcd1

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Purpose. To determine if a previously reported nonsense mutation (G to A transition at nucleotide position 2420) in the canine rod cyclic GMP (cGMP) phosphodiesterase β (PDEB) subunit gene cosegregates with the rod-cone dysplasia 1 disease allele (rcd1) in the rcd1-dog reference colony; to establish the prevalence of this mutation among rcd1-affected Irish setters in the United States; and to screen for this mutation in other forms of canine hereditary progressive retinal atrophy (PRA).

Methods. Exon 21 of canine PDEB, previously reported to contain a nonsense mutation in rcd1-affected dogs, was amplified by polymerase chain reaction from genomic DNA isolated from peripheral blood samples. The mutation was detected in amplified DNA by restriction enzyme digestion and double-stranded conformational polymorphism. Linkage between rcd1 and the PDEB mutation was tested using the computer program LIPED.

Results. Three different rcd1-informative canine pedigrees were tested for the PDEB nonsense mutation. The first was a multigenerational pedigree representing the rcd1 reference colony. The other two pedigrees represented purebred Irish setter breeding lines in which rcd1 was known to be segregating. In all three pedigrees, the same point mutation was present and segregated with no discordance with the rcd1 allele. Linkage analysis established a maximum logarithm of odds (LOD) score of 12.05 at a linkage distance (theta) of 0.0. In a representative sampling of Irish setters in the United States diagnosed clinically as affected with typical rcd1 phenotype, all dogs were demonstrated to have the same (codon 807) PDEB mutation. Three of four Irish setters affected with atypical, relatively slower disease also had this mutation, but one dog did not. This point mutation in the canine PDEB gene was absent in other forms of canine hereditary retinal degeneration.

Conclusions. In three informative pedigrees, the codon 807 mutation in canine PDEB cosegregates with the rcd1 disease allele with zero discordance. A linkage distance (theta) of zero, with an LOD score of 12.05, indicates identity of this mutation and rcd1. This appears to be the only mutation causing rcd1 in the United States. In all other forms of canine hereditary retinal degeneration tested (ud, erd, prcd, rcd2, X-linked PRA, and in one Irish setter with late onset PRA), this PDEB point mutation was absent. Invest Ophthalmol Vis Sci. 1994;35:4291-4299.

Among the hereditary retinal degenerations affecting humans, most of the causative mutations are found at three gene loci: rhodopsin, peripherin-RDS, and PDEB.1-5 The latter gene codes for the beta subunit of rod cyclic GMP (cGMP) phosphodiesterase.6,7 PDEB mutations were initially identified in rd1 mice2-9 and subsequently in rcd1-affected dogs10-12 before they were recognized in human patients with autosomal recessive retinitis pigmentosa (RP).3

Rod-cone dysplasia type 1 (rcd1) is one of several canine retinal dystrophies, collectively termed progressive retinal atrophy (PRA), that afflict different breeds of dogs.13 The rcd1 phenotype is an early-onset disease, originally identified in Irish setters14-16 in the United Kingdom.17,18 Its inheritance has long been established as autosomal recessive14,16 which, coupled

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with its early onset, has encouraged incompletely successful efforts to eradicate the gene from the Irish setter breeding population by test-mating programs.19,20 Crossbreeding experiments have established that red1 and the mutations for two other canine autosomal recessive early-onset retinal degenerations (erd, red2) are mutually nonallelic.13

A specific canine strain, the red1-dog, has been developed in the United States from dogs affected with naturally occurring rod–cone dysplasia type 1. The red1 dog breeding population forms a reference colony in which the disease has been extensively characterized.21-27 The disease results from arrested postnatal development and subsequent degeneration of rod photoreceptor cells.21,27 These pathologic changes are preceded and accompanied by abnormal retinal cGMP metabolism; from 10 days of age, the cGMP content of the red1 retina rises sharply, up to 10-fold higher than normal, with a concomitant deficiency of cGMP phosphodiesterase activity.24-26 Retinal PDEB mRNA levels are specifically reduced in the red1-affected retina before the biochemical or pathologic signs of disease are evident.10

Suber and associates reported recently that affected dogs from the red1 reference colony carry a nonsense amber mutation at codon 807 of the gene for the beta subunit of rod cGMP phosphodiesterase. This mutation has been independently confirmed in a small population of red1-affected Irish setters.12 The deposited sequences for the canine PDEB homolog have been variously named (Genbank: DOGPDBS, accession L13262; EMBL: CFRGMPPB, accession Z29014). For present purposes, the canine homolog of PDEB is simply referred to as canine PDEB. The red1 mutation in canine PDEB, a G to A transition at nucleotide position 2420, would cause premature termination of the phosphodiesterase–beta subunit by 49 amino acid residues.11 The truncated protein would lack the C-terminal domain required for posttranslational processing and membrane association. Except for a limited study on a small, single-generation Irish setter pedigree with incomplete ascertainment, cosegregation of this point mutation with red1 has not yet been established.15 Further, it is not known whether other mutations in the canine PDEB gene might be responsible for red1 in different dogs. This latter question is relevant because multiple PDEB mutations have been identified in different human patients affected with RP.5

In this study, we have tested for cosegregation of the canine PDEB codon 807 mutation with red1 in multigenerational pedigrees from both the reference colony of red1 dogs and the purebred Irish setter breeding population within the United States. Similarly, we analyzed a representative number of purebred red1-affected Irish setters from within the United States to determine whether other PDEB mutations were present. We report here that the previously identified amber mutation at codon 807 of canine PDEB cosegregates with red1, with zero discordance, both in the reference colony of red1 dogs and in purebred Irish setters within the United States. Linkage between red1 and the canine PDEB codon 807 mutation is demonstrated with a recombination fraction of zero and a maximum LOD score of 12.05. Presumably, this is the only causative mutation extant in dogs exhibiting the red1 clinical phenotype. This point mutation in canine PDEB is absent in seven other breeds of dogs affected with other forms of PRA.

MATERIALS AND METHODS

Animals

The red1 reference colony is maintained as part of a National Eye Institute–National Institutes of Health-sponsored project. This and several other well-characterized canine mutant strains are bred and maintained at the Retinal Disease Studies Facility (Kennett Square, PA). The reference colony was originally derived in 1974 from purebred Irish setters affected with red127 and forms the reference colony for all recent research on this model disease. The strain is no longer purebred Irish setter because matings to laboratory-strain beagles and beagle-crossbred dogs (homozygous normal at the red1 locus) have been used in the breeding program. Of the eight foundation dogs shown in Figure 1A, three were purebred, inbred, red1-affected Irish setters (dogs 7, 22, and 23); dog 20 was a purebred homozygous normal Irish setter not closely related to dogs 7, 22, and 23; dog 6 was a purebred laboratory strain beagle; and dogs 8, 21, and 26 were derived by crossbreeding beagles to different strains of dog. It should be noted that, although the general phenotypic characteristics of the dogs in this colony now vary from dog to dog and differ from that of purebred Irish setters, the clinical, electroretinographic, and morphologic expression of red1 disease has remained unchanged from that in purebred Irish setters (Acland and Aguirre, unpublished observations, 1994). Blood and tissue samples from 58 dogs representing several generations of informative breedings within this reference colony were tested in the present study, and data from 42 dogs of this pedigree are shown in Figure 1A.

Blood samples were also analyzed from purebred Irish setters representing the natural breeding population of these dogs in the United States. These samples were collected in two sets. The first set of 41 dogs forms two pedigrees of informative Irish setters whose red1 genotype has been previously established as part of a test-mating program run by the Irish Setter Genetic Registry (Figs. 1B, 1C). The second set of 22 dogs comprises a representative sampling of all other available red1-
Cosegregation of Mutant Canine PDEB With rcdl

affected Irish setters diagnosed clinically within the United States. The dogs in this latter group of purebred Irish setters are not, in general, closely related to each other, to the first set of Irish setters, or to the rcdl reference colony of dogs. In this group, an effort was made to identify those dogs clinically diagnosed with rcdl by fundus examination or electroretinography, but exhibiting a later age of onset or slower rate of progression of the disease, or both.

To determine the specificity of the PDEB codon 807 mutation to rcdl, we also tested seven other breeds of dogs affected with hereditary retinal disorders different from rcdl. From within the Retinal Disease Studies Facility canine models colony, blood or tissue samples were collected from strains representing cone degeneration (cd),29 early retinal degeneration (erd),29 3 breed-specific (miniature poodle, English cocker spaniel, Labrador retriever) allelic variants of progressive rod cone degeneration (prcd),30,31 and X-linked progressive retinal atrophy (XLPRA).32 Samples were also collected from purebred collies affected with rod-cone dysplasia type 2 (rcd2), a disease that is characterized by elevated retinal cGMP levels and deficient cGMP-PDE activity but that is nonallelic to rcdl.x

All procedures involving animals were performed in adherence to the ARVO Resolution for the Use of Animals in Ophthalmic and Vision Research.

Source and Isolation of Genomic DNA

For most assays, genomic DNA was isolated from 200 μl of blood samples essentially as described previously33 using 0.5% Tween 20 and proteinase K. Isolated DNA was cleaned by phenol–chloroform extraction and ethanol precipitation and suspended in 20 μl TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). For some assays from deceased dogs, a small spleen sample that had been stored at −70°C was used instead of blood; splenic DNA was isolated, cleaned, and prepared in the same manner as described above for blood samples.

Amplification of DNA by Polymerase Chain Reaction

Amplification of PDEB Putative Exon 21 (157 bp) for Detection of Single Base Mutation. Polymerase chain reaction (PCR) was performed using 1 to 2 μg genomic DNA and 0.4 μM of each of the following primers: 5'GAGTTTTCCCGTTTCCACGAA (forward) 5'GCTTTCTTGGCTGTCGTCCT (reverse).

All reactions were performed in a volume of 100 μl containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM MgCl2, 10% DMSO, and 0.2 μM each dATP, dCTP, dGTP, dTTP, and 2.5 U Taq DNA polymerase (Life Technologies, Gaithersburg, MD). Reactions were carried out for 30 cycles at an annealing temperature of 54°C for 1 minute, a polymerization temperature of 72°C for 1 minute, and a heat-denaturation temperature of 94°C for 1 minute in a thermal cycler (Thermolyne, Dubuque, IA). After 30 cycles, samples were held at 4°C in the thermal cycler until removed for analysis.

Amplification of Partial PDEB Putative Exon 21 Using Mismatch Primer. PCR designed to introduce an allele-specific restriction site was performed using 1 to 2 μg of genomic DNA, 0.4 μM of 5'-mismatch primer (5'-GACTGCAAGAACAGGAGGACT), and 0.4 μM 3'-primer as above (5'-GCTTTCTTGGCTGTCGTC-
CTGTCCT). In the 5'-mismatch primer, the underlined C replaces A in the PDEB gene. All reactions were carried out as described above, except that the annealing temperature was 60°C.

**Double-Stranded Conformational Polymorphism**

We used the double-stranded conformational polymorphism (DSCP) technique of Ganguly et al.3 for the detection of single-base mutations by heteroduplex formation. For each PCR-amplified test sample DNA containing PDEB exon 21 (157 bp), a 20-μl aliquot portion was placed in the first of two tubes. In the second, a 10-μl aliquot portion of the test sample was mixed with an equivalent amount of known control sample containing only wild type PDEB alleles. Each pair of tubes was heated at 98°C for 5 minutes to separate DNA strands and then annealed at 68°C for 60 minutes. The treated samples were concentrated to 5 μl by speed vac and mixed with 5 μl of 20% ethylene glycol–30% formamide containing 0.25% of xylene cyanol and bromphenol blue. To separate heteroduplex from homoduplex products, samples were run under mildly denaturing condition in a standard 5% polyacrylamide gel (polymerized in 10% ethylene glycol–15% formamide–Tris-taurine buffer [44.5 mM Tris–28.5 mM Taurine–0.5 mM EDTA, pH 9.0]).

The gel was stained with ethidium bromide at 0.5 to 1.0 μg/ml for 10 minutes, destained for 10 minutes, and photographed under standard conditions.

**Restriction Enzyme Digestion**

PCR product generated from exon 21 of PDEB gene, using the mismatch primer set described above, was extracted with phenol–chlorehform, precipitated with ethanol, and suspended in 10 mM Tris-HCl (pH 8.0). Cleared PCR product was digested with either Bfa I or Bsr I under the conditions recommended by the manufacturer (New England Biolabs, Beverly, MA), and the digests were analyzed by electrophoresis in standard 6% polyacrylamide gel using TBE buffer (89 mM Tris-borate, 2 mM EDTA, pH 8.0).

**Linkage Analysis**

Linkage between rd1 and the PDEB mutation was tested using the computer program LIPED.35 Dogs in the pedigrees known from breeding history to be homozygous normal for rd1 are so indicated in Figure 1. However, for the purposes of this analysis, we adopted the conservative approach of assuming the homozygous normal state to be indistinguishable from the heterozygous.

**RESULTS**

**Detection of Nonsense Mutation in Putative Exon 21 of Canine cGMP PDEB Gene**

**DSCP–Heteroduplex Analysis.** As shown in Figure 2A, a test sample produced a single band corresponding to homoduplex double-stranded DNA when it contained only wild type (+/+) lane 1) or mutant alleles (rd1/rd1; lane 3), but it produced two bands when it contained both mutant and wild type alleles (rd1/+; lane 2). In the latter, the more intense lower band holds both possible homoduplexes (that is, rd1/rd1 and +/+), and the upper band represents formation of a heteroduplex (rd1/+). By this analysis, detection of heterozygotes was successful in all cases, but homozygous normal or affected samples could not be differentiated.

To distinguish rd1-affected from normal samples, a test sample was first mixed with a known control (homozygous normal at the PDEB locus) and then

![Figure 2. DSCP analysis and restriction enzyme digestion of PCR-amplified PDEB exon 21. Normal (lane 1), rd1-carrier (lane 2), and rd1-affected (lane 3) samples were analyzed by (A) DSCP of test sample only; (B) DSCP of test sample mixed with normal (+/+) sample; (C) Bsr I digestion, and (D) Bfa I digestion. DSCP = double-stranded conformational polymorphism; PCR = polymerase chain reaction; PDEB = phosphodiesterase β.](http://iovs.arvojournals.org/pdfaccess.ashx?url=data/journals/iovs/933403/)
melted and annealed to induce heteroduplex formation, as described in Materials and Methods. As shown in Figure 2B, an *rdl*-affected sample mixed in this manner with normal produces two bands corresponding to homoduplex and heteroduplex formation. This is similar to the result described above when electrophoresing the *rdl* carrier sample alone.

Thus, DSCP using the test sample, alone and in combination with a known control sample, could distinguish by heteroduplex detection a sequence difference in the putative exon 21 of the PDEB gene between normal, affected, and carrier samples.

**Restriction Enzyme Digestion.** The mismatch primer that we used for creating a restriction site for detection of the nonsense mutation at the cGMP PDEB locus was originally used by Clement et al 12 for *Bfa* I digestion (C'TAG). By examining the sequence created by the mismatch primer, we found that the wild type allele amplified with this mismatch primer creates a *Bsr*I site (ACTGGN'). Thus, PCR-amplified DNA (114 bp) from the wild type allele (+/+) on digestion with *Bsr*I produced 96-bp and 18-bp fragments. Similarly, digestion of PCR-amplified DNA from mutant allele (*rdl/rdl*) with *Bfa*I produced 92-bp and 22-bp fragments. The *rdl* carrier sample, which contains both the wild type and mutant alleles (*rdl/+*), was partially digested by both *Bsr*I and *Bfa*I (Figs. 2C, 2D). Determination of the genotype of the samples is critically dependent on complete digestion of the PCR-amplified *rdl* allele to distinguish reliably an *rdl*-affected sample containing mutation in both the alleles (*rdl/rdl*) from an *rdl*-carrier sample containing one wild type and one mutant allele (*rdl/+*). For this reason, we have used digestion of PCR-amplified DNA samples by both *Bfa*I and *Bsr*I. In this way, the digestion of an unknown sample with both the restriction enzymes separately will distinguish between the normal and the mutant alleles and is not dependent on complete digestion of the PCR product by either enzyme.

**Cosegregation of the cGMP PDEB Nonsense Mutation With *rdl***

Samples analyzed included dogs from three different pedigrees. The first pedigree (Fig. 1A) represents a partial, multigenerational pedigree of the *rdl* reference colony. The second and third pedigrees (Figs. 1B, 1C) represent purebred Irish setters from two different breeding lines in which *rdl* was known to segregate. All the members of the pedigrees were analyzed for the presence of the PDEB mutation by heteroduplex formation and restriction enzyme digestion with *Bsr*I and *Bfa*I. Samples from all the Irish setters that were clinically diagnosed as *rdl* affected were found to have the point mutation in both alleles of PDEB. All identified obligate heterozygotes (*rdl* carrier) were found to contain the mutation in one of the two alleles. Analysis of a selected sample of dogs from the pedigree in Figure 1A is shown by heteroduplex formation and *Bsr*I digestion (Fig. 3). We analyzed 121 Irish setter samples collected from across the United States that included the samples shown in the pedigrees (Fig. 1) and others with no known pedigree information. We found 100% concordance between the *rdl* phenotype and the identified nonsense mutation at nucleotide 2420 (G to A transition) in PDEB.

Four of these samples were from Irish setter dogs whose clinical disease represented a slower disease phenotype than is usually observed in the *rdl* reference colony. One of these four dogs belonged to one of the informative pedigrees studied (Fig. 1B, arrowhead), whereas the remaining three dogs were presumably unrelated; two of these had been obtained as adults from animal shelters. Details of their clinical disease is presented in Table 1. In three of the dogs (Table 1; dogs A, B, and C), we confirmed the presence of the nonsense mutation at nucleotide 2420 in PDEB using heteroduplex analysis and restriction enzyme digestion. In one Irish setter dog whose clinical disease differed from *rdl* by the markedly delayed onset of night blindness (5.5 years rather than 6 weeks of age) and slow disease progression (Table 1, dog D), no abnormalities were identified in codon 807 or exon 21 by re-
TABLE 1. Clinical and Laboratory Observations in Four Irish Setter Dogs With Atypically Slow Retinal Degeneration

<table>
<thead>
<tr>
<th>Dog</th>
<th>Age (years)</th>
<th>Clinical History, Observations</th>
<th>DSCP*</th>
<th>Bfa I Digestion†</th>
<th>Bsr I Digestion†</th>
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</thead>
<tbody>
<tr>
<td>A‡</td>
<td>4</td>
<td>Night blind, good day vision</td>
<td>Heteroduplex</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Deterioration of day vision</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>Complete cataracts; extraction (right eye), improved day vision</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>Night blind; fundus = midstage disease, PRA§</td>
<td>Heteroduplex</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>History of night blindness; good day vision until cataracts developed recently, ERG nonrecordable</td>
<td>Heteroduplex</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>5-month, history of visual impairment; posterior subcapsular cataracts; fundus = midstage disease, PRA§</td>
<td>Homoduplex</td>
<td>—</td>
<td>+</td>
</tr>
</tbody>
</table>

Three dogs (A, B, C) are homozygous for the PDEB codon 807 mutation, but the fourth (D) is wild-type at this locus.

* DSCP heteroduplex analysis = amplified PDEB exon 21, test sample mixed with normal sample.
† Bfa I and Bsr I digestion identifies codon 807 mutant and normal PDEB alleles, respectively; + = complete digestion; — = no digestion.
‡ Dog identified with arrowhead in Figure 1B.
§ Midstage disease; PRA = retinal vascular attenuation; generalized hyperreflectivity of tapetum lucidum secondary to retinal thinning.

PDEB = phosphodiesterase β; DSCP = double-stranded conformational polymorphism.

Restriction enzyme digestion and heteroduplex analysis, respectively (data not shown).

**Linkage Analysis**

No recombinants were observed among 40 individuals from informative litters in pedigrees A, B, and C (Fig. 1), yielding an observed recombination fraction of zero. Results of linkage analysis, using LIPEDE, for a range of estimated recombination fractions (theta) are listed in Table 2. A maximum combined LOD score of 12.05 was obtained for theta of zero, indicating strongly that the best estimate of the linkage distance between **rcdl** and the canine PDEB codon 807 mutation is zero.

**Absence of PDEB Exon 21 Mutations in Other Forms of Canine Hereditary Retinal Degeneration**

Putative exon 21 of canine PDEB was PCR amplified from samples representing dogs affected with seven other hereditary retinal degenerations: early retinal degeneration (**erd**), cone degeneration (**cd**), rod–cone dysplasia type 2 (**rcd2**), XLPRA, and three allelic variants of progressive rod–cone degeneration (**pred**). Amplified DNA was tested for heteroduplex formation by itself (Fig. 4A) and in combination with samples taken from genotypically normal Irish setter (+/+ ) (Fig. 4B) as described in Materials and Methods. Samples from known **rcdl-normal**, **rcdl-carrier**, and **rcdl-affected** dogs also were run simultaneously as controls (Fig. 4). No heteroduplex formation was detected by DSCP in any of the samples from dogs with the other hereditary retinal degenerations, indicating that there were no differences from normal in the nucleotide sequences of the putative exon 21 of the PDEB gene.

**DISCUSSION**

We demonstrate here unequivocally that the previously reported**11** amber mutation in PDEB of **rcdl**-

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**TABLE 2. Linkage Analysis**

<table>
<thead>
<tr>
<th>Theta</th>
<th>0.0</th>
<th>0.001</th>
<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
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<tbody>
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<td>Pedigree A</td>
<td>9.03</td>
<td>9.02</td>
<td>8.34</td>
<td>7.61</td>
<td>6.03</td>
<td>4.23</td>
<td>2.16</td>
</tr>
<tr>
<td>Pedigree B</td>
<td>0.91</td>
<td>0.91</td>
<td>0.75</td>
<td>0.68</td>
<td>0.44</td>
<td>0.23</td>
<td>0.06</td>
</tr>
<tr>
<td>Pedigree C</td>
<td>2.12</td>
<td>2.1</td>
<td>1.93</td>
<td>1.74</td>
<td>1.33</td>
<td>0.87</td>
<td>0.35</td>
</tr>
<tr>
<td>Total</td>
<td>12.05</td>
<td>12.03</td>
<td>11.06</td>
<td>10.03</td>
<td>7.8</td>
<td>5.33</td>
<td>2.57</td>
</tr>
</tbody>
</table>

Two-point linkage analysis of **rcdl** and the codon 807 mutation in canine PDEB. Data comprises LOD scores for linkage between **rcdl** and PDEB mutation, from computer program LIPED. LOD scores were calculated for each pedigree of Figure 1 and summed over all pedigrees. A maximum combined LOD score of 12.05 was obtained for a recombination fraction (theta) of 0.0.

PDEB = phosphodiesterase β; LOD = logarithm of odds.
affected dogs cosegregates with redI in informative pedigrees, with zero discordance. This is true of both the redI reference colony and of a large representative sampling of the Irish setter breed in the United States, yielding a combined LOD score of >12 for a theta of zero. This confirms the demonstration by Clements et al of the same mutation in a small single-generation pedigree of redI-affected Irish setters in Britain.

To establish linkage between this mutation and redI, we analyzed samples from multigenerational pedigrees by DSCP and restriction enzyme digestion. The single base change in PDEB was detected by DSCP, and the result was in full agreement with the clinical diagnosis of the test samples. However, because this method will not distinguish between single base changes at different positions in the test DNA fragment, we also analyzed samples by digestion with two different restriction enzymes separately to determine unequivocally the presence or absence of the reported mutation in the canine PDEB gene.11,12

We analyzed samples from 121 dogs from within the United States. Of these, 99 dogs were members of three separate redI-informative pedigrees, and 22 were Irish setters with no pedigree information. All samples from obligate heterozygotes for redI and all samples from dogs clinically diagnosed as redI affected were found to contain the nucleotide 2420-codon 807 amber mutation in either one or both alleles, respectively, of the canine PDEB gene, strongly indicating that almost completely eliminated the disease from Irish setters in the 1950s. This effort, based on testing candidate animals to redI-affected dogs and on clinical or histopathologic determination of disease presence or absence in their offspring, assigned genotypic status (redI-heterozygous or homozygous normal) to the candidate parent on a statistical basis. Identified carriers were eliminated from the breeding population. Because this approach selects against all allelic mutations, it may have eliminated any other canine PDEB mutations originally present in the Irish setter population.

The results in the dog are similar to those reported for the homologous disorder in the red mouse in that the same molecular defect is uniformly distributed in the affected population. In red, two distinct abnormalities are found—an Xmr-28 retroviral insertion in the first intron,13 and a nonsense mutation in exon 7 where the tyrosine in codon 347 is changed to a stop codon14; both defects seem to be present in all the strains of tested mice that have the red genotype. In contrast, four different mutations (two nonsense and one missense mutations; one base pair deletion) have been reported in humans with autosomal recessive RP. In all cases, the RP-affected patients were compound heterozygotes for two different allelic mutations of the PDEB gene.3

Because redI is the most common form of PRA in Irish setters, we wanted to examine for the presence of this mutation in any available Irish setter with a clinical diagnosis of PRA but whose clinical disease differed from redI because it had a later onset, a slower progression, or both. We analyzed samples from four such Irish setters; three contained the reported point mutation in the PDEB gene, but one did not. The three dogs with atypical disease that did have the PDEB codon 807 mutation demonstrate that, although redI is characteristically an early-onset disorder, there is in fact considerable variability in its clinical manifestation. This issue has long been recognized. In 1953, Parry described two strains of redI-affected Irish setters: In one strain, dogs were consistently day blind by 6 to 8 months of age, but in the other, they were not day blind until they were more than 3 years old. When affected dogs from the two strains were crossmated, all progeny were affected, demonstrating that a single locus was involved.16 In the fourth dog we tested, in which the PDEB codon 807 mutation was absent, disease did not develop until 5.5 years of age. We are presently examining the rest of the PDEB gene from the available genomic DNA to determine if the disease in this animal is the result of a mutation elsewhere in the gene. These results indicate that, even though redI is the most common
and characteristic retinal degeneration in Irish setters, other forms of PRA, particularly the late-onset variety, are likely to be present in the breed, and these are genetically distinct from rd1.

So far, the rd1 mutation in PDEB is the only identified mutation known to cause canine PRA. Thus, it was relevant to examine the presence of this mutation in other breeds of dogs affected with PRA. It has been reported that PRA-affected Tibetan terriers and miniature longhaired dachshunds do not carry this mutation.2 We tested for the presence of this mutation in seven other hereditary retinal degenerations affecting dogs (cd, erd, three allelic variants of prcd, rcd2, and XLPRDA). No evidence of mutation in canine PDEB exon 21 was detectable by DSCP in any of these disorders.

**Key Words**

cGMP phosphodiesterase, dog, PDEB, PRA, progressive retinal atrophy, progressive rod–cone degeneration, retinal degeneration, retinitis pigmentosa, RP, rod–cone dysplasia 1, rod–cone dysplasia 2

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**References**


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