Progressive rod-cone degeneration (PRCD) in selected dog breeds and variability in its phenotypic expression

J. Dostal[†1], A. Hrdlicova[1,2], P. Horak[1]

[1]Institution of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Libechov, Czech Republic
[2]Czech University of Life Sciences in Prague, Czech Republic

ABSTRACT: Progressive rod-cone degeneration (PRCD) is a late onset autosomal photoreceptor degeneration found in canines. PRCD in canines is homologous to one form of retinitis pigmentosa (RP) found in humans and displays phenotypic similarity as well as having the identical causative mutation. The *PRCD* gene was mapped to the centromeric region of canine chromosome 9 (CFA9). We report here a population study of 699 dogs of the following breeds and the following frequencies of the disease-causing mutation: American Cocker Spaniel (0.09), English Cocker Spaniel (0.34), English Springer Spaniel (0.00), Welsh Springer Spaniel (0.00), Flat Coated Retriever (0.00), Golden Retriever (0.00), Chesapeake Bay Retriever (0.14), Nova Scotia Duck Tolling Retriever (0.44), Labrador Retriever (0.07), Poodle Toy (0.45), Poodle Miniature (0.20), Poodle Medium (0.05), Poodle Standard (0.00), Portuguese Water Dog (0.33), Chinese Crested Dog (0.02), Shipperke (0.06), and Australian Cattle Dog (0.00). The disease results in complete blindness in the affected individual in almost every case. The time of onset and disease progression varies between dog breeds as well as between individuals. A modifier gene is likely to segregate in genomic proximity to the *PRCD* gene and may influence phenotypic expression.

Keywords: canine; retinitis pigmentosa; autosomal; recessive; causative mutation

Progressive rod-cone degeneration (PRCD) is an autosomal recessive photoreceptor degeneration of late onset in dogs. This ocular disorder is one of several inherited diseases designated as progressive retinal atrophy (PRA) in many dog breeds. The disease is homologous to human retinitis pigmentosa (RP) that primarily affects the retinal rod photoreceptors. RP is among the most common inherited forms of blindness affecting approximately one in every 4000 people (Phelan and Bok, 2000). The canine PRA models are valuable, as they are homologous to the human RP. Thus, they can aid in the identification of novel genes and metabolic pathways necessary for normal photoreceptor function, valid for both humans and dogs. From a clinical aspect, it is a disease of rod and cone photoreceptors.

The *PRCD* locus was mapped to the centromeric end of canine chromosome 9 (CFA9) in a region showing conservation of synteny with human HSA17q22 (Acland et al., 1998; Acland et al., 1999). Based on clinical similarities of the diseases, as well as chromosomal location, *PRCD* has been proposed as a locus homologous for RP17 and the only animal model for RP17 (Acland et al., 1998).

The order of the clusters, as well as the gene order within each cluster on CFA9 and HSA17q-ter differs, suggesting certain evolutionary rearrangements. In addition to homology with exonic sequences, comparative analysis between canine and human sequences from the PRCD region and human sequence from HSA17q25 revealed conservation outside of exons. A high level of canine/human conservation of non-coding sequences has been previously reported (Dubchak et al., 2000; Frazer et al., 2001). These non-coding sequences close to the 5´-end of gene probably include the regulatory elements of genes mapped to the re-

gion, but others do not have any obvious role. It has been proposed that these conserved canine/human sequences might represent unidentified genes or sequences that regulate processes such as transcription, replication, and chromosome pairing and condensation (Frazer et al., 2001).

To establish more detailed regions of synteny between dog CFA9 and human HSA17q-ter region, a robust gene-enriched map with 34 gene-based markers and 12 microsatellites, with the highest resolution and number of markers for the centromeric end of CFA9 was created (Sidjanin et al., 2003). An approximately 1.5-Mb physical map containing both *GRB2* and *GALK1* genes, which are closest to the *PRCD* locus was also constructed. It generated about 1.2-Mb low-pass canine sequence. The generated low-pass canine sequence was used to order and orient the canine sequence against the human sequence. Canine to human comparative sequence analysis identified 49 transcripts that had been previously mapped to the HSA17q25. Results show, however, that conservation of synteny does not necessarily translate into conserved gene order (Sidjanin et al., 2003).

The *PRCD* gene codes for a 54-amino acid protein in dogs and in humans, and a 53-amino acid protein in the mouse. The first 24 amino acids are highly conserved in 14 vertebrate species. A homologous mutation (TGC to TAC) in the second codon shows complete concordance with the disease in 18 different dog breeds and/or breed varieties. The same homologous mutation was identified in a human patient from Bangladesh with autosomal recessive retinities pigmentosa. This provides strong evidence that mutation in the novel *PRCD* gene causes autosomal recessive retinal degeneration in both dogs and humans (Zangerl et al., 2006).

MATERIAL AND METHODS

Clinical examination of dog retinas was performed by veterinarians

Samples of unclotted blood (aprox. 0.5 ml) from purebred dog breeds in the Czech Republic were collected in K3EDTA test tubes and shipped to the laboratory. Blood samples were kept in a freezer at –18 °C until used.

Genomic DNA was isolated using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) or extracted using QuickGene DNA whole blood kit S (Fujifilm Corporation, Japan) on the GuickGene-Mini 80 apparatus.

For the amplification of the *PRCD* gene fragment harbouring the indel, PCR was carried out using a set of primers as described by Zangerl et al. (2006). Primer sequences used in the study were: PRCD – F, 5'-CCAGTGGCAGCAGGAACC-3', and PRCD – R, 5'-CCGACCTGCTGCCCACGACTG-3'.

Amplification conditions were 2 min at 95 °C, followed by 29 cycles at 95 °C (30 s), 58 °C (30 s) and 68 °C (1 min), with final extension at 72 °C (7 min).

Restriction enzyme digestion of PCR products (25 µl) was performed with *RsaI* endonuclease (BioLabs, New England).

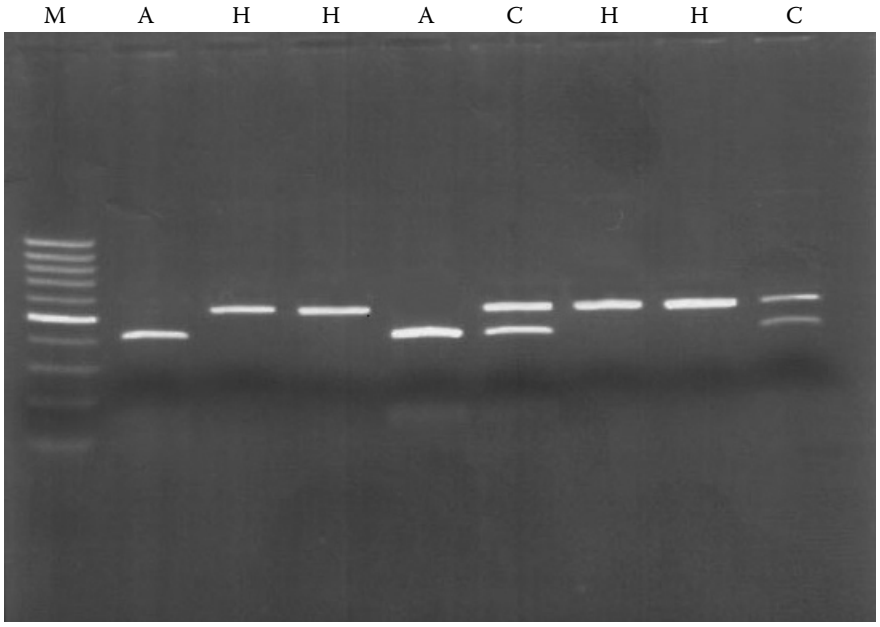

Figure 1. Results of PCR fragment polymorphism in *PRCD* gene after restriction enzyme digestion with *RsaI* endonuclease in 1.5% agarose gel electrophoresis. Samples are designated as: M = marker 100–1000 bp, H = genetically healthy, C = carriers, A = affected dogs

Agarose gel (1.5%) electrophoresis of PCR products was performed in TBE buffer (Bio Basic Inc., Ontario, Canada) composed of 40mM tris, 20mM boric acid and 1mM EDTA, pH 7.6. The gels were screened under UV light and photographed.

RESULTS

Amplification from genomic DNA as described above resulted in a 512-bp PCR product. Restriction enzyme digestion of PCR products with *RsaI* endonuclease specific to the sequence GT/AC results in two bands, 396 bp and 116 bp in the presence of the mutant allele (GTAC). The PCR product of the native allele (GTGC) is not digested by *RsaI* endonuclease and migrates as one band of 512-bp on agarose gel electrophoresis. Genotypes of individual dogs are thus easy to identify.

Results of PCR analysis and identification of genotypes are presented in Figure 1.

Genotypes and allele frequencies of different pure breed dog were calculated. They are presented in Table 1.

DISCUSSION

High frequencies of the mutant allele in the *PRCD* locus were found in English Cocker Spaniels, Nova Scotia Duck Tolling Retrievers, and Miniature Poodles. In contrast, no disease alleles were observed in English Springer Spaniels, Welsh Springer Spaniels, Flat Coated Retrievers, Golden Retrievers, Poodle Standards, and Australian Cattle Dogs. Three samples of analyzed blood from Portuguese Water Dogs are not representative but indicate a higher frequency of the mutant allele in this breed. In the Schipperke breed we identified only one heterozygous individual among eight studied dogs and no affected ones were found. This is the first description of the disease-associated mutation in the

Table 1. Genotypes and allele frequencies in studied dog breeds

Breed	*n*	Genotype frequencies			Allele frequencies	
		healthy *PRCD/PRCD*	carrier *PRCD/prcd*	affected *prcd/prcd*	*PRCD*	*prcd*
American Cocker Spaniel	55	46	8	1	0.91	0.09
English Cocker Spaniel	135	61	58	16	0.66	0.34
English Springer Spaniel	18	18	0	0	1.00	0.00
Welsh Springer Spaniel	11	11	0	0	1.00	0.00
Flat Coated Retriever	25	25	0	0	1.00	0.00
Golden Retriever	57	57	0	0	1.00	0.00
Chesapeake Bay Retriever	7	5	2	0	0.86	0.14
Nova Scotia Duck Tolling Retriever	44	14	21	9	0.56	0.44
Labrador Retriever	8	51	6	1	0.93	0.07
Poodle Toy	59	23	19	17	0.55	0.45
Poodle Miniature	65	43	18	4	0.80	0.20
Poodle Medium	21	19	2	0	0.95	0.05
Poodle Standard	4	4		0	1.00	0.00
Portuguese Water Dog	3	1	2	0	0.66	0.33
Chinese Crested Dog	99	96	3	0	0.98	0.02
Schipperke	8	7	1	0	0.94	0.06
Australian Cattle Dog	33	33	0	0	1.00	0.00
Total	702	514	140	48		

"Normal" allele is designated *PRCD*, mutant allele responsible for the disease is designated *prcd*

Table 2. Expression of progressive rod-cone degeneration compared of age of diagnosis using ophthalmology and electroretinography (ERG) in selected breeds according to Aguirre and Acland (2006)

Breed	Age of diagnosis	
	ophthalmoscopy	ERG
Poodle Toy and Miniature	3–5 years	9 months
American Cocker Spaniel	3–5 years	9 months
Portuguese Water Dog	3–5 years	1.5 years
Labrador Retrievers	4–6 years	1.5 years
English Cocker Spaniel	8–12 years	2.5 years and more

PRCD gene in this breed, and for this reason the manifestation of the disease in Schipperke dogs cannot be described.

The phenotypic expression of the disease varies greatly within breeds and between individuals (Aguirre and Acland, 2006). The reported variation of PRCD disease in selected breeds is shown in Table 2.

Previous research uncovered one exception in the phenotypic expression of the PRCD disease in an American Eskymo dog that was homozygous affected (AA), but clinically normal at the age of 13 years (Zangerl et al., 2006). In English Cocker Spaniels the age of early diagnosis varies from 3.1 to 13.5 years in dogs having the same mutation in the canine *PRCD* gene. For this reason, it has been proposed that genetic modifier(s) may play a crucial role in expression of the disease.

In the population described here one American Cocker Spaniel of the age of 11 years, one Poodle Toy at the age of 10 years, and two English Cocker Spaniels of the age of 10 and 11 years genetically "affected" – homozygous (AA) were clinically normal. These results are in agreement with Aguirre and Acland (2006) who propose an effect of some modifier(s) gene(s) on the expression of PRCD disease.

On the other hand 4 related Chinese Crested Dogs in one kennel, and one Poodle Toy with a clear PRA disorder were genetically *PRCD* homozygous (GG) - clear. This indicates the genetic contribution of some other causative mutation.

Selection against PRCD disease in Nova Scotia Duck Tolling Retrievers (frequency of the mutant allele is 0.44) complicates the finding of another mutation responsible for the development of Collie eye anomaly (CEA). The frequency of the CEA mutant allele is 0.244 (Dostal et al., 2010). To select against both mutations in a population of the breed requires a thoroughly defined breeding policy and consideration of what the population needs, instead of simply taking into account the desires of breeders. The Europe-wide cooperation of Clubs and breeders is also very important because the population size is not very large.

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Corresponding Author:

Ing. Anna Hrdlicova, Institution of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Libechov, Rumburska 89, 277 21 Libechov, Czech Republic

Tel. +420 315 639 540, Fax +420 315 639 510, E-mail: hrdlicova@iapg.cas.cz