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A novel mutation in *PDE6B* in Spanish Water Dogs with early-onset progressive retinal atrophy

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Abstract

Objective: To identify the underlying mutation in a recently identified early-onset progressive retinal atrophy (PRA) in the Spanish Water Dog (SWD) breed.

Animal Studied: Eighteen SWDs were used in this study. Six SWDs diagnosed with PRA and 12 phenotypically normal SWDs.

Procedures: An exclusion analysis using an established microsatellite panel to screen PRA candidate genes was combined with whole genome sequencing of two affected SWD siblings and two phenotypically normal SWDs (a sibling and the dam).

Results: A 6-bp deletion was identified in exon 19 of *PDE6B* removing two highly conserved amino acids from the enzymatic domain of the PDE6B protein (c.2218-2223del; p.Phe740_Phe741del). This segregated with the disease status in the small study pedigree.

Conclusions: Identification of this novel *PDE6B* mutation adds to the already described *PDE6B* mutations responsible for PRA in the Irish Setter, Sloughi, and American Staffordshire Terrier dog breeds. A DNA-based test was designed to allow breeders to genotype their animals and make informed breeding decisions in the effort to eradicate PRA from the SWD breed.

KEYWORDS

canine model, inherited retinal disease, phosphodiesterase 6B, progressive retinal atrophy, retinitis pigmentosa

1 | INTRODUCTION

Progressive retinal atrophy (PRA) is a group of inherited retinal degenerations reported in over 100 breeds of dog.[1] It is characterized by progressive, bilateral retinal thinning and retinal vascular attenuation and culminates in loss of vision. Typical PRA is characterized by an initial loss of rod photoreceptors followed by the cone photoreceptors. Mutations in over 20 genes have been identified as causal for PRA in dogs.[2,3]

Progressive retinal atrophy occurs in the Spanish Water Dog (SWD) breed. They are one of the many breeds with PRA due to a mutation in the progressive rod-cone degeneration gene (*PRCD*). Typically *PRCD*-PRA is a relatively late-onset form of PRA.[4] A DNA test for the *PRCD* mutation has been available and used by the SWD breed club for over 10 years to make breeding decisions (swdclub.org). More recently, a family of SWDs presented with a recessively inherited early-onset form of PRA which genetic testing showed was not due to the *PRCD* mutation (Figure 1). The affected dogs were identified with vision problems from as early as 1.5 years of age, and by 4.5 years of age, the condition was advanced with owners reporting significant loss of vision.

In this study, we report a novel mutation in *PDE6B* resulting in the deletion of two highly conserved amino acids

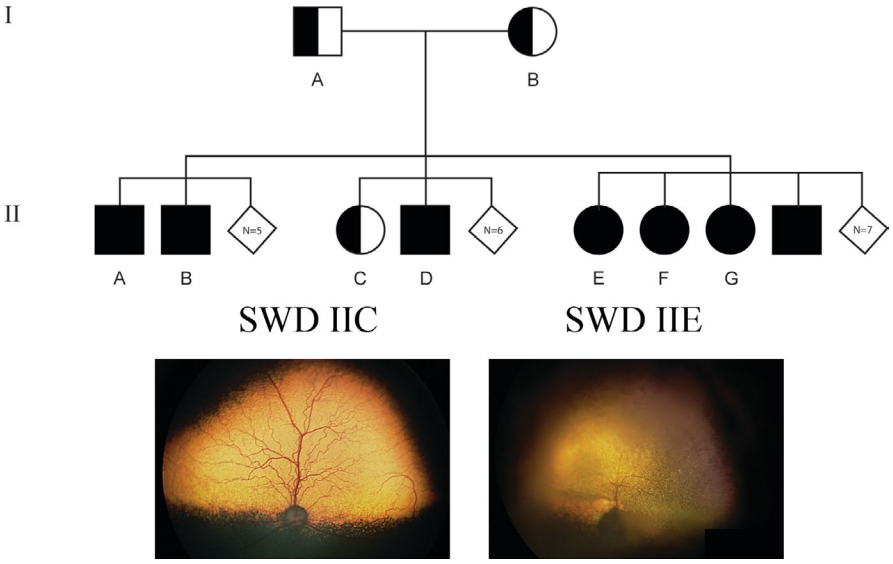

FIGURE 1 Early-onset progressive retinal atrophy in Spanish Water Dogs (SWD). (Top) Pedigree of family group with segregating PRA. Only dogs that we received DNA samples from are indicated with letters. Diamond symbols indicate the number of animals from the litter that we have no information or DNA samples. (Bottom) Fundus images from a phenotypically normal (SWD IIC, ~6-y-old) and an affected (SWD IIE, ~4.5-y-old) SWD. Note the severe vascular attenuation and tapetal hyperreflectivity in the affected dog fundus

in the functional domain of the protein responsible for hydrolyzing cyclic guanosine monophosphate (cGMP) in the rod phototransduction cascade. This mutation segregates with the PRA phenotype within the affected family.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Blood or cheek swabs were collected and donated with owner consent for DNA isolation. DNA isolated from blood was used for whole genome sequencing (WGS) from the dam (SWD IB), two affected siblings (SWD IIE, IIF), and a phenotypically normal sibling (SWD IIC) (Figure 1). Author SPJ performed eye examinations on three of the four dogs chosen for WGS (SWD IB, IIC, IIE). Fundus images were collected from an affected ~4.5-year-old dog (SWD IIE) and a phenotypically normal ~6-year-old dog (SWD IIC) using a RetCam II Video Fundus Camera (Clarity Medical Systems; Figure 1).

All procedures were in compliance with the Association for Research in Vision and Ophthalmology (ARVO) statement for the Use of Animals in Ophthalmic and Vision Research and approved by the Michigan State University Institutional Animal Care and Use Committee (AUF number 05/14-090-00).

2.2 | Progressive retinal atrophy panel and exclusion analysis

A previously developed panel of microsatellite markers flanking 45 PRA candidate genes was used to initially screen three affected SWDs for an association between the markers and the phenotype.[5] Genes were excluded if (a) one affected dog was heterozygous for both markers and/or (b) more than one affected dog was heterozygous for one marker. Allele sizes had to be more than one repeat size different to be considered separate alleles, (see[5-7] for detailed descriptions of exclusion analysis). After the first round of exclusions, the remaining unexcluded genes were screened with two additional affected SWDs and three phenotypically normal SWDs (dam, sire, and sibling).

2.3 | Whole genome sequencing

DNA from two affected siblings, a phenotypically normal sibling and dam (obligate carrier) were sequenced to ~30X coverage (Novogene Co. Ltd) using the Illumina Novaseq6000 platform on paired-end 150 bp reads prepared with the NEB Next UltraII DNA Library Prep Kit.

Raw reads from the SWD samples were trimmed using TrimGalore (https://github.com/FelixKrueger/TrimGalore) and aligned to the CanFam3.1 reference genome with HiSat2.[8] Variations between the samples and the reference genome were called by FreeBayes.[9] Raw data from all 4 SWDs are available from the NCBI Sequence Read Archive (SRA) (BioProject:PRJNA630105, accession numbers: SAMN14824620, SAMN14824621, SAMN14824622, SAMN14824623).

2.4 | Variant analysis

Variant files (vcfs) were analyzed using Golden Helix VarSeq software (Golden Helix). Variants that were unique and homozygous in the affected SWDs were identified when compared to vcfs from 64 dogs from various breeds (including the two phenotypically normal SWDs sequenced in this study). Specifically, the VarSeq filters

were set to: SWD IIE and IIF must be homozygous for the variant, SWD IB must be heterozygous for the variant, SWD IIC must be heterozygous or reference for variant, all other dogs must be reference and the variant must be in an exon of a known PRA candidate gene (Table S1). Variant lists containing variants outside of exons in PRA candidate genes and variants that are homozygous in the affected SWDs and not homozygous in any control dogs can be viewed in Tables S2 and S3.

2.5 | Sanger sequencing

The variant identified in exon 19 of *PDE6B* (CanFam3.1 accession# GCA_000002285.2; chr3:91,749,865-91,749,870) was PCR amplified (forward primer –5′-CTCGTGTCACATGACCAACC-3′, reverse primer – 5′-ATTTCTGACCAAGCGCTGAC-3′) and confirmed via Sanger sequencing.

2.6 | Genotyping assay

A genotyping assay was developed to rapidly screen future SWDs for the presence of the mutation. Primers were designed around the mutation to amplify a small product size (139 bp, forward primer –5′-CTCACAATAGGCTGCTGATCC-3′ and reverse primer – 5′-ATTTCTGACCAAGCGCTGAC-3′) to visualize directly on agarose gel. The 6-bp deletion can be appreciated with a 4% agarose gel run at 145 volts for 2.5 hours in chilled running buffer (Figure S1).

3 | RESULTS

3.1 | Pedigree information and disease phenotype

DNA samples were collected from a family of SWDs in which the early-onset non-*PRCD* form of PRA was segregating (Figure 1). The dam (SWD IB) and one of the offspring (SWD IIC) were examined by SPJ and were phenotypically normal (~8 and ~6 years of age, respectively). The sire had a clear eye certification examination at ~ 2.5 years of age. An autosomal recessive mode of inheritance was assumed due to the normal phenotype in the parents of the affected dogs. The age at ophthalmoscopic diagnosis by a boarded veterinary ophthalmologist ranged from ~1.5-4.5 years old although owners reported noticing changes in their dogs' vision before the official diagnoses. SWD puppies homozygous for the mutation were not available for functional testing to assess whether the *PDE6B* mutation identified completely ablated rod function. Fundus images from an affected ~4.5-year-old dog (SWD IIE) showed advanced retinal degeneration typical of PRA, including vascular attenuation and tapetal hyperreflectivity (Figure 1).

3.2 | Progressive retinal atrophy panel

A marker panel consisting of 90 microsatellites flanking 45 PRA candidate genes was utilized to rapidly screen these genes for association with PRA in the affected SWDs.[5] Initially, three affected dogs were genotyped in the panel and 39/45 genes were excluded (data not shown). A gene was excluded if there was at least 1 dog genotyped with an allele size difference greater than ±1 repeat in at least 1 of the markers. The six unexcluded genes were as follows: *C2orf71*, *PDE6B*, *RPE65*, *SPATA7*, *TTC8,* and *USH2A*.

The six unexcluded genes were then screened in two additional affected SWDs and three phenotypically normal SWDs. Only two candidate genes could not be excluded that showed reasonable variability in the control SWDs; *PDE6B;* and *USH2A*.

3.3 | Identification of candidate variants on whole genome sequencing

Two affected siblings, a phenotypically normal sibling and the dam were sequenced to an average of ~30X coverage. Over 5 million variants per dog were called. After applying the filters via Golden Helix VarSeq (see Materials and Methods), only 1 variant fit the criteria: a 6-bp deletion in exon 19 of *PDE6B* (CanFam3.1; chr3:91,749,865-91,749,870; GCA_000002285.2:c.2218-2223del; p.Phe740_Phe741del). This in-frame mutation deletes two highly conserved amino acids within the catalytic domain of PDE6B (Figure S2). The effect of the mutation is predicted to be deleterious (Provean score −21.4; deleterious equals any number below −2.5).[10] Sanger sequencing confirmed the genotypes in the four samples used for WGS and was then used to screen 14 additional SWDs. All affected dogs were homozygous for the mutation, the dam, and sire were heterozygous (as expected for obligate carriers), and no phenotypically normal dogs were homozygous for the mutation (N = 9). There were no variants in *USH2A* that fit the filtering criteria.

4 | DISCUSSION

There is a history of PRA in SWDs. They are one of the >60 breeds of dogs that carry the mutation in *PRCD* resulting in a late-onset PRA within the breed.[4,11,12] The genetic test for *PRCD* has been available to the SWD breeders for over 10 years, and it has been utilized to aid in breeding programs to remove the mutation from the SWD population (swdclub.org).

Recently, a new form of PRA, independent of the *PRCD*-PRA, was identified in a family group of SWDs. The same breeding pair was bred three times resulting in 26 offspring, at least seven of which were diagnosed with PRA. A 6-bp deletion in exon 19 of *PDE6B* was identified that segregates with the recessively inherited newly identified form of PRA in SWDs.

There are three other breeds of dogs that have PRA as a result of a mutation in *PDE6B*. Interestingly, the mutations in the three other breeds are all in exon 21. Irish Setter dogs have a nonsense mutation (c.2420G > A, p.Trp807Ter) which results in loss of rod and many cone photoreceptors by 1 year of age.[13,14] Sloughi dogs have an 8-bp insertion resulting in a frameshift and a premature stop codon (c.2449_2456insTGAAGTCC, p.Lys816Terfs817). There are limited available details of the resulting phenotype, but it is described as a severe early-onset PRA.[15] American Staffordshire Terriers have an in-frame deletion resulting in loss of 1 amino acid (c.2404-2406del, p.Asn802del). The resulting phenotype has a slower onset than that of the Irish Setter, but significant loss of photoreceptors is evident by 20 months of age.[16]

PDE6B is one of two active subunits of the PDE6 holoenzyme complex which is an essential component of the rod phototransduction cascade and both PDE6A and PDE6B subunits are needed to form the active phosphodiesterase. The PDE6 holoenzyme is activated by the G-protein, transducin, by cleaving of the two inhibitory gamma subunits. The activated PDE6 hydrolyzes cGMP. The reduction in cGMP levels leads to closure of cGMP-gated channels in the rod outer segment membrane and hyperpolarization of the cell.[17] Loss of normal function of PDE6 in the retinal degeneration 1 (*rd1*) mouse and the Irish Setter dog results in accumulation of cGMP which is hypothesized to lead to opening of larger proportions of the cGMP-gated channels than normal allowing an influx of cations into the rod cell affecting mitochondria and triggering apoptosis. The resulting death of rod photoreceptors leads to secondary loss of the cones which cannot survive without a population of surrounding rods.[18-21] *PDE6B* mutations account for 4%-5% of autosomal recessive retinitis pigmentosa (RP) in humans which is the commonest phenotype for *PDE6B* mutations, although a subset of mutations can cause an autosomal dominant congenital stationary night blindness.[22,23] Two naturally occurring *Pde6b* mouse models (*rd1* and *rd10*) exist that have been used extensively to understand the role of PDE6B in photoreceptors and the retina. The *rd1* mice have a nonsense mutation (p.Tyr347Ter) that results in a severe, early-onset retinal degeneration in which the photoreceptors degenerate before they are fully developed.[24] The *rd10* mice have a missense mutation (p.Arg560Cys) with a comparatively slower disease progression.[25] A genotype-phenotype study was conducted using mutagenized heterozygous *rd1* mice to characterize the effect of compound heterozygous mutations in *Pde6b*. Seven mutations were identified, 4 had similar phenotypes to *rd1* mice (mutations predicted to result in protein loss of function) and 3 had milder phenotypes compared to *rd1* mice (two missense mutations and a splice site mutation).[26]

Tissue is not available from the PRA-affected pet SWDs to allow us to perform immunohistochemistry or Western blot to see if the mutated protein is unstable but we predict that the mutation will have a marked effect on the normal enzymatic function. The deleted amino acids (p.Phe740_Trp741del) in SWDs are within the highly conserved functional PDE domain of PDE6B (and other PDE6 proteins) (Figure S2). Phe742 in the other PDE6 active subunit (PDE6A) (equivalent to Phe740 in PDE6B) is in the catalytic pocket of the enzyme and has hydrophobic interactions with cGMP.[27] Given the deletion of the two conserved amino acids in the functional domain, the loss of Phe740 which is homologous to Phe742 in PDE6A and the established role of mutations in *PDE6B* in PRA and RP, it is highly likely that this 6-bp deletion is deleterious and is the causative mutation underlying the newly identified early-onset form of PRA in SWDs. The SWD is one of a growing number of dog breeds in which more than one form of PRA has been identified.[4]

The identification of this novel mutation in *PDE6B* which segregates with early-onset PRA in SWDs will allow for a DNA-based genotyping assay for breeders to use in an effort to eradicate PRA from the breed.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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