HHS Public Access

Author manuscript

Dobutsu Iden Ikushu Kenkyu. Author manuscript; available in PMC 2015 June 25.

Published in final edited form as:

Dobutsu Iden Ikushu Kenkyu. 2014 ; 42(2): 79–89. doi:10.5924/abgri.42.79.

Inherited retinal diseases in dogs: advances in gene/mutation discovery

Keiko Miyadera

School of Veterinary Medicine, University of Pennsylvania, 3900 Delancey St, Philadelphia PA 19104 USA

Abstract

1. Inherited retinal diseases (RDs) are vision-threatening conditions affecting humans as well as many domestic animals. Through many years of clinical studies of the domestic dog population, a wide array of RDs has been phenotypically characterized. Extensive effort to map the causative gene and to identify the underlying mutation followed. Through candidate gene, linkage analysis, genome-wide association studies, and more recently, by means of next-generation sequencing, as many as 31 mutations in 24 genes have been identified as the underlying cause for canine RDs. Most of these genes have been associated with human RDs providing opportunities to study their roles in the disease pathogenesis and in normal visual function. The canine model has also contributed in developing new treatments such as gene therapy which has been clinically applied to human patients. Meanwhile, with increasing knowledge of the molecular architecture of RDs in different subpopulations of dogs, the conventional understanding of RDs as a simple monogenic disease is beginning to change. Emerging evidence of modifiers that alters the disease outcome is complicating the interpretation of DNA tests. In this review, advances in the gene/mutation discovery approaches and the emerging genetic complexity of canine RDs are discussed.

1. Introduction

Inherited retinal diseases (RDs) in humans comprise a spectrum of clinically heterogeneous vision-threatening conditions such as retinitis pigmentosa (RP), cone-rod dystrophy (CRD) and Leber's congenital amaurosis (LCA). Since the mapping of the *RHO* (rhodopsin) gene in a form of RP (Farrar *et al.* 1990), 221 genes have been associated with human RDs (RetNet; www.sph.uth.tmc.edu/RetNet/), and the number of genes continues to grow. Animal models have been indispensable in validating the physiologic and pathologic mechanisms of these genes, most extensively, as genetically modified mice. To a lesser degree but with no less significance, dogs also have received much attention over the years as a unique, naturally-occurring model of RDs. In this review, I will discuss the current knowledge of the molecular basis of canine RDs and advances in the gene/mutation discovery approach.

Correspondence: Keiko Miyadera, School of Veterinary Medicine, University of Pennsylvania, 3900 Delancey St, Philadelphia PA 19104 USA, kmiya@vet.upenn.edu.

2. Phenotypic characteristics of canine RDs

The most commonly diagnosed canine RD is progressive retinal atrophy (PRA) which is considered homologous to RP in humans. Dogs affected with PRA initially present night blindness, eventually progressing into total vision loss. While PRA has been documented in numerous canine breeds, it was once thought to be a single disease caused by the same mutation across breeds. However, breed-specific clinical phenotype (e.g. age of onset, rate of progression) together with the evidence from diligent mating trials indicated that multiple independent forms of PRAs exist, and that each form is specific to certain breed(s). Inherited canine RDs may be classified as “progressive” that undergo programmed degenerative changes during life, “stationary” that are congenital functional abnormalities where the functional defect of the retina, often caused by a total absence of a functional retina protein is present at birth and do not change throughout life, or “developmental” that are (Table 1). Most canine RDs are autosomal recessive, while autosomal dominant (Kijas *et al.* 2002) and X-linked PRAs have been identified (Acland *et al.* 1994; Zhang *et al.* 2002). Notably, all canine RDs molecularly characterized to date have been reported as a monogenic trait (i.e. caused by a mutation in a single gene), following a Mendelian inheritance.

2.1. Progressive disorders

PRA and CRD are progressive RDs, primarily affecting the photoreceptors. PRA is characterized by initial degeneration of rod photoreceptors causing night blindness, progressing to total blindness. Affected dogs with visual deficits have characteristic fundus changes starting from tapetal hyperreflectivity, attenuation of retinal blood vessels, then pale optic disc. CRD is less common, and predominantly affects cone photoreceptors while rods are affected later or to a lesser degree. Early clinical signs therefore can be day vision problems. However, diagnosis is often made when there is already extensive day and light visual deficit with end-stage fundic changes similar to PRA. As such, CRD may be indistinguishable from PRA at the clinical level, thus often being referred to as PRA.

While dogs affected with PRA/CRD show similar fundic changes at the end-stage of the disease, the age of onset and the rate of progression may vary, depending on the underlying gene/mutation, thereby depending on the breed. Early-onset PRA/CRD results from abnormal retinal development or progressive degeneration starting during or soon after retinogenesis. This is followed by a rapid progression toward end-stage retinal degeneration, and can be clinically evident in young adult dogs. Late-onset PRA/CRD is characterized by pathological changes after normal development of the retina. As such, they tend to represent defects in pathways critical for the long-term maintenance of normal photoreceptors. Visual deficits may not become apparent until well after reproductive maturity, and further progression is typically much slower than in early-onset PRA/CRD, and may not become clinically evident until later in life.

2.2. Stationary disorders

Certain forms of RD show no or very limited progression. Examples of such stationary disorders are achromatopsia (cone degeneration) (Sidjanin *et al.* 2002) and canine LCA

(cLCA), previously known as congenital stationary night blindness (CSNB) (Narfström *et al.* 1989).

2.3. Developmental disorders

Retinal dysplasia is a developmental disorder characterized by a defect in retinal differentiation. Syndromic retinal dysplasia in Labrador Retrievers and Samoyeds are oculoskeletal disorders that model autosomal recessive Stickler syndrome in humans (Goldstein *et al.* 2010a). Another developmental disorder is Collie eye anomaly (CEA) variably affecting the retina-choroid-scleral complex ranging from choroidal hypoplasia, scleral coloboma to retinal detachment. CEA are considered homologous to macular coloboma in humans. Due to high worldwide prevalence of CEA in Collies (70–90%), it is the most common inherited canine RDs, yet impairment of vision is uncommon (Barnett and Stades 1979; Roberts 1969).

3. Genes associated with RDs in humans and dogs

The phototransduction cascade and the retinoid cycle are the two critical functional pathways involved in vision. Mutations in genes participating in these pathways may be associated with RDs, and many of the protein products are localized to the outer retina where these pathways take place. More recently, genes associated with the morphology and function of the photoreceptor connecting cilia have been associated with RDs, and their roles in normal and diseased retina have been a major focus of study (Estrada-Cuzcano *et al.* 2012). Of the ~23 genes associated with canine RDs to date, at least 6 genes have implications of a primary role in cilia trafficking. The complex gene-gene interaction associated with cilia trafficking remains to be fully unraveled.

4. Advances in canine RD gene/mutation discovery

The gene/mutation discovery approaches for inherited canine traits have seen considerable development over the past 20 years. Initial attempts were focused on candidate genes, followed by the introduction of linkage mapping, positional cloning strategies, and microarray-based genome-wide association study (GWAS). More recently, next-generation sequencing (NGS) has been implemented with increasing availability and affordability. In dogs, due to the “breed barrier” which prevents admixture of different breeds to establish and maintain distinct breeds, the genetic makeup within the same breed is typically uniform where many sequence variants are ‘fixed’. Such within-breed genetic uniformity has been both an advantage and a disadvantage in investigating canine genetic traits (Miyadera *et al.* 2012a).

4.1. Candidate gene analysis

Candidate genes are selected based on existing knowledge of the biochemistry or associated diseases, or on information from other species such as humans and mice. Early studies have largely relied on a candidate gene approach largely because of the lack of alternative canine genomic tools.

The first canine RD mutation was found by screening a candidate gene *PDE6B* for rcd1 in Irish Setters (Clements *et al.* 1993; Suber *et al.* 1993), based on phenotypic and biochemical similarity to the *rd* mice with a *PDE6B* mutation (Bowes *et al.* 1990; Farber and Lolley 1974). Candidate gene approaches have since identified mutations for rcd1*a* in Sloughi dogs (*PDE6B*) (Dekomien *et al.* 2000), for rcd3 in Cardigan Welsh Corgis (*PDE6A*) (Petersen-Jones *et al.* 1999), and ADPRA in Bullmastiffs and English Mastiffs (*RHO*) (Kijas *et al.* 2002). However, while successes are often highlighted, there have been too many failed candidate gene screening attempts mostly unpublished, questioning its efficacy as the primary approach for mutation discovery in canine RDs (Aguirre-Hernandez and Sargan 2005). Further, while there are hundreds of genes so far associated with human RDs, screening for all the candidate genes has become unrealistic. Still, phenotype-based selection of *BEST1* in CMR1, CMR2, and CMR3 has proven to be a success (Guziewicz *et al.* 2007; Zangerl *et al.* 2010) guided by the high clinical and pathologic resemblances to Best macular dystrophy in humans.

4.2. Whole-genome scan

Unlike candidate gene studies which limit the inspection to certain genes of interest, a whole genome scan interrogates the entire genome necessitating no prior assumption or knowledge of the target locus. The current tools of gene/mutation discovery are based on the 7.5X sequence of the Boxer dog (Lindblad-Toh *et al.* 2005), the earlier 1.5X Poodle genome sequence (Kirkness *et al.* 2003), as well as single nucleotide polymorphisms (SNPs) information across multiple breeds.

Linkage mapping and positional cloning strategies—A linkage analysis (LA) mapping utilizes informative families containing affected and non-affected dogs. Co-segregation of genetic markers with the phenotype is analyzed to identify recombination, or lack thereof. Subsequently, the region of interest may be further narrowed by fine-mapping, followed by positional candidate gene analysis.

Genome-wide association studies (GWAS)—With the development of microarray-based DNA chips, high-throughput SNP analysis has become feasible in both humans and domestic animals (Andersson 2009). In a GWAS approach, SNP markers pre-determined across the genome are used to assess association with the disease. For canine traits, the 170K canine SNP chip (Illumina) is the currently most commonly used platform. It has been postulated that 20 cases and 20 controls, and 50 cases and 50 controls should be sufficient for mapping autosomal recessive and dominant traits, respectively using 10,000–30,000 SNPs chips (Lindblad-Toh *et al.* 2005), due to the conveniently long linkage disequilibrium within a given breed (0.5–5Mb) (Gray *et al.* 2009; Sutter *et al.* 2004). Since the first report of CRD in Standard Wirehaired Dachshunds (Wiik *et al.* 2008), at least 10 forms of canine RDs have been mapped by GWAS (Table 1). These studies typically map a relatively large chromosomal region, followed by positional candidate gene screening with/without fine mapping.

4.3. Next-generation sequencing

More recently, the next-generation sequencing (NGS) technique has been applied to canine RD studies aiding in mapping, fine mapping and functional analysis. NGS is based on a massively-parallel, high-throughput sequencing which produces millions of short sequences to be analyzed by extensive bioinformatics. A targeted-NGS approach is used where a genetic locus has been mapped by a whole genome scan. The genomic area of interest is then captured using custom-designed probes, and the enriched DNA is subjected to NGS. GWAS-guided fine mapping by targeted-NGS has so far identified mutations associated with GR_PRA2 in Golden Retrievers (Downs *et al.* 2014) and PRA3 in Tibetan Spaniels/ Terriers (Downs & Mellersh 2014). Exome-sequencing is a form of targeted-NGS which captures only the predetermined known canine exon regions for NGS. Ahonen *et al.* (2013) used exome-sequencing as a parallel approach to identify the *CNGB1* mutation associated with Papillon PRA. Other NGS applications include RNA-seq and whole-genome sequencing, each of which has already proven useful in mapping and mutation screening of non-ocular diseases in dogs (Forman *et al.* 2012; Drogemuller *et al.* 2013; Guo *et al.* 2014). RNA-seq uses tissue-specific RNA as the template and provides a complete snapshot of transcripts expressed in the tissue of interest, allowing assessment of the level of expression (quantitative) as well as structural and sequence changes (qualitative).

5. Emerging complexity of canine RDs

5.1. Multiple forms of RD per breed

After a DNA test for certain RD has become available, some of the target breed populations have been found to contain dogs that are tested as genetically tested “clear” but are in fact clinically affected with RD (Table 2). This indicates the presence of alternative form(s) of RD independent of the already known RD. Such genetic heterogeneity has been a dilemma for the DNA test providers as well as the users alike, and those dogs with discordant DNA test results become subjects for further investigation to identify the missing RD gene/ mutation for the breed.

Early-onset PRA in Portuguese Water Dogs (PWD)—PWDs are one of many breeds affected with PRCD-PRA. PRCD in this breed is a late-onset form of PRA typically presenting with night blindness at mid age gradually progressing into total blindness. The author and colleagues have recently identified a new form of PRA in PWDs that are not associated with PRCD. It presents as an early-onset PRA where night blindness is recognized at 1–2 years of age followed by rapid progression to total blindness. Affected cases and controls have been identified for a GWAS.

5.3. Modifiers

While RDs have largely been considered as simple monogenic traits, there is increasing evidence to suggest that more than one gene mutation could be affecting the phenotypic outcome. This is not surprising considering the number of genes (>220) associated with human RDs to date. Genetic polymorphisms in any of these genes albeit non-pathogenic on their own, could potentially function as modifiers altering the effect of a primary mutation. PRCD-PRA affecting a variety of dog breeds show variable yet breed-specific ages of onset.

This is thought to be largely attributed to breed-specific genetic backgrounds that are collections of genetic polymorphisms across the genome. Modifier genes may or may not be retina-specific, and could be genes interacting with any retinal gene, further inflating the number of potential modifier genes.

The author has previously demonstrated the presence of a genetic modifier in cone-rod dystrophy 1 (cord1) in Miniature Longhaired Dachshunds (MLHDs). The condition was first described as an autosomal recessive CRD with an early-onset (age <1 year) in a MLHD research colony in the UK (Curtis & Barnett 1993). This disease was found to segregate completely with a mutation in *RPGRIP1*, at least within this closed research colony (Mellersh *et al.* 2006). However, when Japanese pet MLHDs were screened, the age of onset was much broader (4 months - 15 years, mean ± SD: 4.6 ± 3.4 years) compared to the UK colony (Miyadera *et al.* 2009). Furthermore, the Japanese pet MLHDs showed significant phenotype-genotype discordance; 20% of blind cases were not homozygous for the mutation, while 16% of the apparently normal dogs were homozygous affected genetically. To account for this disparity, a GWAS was carried out using 80 *RPGRIP1*$^{-/-}$ (homozygous mutant) dogs differing by the clinical phenotypes; this led to the identification of an independently segregating homozygous modifier locus on chromosome 15 (Miyadera *et al.* 2012b). The UK research colony turned out to be ‘fixed’ for this modifier locus, thus being masked in the initial mapping study by Mellersh *et al.* (2006). The gene/mutation underlying this modifier and its interaction with RPGRIP1 is current our focus of study.

7. DNA tests

DNA tests for most canine RDs whose underlying mutation is known are available commercially (Mellersh 2012). Unlike in the human population with a huge amount of genetic heterogeneity requiring screening of an extensive panel of genes and mutations (Stone 2007; Zernant *et al.* 2005), in dogs, testing of only the known mutation(s) associated with the respective breed is often sufficient. Notably, a mix dog produced from different canine breeds which happen to harbor the same RD mutation can be affected with that RD such as PRCD-PRA seen among the increasingly popular ‘designer dogs’ (e.g. Labradoodles, Goldendoodles). Meanwhile, new breeds affected with the existing forms of RDs are found each year and added to the list.

The advantage of DNA tests is its ability to detect unaffected carriers and subclinical cases prior to disease onset. It is important to note that other unidentified forms of RDs will not be screened with the existing DNA tests. In many situations, following identification of a mutation, coordinated effort is implemented by the breeding community leading to a substantial decrease in the disease and mutant allele frequencies in the breed. Meanwhile, other non-RD diseases/traits should also be taken into account when considering breeding strategies to assure the overall health of the breed while nurturing desirable traits. This is important as the selective pressure by DNA tests can significantly reduce the genetic diversity when the mutation frequency is high in the breed. In the author’s opinion, the aim of DNA tests are not to eliminate certain dogs from breeding, but to safely include them by identifying genetically ‘clear’ dogs that can be bred to. Where maintaining the genetic

diversity of the breed population is a concern, otherwise desirable dogs may potentially be used for breeding as long as a DNA-test is performed and breeding is planned accordingly.

8. Prospects

While many successful mapping studies have benefited from purpose-bred research colonies, cases and controls recruited from the general pet population have contributed significantly to the effort. Still, research colonies remain essential in providing adequate resources for functional studies and developing new therapies. Identification of genes/mutations underlying canine RDs have provided excellent testing grounds for gene replacement therapy, one of the approaches under active development to prevent, stabilize, or reverse the retinal degenerative process in humans and animals.

cLCA in Briards with an *RPE65* mutation has been treated successfully with subretinal injection of a recombinant adeno-associated virus 2 carrying wild-type *RPE65* (Acland *et al.* 2001; Narfström *et al.* 2003). The single injection was safe, effective and stable with functional improvement up to four years (Acland *et al.* 2005; Narfstrom *et al.* 2008). The treatment was subsequently applied to human LCA patients carrying *RPE65* mutations leading to significant restorations of vision (Bainbridge *et al.* 2008; Hauswirth *et al.* 2008; Maguire *et al.* 2008). Successful gene therapy using the dog model has also been achieved in achromatopsia (Komaromy *et al.* 2010) and CMR (Guziewicz, personal communication).

9. Conclusion

Since the first identification of the *PDE6B* mutation in 1993, significant progress has been made in the molecular study of canine RDs thanks to the development of genetic resources and tools. Phenotypic and genetic similarities between canine and human RDs have provided an excellent opportunity to study the molecular mechanisms across species. Molecular characterization of canine RDs is likely to become even faster and affordable over the coming years, with increasing feasibility of the NGS approach. Due to the history of breed development, the genomic background within each dog breed is relatively uniform compared to the extremely heterogeneous human population as a whole. Identification of primary mutations and manageable number of minor alleles or modifiers should therefore be more advantageous using the canine population, and will continue to contribute in the understanding of RDs in dogs and in humans.

Acknowledgments

I wish to thank Dr. Gustavo Aguirre, University of Pennsylvania for valuable discussions. The author is supported by the Career Development Award for Veterinary Residents from the Foundation Fighting Blindness.

References

Acland GM, Aguirre GD, Bennett J, Aleman TS, Cideciyan AV, Bennicelli J, Dejneka NS, Pearce-Kelling SE, Maguire AM, Palczewski K, Hauswirth WW, Jacobson SG. Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness. Molecular Therapy. 2005; 12:1072–1082. [PubMed: 16226919]

Acland GM, Aguirre GD, Ray J, Zhang Q, Aleman TS, Cideciyan AV, Pearce-Kelling SE, Anand V, Zeng Y, Maguire AM, Jacobson SG, Hauswirth WW, Bennett J. Gene therapy restores vision in a canine model of childhood blindness. Nature Genetics. 2001; 28:92–95. [PubMed: 11326284]

Acland GM, Blanton SH, Hershfield B, Aguirre GD. XLPRA: a canine retinal degeneration inherited as an X-linked trait. American Journal Medical Genetics. 1994; 52:27–33.

Aguirre-Hernandez J, Sargan DR. Evaluation of candidate genes in the absence of positional information: a poor bet on a blind dog! Journal of Heredity. 2005; 96:475–484. [PubMed: 16135711]

Aguirre GD, Baldwin V, Pearce-Kelling S, Narfstrom K, Ray K, Acland GM. Congenital stationary night blindness in the dog: common mutation in the RPE65 gene indicates founder effect. Molecular Vision. 1998; 4:23–29. [PubMed: 9808841]

Ahonen SJ, Arumilli M, Lohi H. A CNGB1 frameshift mutation in Papillon and Phalène dogs with progressive retinal atrophy. PLoS One. 2013 Aug 28.8(8):e72122. [PubMed: 24015210]

Andersson L. Genome-wide association analysis in domestic animals.: a powerful approach for genetic dissection of trait loci. Genetica. 2009; 136:341–349. [PubMed: 18704695]

Bainbridge JW, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, Viswanathan A, Holder GE, Stockman A, Tyler N, Petersen-Jones S, Bhattacharya SS, Thrasher AJ, Fitzke FW, Carter BJ, Rubin GS, Moore AT, Ali RR. Effect of gene therapy on visual function in Leber's congenital amaurosis. New England Journal of Medicine. 2008; 358:2231–2239. [PubMed: 18441371]

Barnett K, Stades F. Collie eye anomaly in the Shetland sheepdog in the Netherlands. Journal of Small Animal Practice. 1979; 20:321–329. [PubMed: 120471]

Bowes C, Li T, Danciger M, Baxter LC, Applebury ML, Farber DB. Retinal degeneration in the rd mouse Is caused by a defect in the β subunit of rod cGMP-phosphodiesterase. Nature. 1990; 347:677–680. [PubMed: 1977087]

Clements PJM, Gregory CY, Peterson-Jones SM, Sargan DR, Bhattacharya SS. Confirmation of the rod cGMP phosphodiesterase β subunit (PDEβ) nonsense mutation in affected rcd-1 Irish setters in the UK and development of a diagnostic test. Current Eye Research. 1993; 12:861–866. [PubMed: 8261797]

Curtis R, Barnett KC. Progressive retinal atrophy in miniature longhaired dachshund dogs. British Veterinary Journal. 1993; 149:71–85. [PubMed: 8439801]

Dekomien G, Runte M, Godde R, Epplen JT. Generalized progressive retinal atrophy of Sloughi dogs is due to an 8-bp insertion in exon 21 of the PDE6B gene. Cytogenetics Cell Genetics. 2000; 90:261–267. [PubMed: 11124530]

Dekomien G, Vollrath C, Petrasch-Parwez E, Boeve MH, Akkad DA, Gerding WM, Epplen JT. Progressive retinal atrophy in Schapendoes dogs: mutation of the newly identified CCDC66 gene. Neurogenetics. 2010; 11:163–174. [PubMed: 19777273]

Downs LM, Wallin-Hakansson B, Boursnell M, Marklund S, Hedhammar A, Truve K, Hubinette L, Lindblad-Toh K, Bergstrom T, Mellersh CS. A Frameshift Mutation in Golden Retriever Dogs with Progressive Retinal Atrophy Endorses SLC4A3 as a Candidate Gene for Human Retinal Degenerations. PLoS One. 2011; 6:e21452. [PubMed: 21738669]

Downs LM, Bell JS, Freeman J, Hartley C, Hayward LJ, Mellersh CS. Late-onset progressive retinal atrophy in the Gordon and Irish Setter breeds is associated with a frameshift mutation in C2orf71. Animal Genetics. 2013; 44(2):169–77. [PubMed: 22686255]

Downs LM, Wallin-Håkansson B, Bergström T, Mellersh CS. A novel mutation in TTC8 is associated with progressive retinal atrophy in the golden retriever. Canine Genetics and Epidemiology. 2014; 1:4.

Downs LM, Mellersh CS. An Intronic SINE Insertion in FAM161A that Causes Exon-Skipping Is Associated with Progressive Retinal Atrophy in Tibetan Spaniels and Tibetan Terriers. PLoS One. 2014; 9:4, e93990.

Drögemüller M, Jagannathan V, Howard J, Bruggmann R, Drögemüller C, Ruetten M, Leeb T, Kook PH. A frameshift mutation in the cubilin gene (CUBN) in Beagles with Imerslund-Gräsbeck syndrome (selective cobalamin malabsorption). Animal Genetics. 2014; 45(1):148–50. [PubMed: 24164695]

Estrada-Cuzcano A, Roepman R, Cremers FP, den Hollander AI, Mans DA. Non-syndromic retinal ciliopathies: translating gene discovery into therapy. Human Molecular Genetics. 2012; 21(R1):R111–24. [PubMed: 22843501]

Farber D, Lolley R. Cyclic guanosine monophosphate: elevation in degenerating photoreceptor cells of the C3H mouse retina. Science. 1974; 186:449–451. [PubMed: 4369896]

Farrar GJ, McWilliam P, Bradley DG, Kenna P, Lawler M, Sharp EM, Humphries MM, Eiberg H, Conneally PM, Trofatter JA, et al. Autosomal dominant retinitis pigmentosa: linkage to rhodopsin and evidence for genetic heterogeneity. Genomics. 1990; 8:35–40. [PubMed: 2081598]

Forman OP, De Risio L, Stewart J, Mellersh CS, Beltran E. Genome-wide mRNA sequencing of a single canine cerebellar cortical degeneration case leads to the identification of a disease associated SPTBN2 mutation. BMC Genetics. 2012; 13:55.10.1186/1471-2156-13-55 [PubMed: 22781464]

Goldstein O, Guyon R, Kukekova A, Kuznetsova TN, Pearce-Kelling SE, Johnson J, Aguirre GD, Acland GM. COL9A2 and COL9A3 mutations in canine autosomal recessive oculoskeletal dysplasia. Mammalian Genome. 2010a; 21:398–408. [PubMed: 20686772]

Goldstein O, Kukekova AV, Aguirre GD, Acland GM. Exonic SINE insertion in STK38L causes canine early retinal degeneration (erd). Genomics. 2010b; 96:362–368. [PubMed: 20887780]

Goldstein O, Mezey JG, Boyko AR, Gao C, Wang W, Bustamante CD, Anguish LJ, Jordan JA, Pearce-Kelling SE, Aguirre GD, Acland GM. An ADAM9 mutation in canine cone-rod dystrophy 3 establishes homology with human cone-rod dystrophy 9. Molecular Vision. 2010c; 16:1549–1569. [PubMed: 20806078]

Goldstein O, Jordan JA, Aguirre GD, Acland GM. A non-stop S-antigen gene mutation is associated with late onset hereditary retinal degeneration in dogs. Molecular Vision. 2013a; 19:1871–1884. [PubMed: 24019744]

Goldstein O, Mezey JG, Schweitzer PA, Boyko AR, Gao C, Bustamante CD, Jordan JA, Aguirre GD, Acland GM. IQCB1 and PDE6B mutations cause similar early onset retinal degenerations in two closely related terrier dog breeds. Investigative Ophthalmology & Visual Science. 2013b; 54(10): 7005–19. [PubMed: 24045995]

Gray MM, Granka JM, Bustamante CD, Sutter NB, Boyko AR, Zhu L, Ostrander EA, Wayne RK. Linkage disequilibrium and demographic history of wild and domestic canids. Genetics. 2009; 181:1493–1505. [PubMed: 19189949]

Guo J, Johnson GS, Brown HA, Provencher ML, Ronaldo C, Mhlanga-mutangadura T, Taylor JF, Schnabel RD, O'Brien DP, Katz ML. A CLN8 nonsense mutation in the whole genome sequence of a mixed breed dog with neuronal ceroid lipofuscinosis and Australian Shepherd ancestry. Molecular Genetics and Metabolism. 2014; 112:302–309. [PubMed: 24953404]

Guziewicz KE, Zangerl B, Lindauer SJ, Mullins RF, Sandmeyer LS, Grahn BH, Stone EM, Acland GM, Aguirre GD. Bestrophin gene mutations cause canine multifocal retinopathy: a novel animal model for best disease. Investigative Ophthalmology & Visual Science. 2007; 48:1959–1967. [PubMed: 17460247]

Hauswirth WW, Aleman TS, Kaushal S, Cideciyan AV, Schwartz SB, Wang L, Conlon TJ, Boye SL, Flotte TR, Byrne BJ, Jacobson SG. Treatment of leber congenital amaurosis due to RPE65 mutations by ocular subretinal injection of adeno-associated virus gene vector: short-term results of a phase I trial. Human Gene Therapy. 2008; 19:979–990. [PubMed: 18774912]

Kijas JW, Cideciyan AV, Aleman TS, Pianta MJ, Pearce-Kelling SE, Miller BJ, Jacobson SG, Aguirre GD, Acland GM. Naturally occurring rhodopsin mutation in the dog causes retinal dysfunction and degeneration mimicking human dominant retinitis pigmentosa. Proceedings National Acadademy of Sciences, USA. 2002; 99:6328–6333.

Kirkness EF, Bafna V, Halpern AL, Levy S, Remington K, Rusch DB, Delcher AL, Pop M, Wang W, Fraser CM, Venter JC. The dog genome: survey sequencing and comparative analysis. Science. 2003; 301:1898–1903. [PubMed: 14512627]

Komaromy AM, Alexander JJ, Rowlan JS, Garcia MM, Chiodo VA, Kaya A, Tanaka JC, Acland GM, Hauswirth WW, Aguirre GD. Gene therapy rescues cone function in congenital achromatopsia. Human Molecular Genetics. 2010; 19:2581–2593. [PubMed: 20378608]

Kukekova AV, Goldstein O, Johnson JL, Richardson MA, Pearce-Kelling SE, Swaroop A, Friedman JS, Aguirre GD, Acland GM. Canine RD3 mutation establishes rod-cone dysplasia type 2 (rcd2) as ortholog of human and murine rd3. Mammalian Genome. 2009; 20:109–123. [PubMed: 19130129]

Lindblad-Toh K, Wade CM, Mikkelsen TS, Karlsson EK, Jaffe DB, Kamal M, Clamp M, Chang JL, Kulbokas EJ, Zody MC, Mauceli E, Xie X, Breen M, Wayne RK, Ostrander EA, Ponting CP, Galibert F, Smith DR, Dejong PJ, Kirkness E, Alvarez P, Biagi T, Brockman W, Butler J, Chin CW, Cook A, Cuff J, Daly MJ, Decaprio D, Gnerre S, Grabherr M, Kellis M, Kleber M, Bardeleben C, Goodstadt L, Heger A, Hitte C, Kim L, Koepfli KP, Parker HG, Pollinger JP, Searle SM, Sutter NB, Thomas R, Webber C, Baldwin J, Abebe A, Abouelleil A, Aftuck L, Ait-Zahra M, Aldredge T, Allen N, An P, Anderson S, Antoine C, Arachchi H, Aslam A, Ayotte L, Bachantsang P, Barry A, Bayul T, Benamara M, Berlin A, Bessette D, Blitshteyn B, Bloom T, Blye J, Boguslavskiy L, Bonnet C, Boukhgalter B, Brown A, Cahill P, Calixte N, Camarata J, Cheshatsang Y, Chu J, Citroen M, Collymore A, Cooke P, Dawoe T, Daza R, Decktor K, Degray S, Dhargay N, Dooley K, Dooley K, Dorje P, Dorjee K, Dorris L, Duffey N, Dupes A, Egbiremolen O, Elong R, Falk J, Farina A, Faro S, Ferguson D, Ferreira P, Fisher S, Fitzgerald M, Foley K, Foley C, Franke A, Friedrich D, Gage D, Garber M, Gearin G, Giannoukos G, Goode T, Goyette A, Graham J, Grandbois E, Gyaltsen K, Hafez N, Hagopian D, Hagos B, Hall J, Healy C, Hegarty R, Honan T, Horn A, Houde N, Hughes L, Hunnicutt L, Husby M, Jester B, Jones C, Kamat A, Kanga B, Kells C, Khazanovich D, Kieu AC, Kisner P, Kumar M, Lance K, Landers T, Lara M, Lee W, Leger JP, Lennon N, Leuper L, Levine S, Liu J, Liu X, Lokyitsang Y, Lokyitsang T, Lui A, Macdonald J, Major J, Marabella R, Maru K, Matthews C, McDonough S, Mehta T, Meldrim J, Melnikov A, Meneus L, Mihalev A, Mihova T, Miller K, Mittelman R, Mlenga V, Mulrain L, Munson G, Navidi A, Naylor J, Nguyen T, Nguyen N, Nguyen C, Nguyen T, Nicol R, Norbu N, Norbu C, Novod N, Nyima T, Olandt P, O'Neill B, O'Neill K, Osman S, Oyono L, Patti C, Perrin D, Phunkhang P, Pierre F, Priest M, Rachupka A, Raghuraman S, Rameau R, Ray V, Raymond C, Rege F, Rise C, Rogers J, Rogov P, Sahalie J, Settipalli S, Sharpe T, Shea T, Sheehan M, Sherpa N, Shi J, Shih D, Sloan J, Smith C, Sparrow T, Stalker J, Stange-Thomann N, Stavropoulos S, Stone C, Stone S, Sykes S, Tchuinga P, Tenzing P, Tesfaye S, Thoulutsang D, Thoulutsang Y, Topham K, Topping I, Tsamla T, Vassiliev H, Venkataraman V, Vo A, Wangchuk T, Wangdi T, Weiand M, Wilkinson J, Wilson A, Yadav S, Yang S, Yang X, Young G, Yu Q, Zainoun J, Zembek L, Zimmer A, Lander ES. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature. 2005; 438:803–819. [PubMed: 16341006]

Maguire AM, Simonelli F, Pierce EA, Pugh EN Jr, Mingozzi F, Bennicelli J, Banfi S, Marshall KA, Testa F, Surace EM, Rossi S, Lyubarsky A, Arruda VR, Konkle B, Stone E, Sun J, Jacobs J, Dell'Osso L, Hertle R, Ma JX, Redmond TM, Zhu X, Hauck B, Zelenaia O, Shindler KS, Maguire MG, Wright JF, Volpe NJ, McDonnell JW, Auricchio A, High KA, Bennett J. Safety and efficacy of gene transfer for Leber's congenital amaurosis. New England Journal of Medicine. 2008; 358:2240–2248. [PubMed: 18441370]

Mellersh CS, Boursnell ME, Pettitt L, Ryder EJ, Holmes NG, Grafham D, Forman OP, Sampson J, Barnett KC, Blanton S, Binns MM, Vaudin M. Canine RPGRIP1 mutation establishes cone-rod dystrophy in miniature longhaired dachshunds as a homologue of human Leber congenital amaurosis. Genomics. 2006; 88:293–301. [PubMed: 16806805]

Mellersh C. DNA testing and domestic dogs. Mammalian Genome. 2012; 23(1–2):109–23. [PubMed: 22071879]

Miyadera K, Kato K, Aguirre-Hernandez J, Tokuriki T, Morimoto K, Busse C, Barnett K, Holmes N, Ogawa H, Sasaki N, Mellersh CS, Sargan DR. Phenotypic variation and genotype-phenotype discordance in canine cone-rod dystrophy with an RPGRIP1 mutation. Molecular Vision. 2009; 15:2287–2305. [PubMed: 19936303]

Miyadera K, Acland GM, Aguirre GD. Genetic and phenotypic variations of inherited retinal diseases in dogs: the power of within- and across-breed studies. Mammalian Genome. 2012a; 23(1–2):40–61. [PubMed: 22065099]

Miyadera K, Kato K, Boursnell M, Mellersh CS, Sargan DR. Genome-wide association study in RPGRIP1(−/−) dogs identifies a modifier locus that determines the onset of retinal degeneration. Mammalian Genome. 2012b; 23(1–2):212–23. [PubMed: 22193413]

Narfström K, Katz ML, Bragadottir R, Seeliger M, Boulanger A, Redmond TM, Caro L, Lai C-M, Rakoczy PE. Functional and structural recovery of the retina after gene therapy in the RPE65 null

mutation dog. Investigative Ophthalmology & Visual Science. 2003; 44:1663–1672. [PubMed: 12657607]

Narfstrom K, Seeliger M, Lai CM, Vaegan, Katz M, Rakoczy EP, Reme C. Morphological aspects related to long-term functional improvement of the retina in the 4 years following rAAV-mediated gene transfer in the RPE65 null mutation dog. Advances in Experimental Medicine and Biology. 2008; 613:139–146. [PubMed: 18188938]

Narfström K, Wrigstad A, Nilsson SEG. The Briard dog: a new animal model of congenital stationary night blindness. British Journal of Ophthalmology. 1989; 73:750–756. [PubMed: 2804031]

Parker HG, Kukekova AV, Akey DT, Goldstein O, Kirkness EF, Baysac KC, Mosher DS, Aguirre GD, Acland GM, Ostrander EA. Breed relationships facilitate fine-mapping studies: a 7.8-kb deletion cosegregates with Collie eye anomaly across multiple dog breeds. Genome Research. 2007; 17:1562–1571. [PubMed: 17916641]

Petersen-Jones SM, Entz DD, Sargan DR. cGMP phosphodiesterase-α mutation causes progressive retinal atrophy in the cardigan Welsh corgi dog. Investigative Ophthalmology & Visual Science. 1999; 40:1637–1644. [PubMed: 10393029]

Roberts S. The collie eye anomaly. Journal of the American Veterinary Medical Association. 1969; 155:859–878.

Sidjanin DJ, Lowe JK, McElwee JL, Milne BS, Phippen TM, Sargan DR, Aguirre GD, Acland GM, Ostrander EA. Canine CNGB3 mutations establish cone degeneration as orthologous to the human achromatopsia locus ACHM3. Human Molecular Genetics. 2002; 11:1823–1833. [PubMed: 12140185]

Stone EM. Leber congenital amaurosis - a model for efficient genetic testing of heterogeneous disorders: LXIV Edward Jackson Memorial Lecture. American Journal of Ophthalmology. 2007; 144:791–811. [PubMed: 17964524]

Suber ML, Pittler SJ, Qin N, Wright GC, Holcombe V, Lee RH, Craft CM, Lolley RN, Baehr W, Hurwitz RL. Irish setter dogs affected with rod/cone dysplasia contain a nonsense mutation in the rod cGMP phosphodiesterase β-subunit gene. Proceedings National Acadademy of Sciences, USA. 1993; 90:3968–3972.

Sutter NB, Eberle MA, Parker HG, Pullar BJ, Kirkness EF, Kruglyak L, Ostrander EA. Extensive and breed-specific linkage disequilibrium in Canis familiaris. Genome Research. 2004; 14:2388–2396. [PubMed: 15545498]

Veske A, Nilsson SEG, Narfström K, Gal A. Retinal dystrophy of Swedish briard/briard-beagle dogs is due to a 4-bp deletion in RPE65. Genomics. 1999; 57:57–61. [PubMed: 10191083]

Wiik AC, Wade C, Biagi T, Ropstad EO, Bjerkas E, Lindblad-Toh K, Lingaas F. A deletion in nephronophthisis 4 (NPHP4) is associated with recessive cone-rod dystrophy in standard wire-haired dachshund. Genome Res. 2008; 18(9):1415–21. [PubMed: 18687878]

Winkler PA, Ekenstedt KJ, Occelli LM, Frattaroli AV, Bartoe JT, Venta PJ, Petersen-Jones SM. A large animal model for CNGB1 autosomal recessive retinitis pigmentosa. PLoS One. 2013 Aug 19.8(8):e72229. [PubMed: 23977260]

Zangerl B, Goldstein O, Philp AR, Lindauer SJ, Pearce-Kelling SE, Mullins RF, Graphodatsky AS, Ripoll D, Felix JS, Stone EM, Acland GM, Aguirre GD. Identical mutation in a novel retinal gene causes progressive rod-cone degeneration in dogs and retinitis pigmentosa in humans. Genomics. 2006; 88:551–563. [PubMed: 16938425]

Zangerl B, Wickstrom K, Slavik J, Lindauer SJ, Ahonen S, Schelling C, Lohi H, Guziewicz KE, Aguirre GD. Assessment of canine BEST1 variations identifies new mutations and establishes an independent bestrophinopathy model (CMR3). Molecular Vision. 2010; 16:2791–2804. [PubMed: 21197113]

AI, Perrault I, Preising MN, Lorenz B, Kaplan J, Cremers FP, Maumenee I, Koenekoop RK, Allikmets R. Genotyping microarray (disease chip) for Leber congenital amaurosis: detection of modifier alleles. Investigative Ophthalmology & Visual Science. 2005; 46:3052–3059. [PubMed: 16123401]

Zhang Q, Acland GM, Wu WX, Johnson JL, Pearce-Kelling S, Tulloch B, Vervoort R, Wright AF, Aguirre GD. Different RPGR exon ORF15 mutations in Canids provide insights into

photoreceptor cell degeneration. Human Molecular Genetics. 2002; 11:993–1003. [PubMed: 11978759]

Table 1

Canine retinal diseases and associated genes

Disease Name	Disease Symbol	Breed	Gene	Gene Function	Approach	References
Progressive Disorders						
Progressive Retinal Atrophy (PRA)						
Rod-cone dysplasia 1	rcd1	Irish Setter	*PDE6B*	Phototransduction	Candidate gene	(Clements *et al.* 1993; Suber *et al.* 1993)
Rod-cone dysplasia 1a	rcd1*a*	Sloughi	*PDE6B*	Phototransduction	Candidate gene	(Dekomien *et al.* 2000)
Rod-cone dysplasia 2	rcd2	Collie	*RD3*	Phototransduction	LA	(Kukekova *et al.* 2009)
Rod-cone dysplasia 3	rcd3	Cardigan Welsh Corgi	*PDE6A*	Phototransduction	Candidate gene	(Petersen-Jones *et al.* 1999)
Rod-cone dysplasia 4	rcd4	Setters and others[a]	*C2orf71*	N.D.	GWAS	(Downs *et al.* 2013)
Early retinal degeneration	erd	Norwegian Elkhound	*STK38L*	N.D.	LA	(Goldstein *et al.* 2010b)
Progressive rod-cone degeneration	PRCD	Multiple breeds[b]	*PRCD*	N.D.	LA	(Zangerl *et al.* 2006)
Golden Retriever PRA 1	GR_PRA1	Golden Retriever	*SLC4A3*	Anion exchange	GWAS	(Downs *et al.* 2011)
Golden Retriever PRA 2	GR_PRA2	Golden Retriever	*TTC8*	Cilia formation	GWAS, targeted-NGS	(Downs *et al.* 2014)
X-linked PRA 1	XLPRA1	Samoyed, Siberian Husky	*RPGR*	Cilia trafficking	LA	(Zhang *et al.* 2002)
X-linked PRA 2	XLPRA2	Mongrel	*RPGR*	Cilia trafficking	LA	(Zhang *et al.* 2002)
Autosomal dominant PRA	ADPRA	Bullmastiff, English Mastiff	*RHO*	Phototransduction	Candidate gene	(Kijas *et al.* 2002)
Generalized PRA	gPRA^{CCDC66}	Schapendoes	*CCDC66*	N.D.	LA	(Dekomien *et al.* 2010)
Italian Greyhound PRA	IG_PRA1	Italian Greyhound	-	-	-	(Goldstein, personal communication)
Papillon PRA	Pap_PRA1	Papillon	*CNGB1*	Phototransduction		(Ahonen *et al.* 2013; Winkler *et al.* 2013)
Basenji PRA	Bas_PRA1	Basenji	*SAG*	Phototransduction	GWAS	(Goldstein *et al.* 2013a)
PRA3	PRA3	Tibetan Spaniel/Terrier	*FAM161A*	Cilia trafficking	GWAS, targeted-NGS	(Downs and Mellersh. 2014)
Cone-Rod Dystrophy (CRD)						
Cone-rod dystrophy	crd1	American Staffordshire Terrier	*PDE6B*	Phototransduction	GWAS	(Goldstein *et al.* 2013b)
Cone-rod dystrophy	crd2	American Pit Bull/Staffordshire Terrier	*IQCB1*	Cilia trafficking	GWAS	(Goldstein *et al.* 2013b)
Cone-rod dystrophy	crd3	Glen of Imaal Terrier	*ADAM9*	N.D.	GWAS (pet, colony)	(Goldstein *et al.* 2010c)
Cone-rod dystrophy	cord1	Miniature Longhaired/Smooth/Wirehaired Dachshunds	*RPGRIP1*	Cilia trafficking	LA GWAS (modifier)	(Mellersh *et al.* 2006; Miyadera *et al.* 2012)
Cone-rod dystrophy	CRD^{NPHP4}	Standard Wirehaired Dachshund	*NPHP4*	Cilia trafficking	GWAS	(Wiik *et al.* 2008)

Disease Name	Disease Symbol	Breed	Gene	Gene Function	Approach	References
Stationary Disorders						
Cone degeneration (achromatopsia, day blindness)	CD	Alaskan Malamute, Miniature Australian shepherd, Siberian Husky	*CNGB3*	Phototransduction	LA	(Sidjanin *et al.* 2002)
Cone degeneration (achromatopsia, day blindness)	CD	German Shorthaired Pointer	*CNGB3*	Phototransduction	LA	(Sidjanin *et al.* 2002)
Canine LCA	cLCA	Briard	*RPE65*	Visual cycle	Candidate gene	(Aguirre *et al.* 1998; Veske *et al.* 1999)
Developmental Disorders						
Retinal dysplasia/Oculo-skeletal dysplasia 1	RD/OSD1	Labrador Retriever	*COL9A3*	Collagen formation	LA	(Goldstein *et al.* 2010a)
Retinal dysplasia/Oculo-skeletal dysplasia 2	RD/OSD2	Samoyed	*COL9A2*	Collagen formation	LA	(Goldstein *et al.* 2010a)
Collie eye anomaly	CEA	Collies and other breeds[c]	*NHEJ1*	N.D.	LA	(Parker *et al.* 2007)
Others						
Canine multifocal retinopathy 1	CMR1	Multiple breeds[d]	*BEST1*	Epithelial transport	Candidate gene	(Guziewicz *et al.* 2007)
Canine multifocal retinopathy 2	CMR2	Coton de Tulear	*BEST1*	Epithelial transport	Candidate gene	(Guziewicz *et al.* 2007)
Canine multifocal retinopathy 3	CMR3	Lapponian Herder	*BEST1*	Epithelial transport	Candidate gene	(Zangerl *et al.* 2010)

Modified from Miyadera et al. (2012a). The Approach column shows the method used to identify the chromosomal location and/or the gene, and the type of sample population used. **LA**, linkage analysis; **N.D.**, not determined; **GWAS**, genome-wide association study.

[a] rcd4-affected breeds: English/Gordon/Irish/Llwellyn Setters, Polish Lowland Sheepdog, Tibetan Terrier.

[b] PRCD-affected breeds: American Cocker Spaniel, American Eskimo Dog, Australian Cattle Dog, Australian Shepherd, Australian Stumpy Tail Cattle Dog, Bolonka Zwetna, Chesapeake Bay Retriever, Chinese Crested, English Cocker Spaniel, English Shepherd, Entlebucher Mountain Dog, Finnish Lapphund, Giant Schnauzer, Golden Retriever, Karelian Bear Dog, Kuvasz, Labrador Retriever, Lapponian Herder, Norwegian Elkhound, Poodle (Miniature/Toy/Standard), Nova Scotia Duck Tolling Retriever, Portuguese Water Dog, Spanish Water Dog, Swedish Lapphund, Yorkshire Terrier.

[c] CEA-affected breeds: Australian Shepherd, Bearded Collie, Boykin Spaniel, Border Collie, Hokkaido Dog, Lancashire Heeler, Nova Scotia Duck Tolling Retriever, Rough/Smooth Collie, Shetland Sheepdog, Silken Windhound, Longhaired Whippet.

[d] CMR1-affected breeds: American Bulldog, Australian Shepherd, Boerboel, Bullmastiff, Cane Corso, Dogue de Bordeaux, Great Pyrenees, Old English Mastiff, Perro de Presa Canario, English/American Bulldog.

Table 2

Examples of breeds affected with more than one confirmed RDs

Breed	Disease 1	Disease 2	Disease 3	Disease 4
Australian Shepherd	prcd-PRA	CEA	CMR1	CD
Golden Retriever	prcd-PRA	GR_PRA1	GR_PRA2	-
Golden Doodle	prcd-PRA	GR_PRA1	GR_PRA2	-
Norwegian Elkhound	prcd-PRA	rd	erd	-
Lapponian Herder	prcd-PRA	CMR3	-	-
Irish Setter	rcd1-PRA	rcd4-PRA	-	-
Labrador Retriever	prcd-PRA	RD/OSD1	-	-
Nova Scotia Duck Tolling Retriever	prcd-PRA	CEA	-	-
Miniature Wirehaired Dachshund	CRD^{NPHP4}	cord1	-	-
Bullmastiff/English Mastiff	ADPRA	CMR1	-	-
Samoyed	XLPRA1	RD/OSD2	-	-
Tibetan Terrier	rcd4-PRA	PRA3	-	-
Collie	rcd2-PRA	CEA	-	-
Portuguese Water Dog	prcd-PRA	early-onset PRA	-	-