Published in final edited form as:
Mol Genet Genomics. 2010 October ; 284(4): 243–250. doi:10.1007/s00438-010-0560-5.

Identification of genetic variation and haplotype structure of the canine *ABCA4* gene for retinal disease association studies

B. Zangerl
Section of Ophthalmology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Ryan Veterinary Hospital, #2050, 3900 Delancey Street, Philadelphia, PA 19104, USA

S. J. Lindauer
Section of Ophthalmology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

G. M. Acland
J.A. Baker Institute, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA

G. D. Aguirre
Section of Ophthalmology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Abstract

Over 200 mutations in the retina specific member of the ATP-binding cassette transporter super-family (*ABCA4*) have been associated with a diverse group of human retinal diseases. The disease mechanisms, and genotype–phenotype associations, nonetheless, remain elusive in many cases. As orthologous genes are commonly mutated in canine models of human blinding disorders, canine *ABCA4* appears to be an ideal candidate gene to identify and study sequence changes in dogs affected by various forms of inherited retinal degeneration. However, the size of the gene and lack of haplotype assignment significantly limit targeted association and/or linkage approaches. This study assessed the naturally observed sequence diversity of *ABCA4* in the dog, identifying 80% of novel variations. While none of the observed polymorphisms have been associated with blinding disorders to date, breed and potentially disease specific haplotypes have been identified. Moreover, a tag SNP map of 17 (15) markers has been established that accurately predicts common *ABCA4* haplotypes (frequency > 5%) explaining >85% (>80%) of the observed genetic diversity and will considerably advance future studies. Our sequence analysis of the complete canine *ABCA4* coding region will clearly provide a baseline and tools for future association studies and comparative genomics to further delineate the role of *ABCA4* in canine blinding disorders.

Keywords

ABCA4; Haplotype structure; Genetic variation; Progressive retinal atrophy; Canine

bzangerl@vet.upenn.edu .

Electronic supplementary material The online version of this article (doi:10.1007/s00438-010-0560-5) contains supplementary material, which is available to authorized users.

Introduction

The ATP-binding cassette (ABC) transporter A-subfamily consists of a large number of transporter proteins, many of which are associated with inherited disorders (Holland et al. 2003; Albrecht and Viturro 2007). Only one member, *ABCA4*, is specific to the retina, and is expressed in the photoreceptor outer segments (Sun and Nathans 1997). More than 500 putative mutations are proposed for *ABCA4* in humans. To date, over 200 of these have been implicated with disease phenotypes (OMIM #601691), the vast majority of which are linked to Stargardt disease (STGD), a juvenile form of macular degeneration (Sun et al. 2000; Webster et al. 2001; Klevering et al. 2005). Individual mutations, however, also cause cone-rod dystrophy (Ducroq et al. 2002), and retinitis pigmentosa (RP) (Cremers et al. 1998); involvement with age-related macular degeneration has been suggested by several reports (Allikmets et al. 1997; Shroyer et al. 2001). Many of these studies are based on allele association with the retinal phenotype, yet the contribution of individual sequence changes and the mechanism leading from mutation to disease is often unclear.

In humans, individual defects are frequently limited to small cohorts or families, barely sufficient to securely associate genotype to phenotype (Stone 2003), and in-depth clinical investigations are limited to non-invasive procedures that depend on patient availability (Kitiratschky et al. 2008; Cideciyan et al. 2009). Progress in understanding the disease has resulted from a series of elegant biochemical and expression studies (Sun et al. 1999, 2000; Sun and Nathans 2001) demonstrating that the ABCA4 candidate ligand is all-*trans*-retinal, a chromophore implicated in facilitating light damage of the retina (Sun et al. 1999). In short, by transporting either all-*trans*-retinal or the Schiff base adduct of all-*trans*-retinal and phosphatidylethanolamine from the luminal to the cytosolic disc membrane, ABCA4 likely accelerates the reduction to all-*trans*-retinol, which is thought to be the rate-limiting step in the visual cycle.

Development of an *Abcr* knockout mouse provided additional insights into the role of this pathway in vivo, but failed to display all features of the human disease (Weng et al. 1999; Molday et al. 2009). Notably, the murine model accumulates A2E deposits, a major lipofuscin fluorophore and toxic all-*trans*-retinal derivate, in the retinal pigment epithelium (RPE), which can be blocked through administration of isotretinoin (Radu et al. 2003). Since accumulation of lipofuscin pigments is assumed to be the primary pathologic defect in Stargardt disease, these studies grant hope for potential therapeutic intervention. Retinal degeneration, however, is not reproduced in pigmented *abca4*$^{-/-}$ strains, and is difficult to distinguish from light and/or age dependent damage in albino mice (Radu et al. 2008). While progress has been achieved by addressing these questions by in vitro experiments (Liu et al. 2008), to date, there are no other animal models available to study the specific role of *ABCA4* mutations on RPE and/or photoreceptor degeneration.

Thus far, studies in canine models based on few polymorphic markers in informative families, have excluded *ABCA4* from association with two autosomal recessive, non-allelic forms of canine cone-rod degeneration (*crd1*, *crd2*) (Kijas et al. 2004) and cone-rod dystrophy in the standard wire haired dachshund (Wiik et al. 2008). The large size of the *ABCA4* coding region and the population structure of the dog that is segregated into genetically isolated breeds (Quignon et al. 2007) pose significant limitations for the identification of variations that are potentially important in only a few breeds. Thus, a study excluding *ABCA4* from association with various forms of progressive retinal atrophy (PRA), the canine RP homologous group of diseases, in 17 breeds was based on identification of only 18 polymorphisms by SSCP (Lippmann et al. 2006). While association studies would be more appropriate for studies of *ABCA4* in the dog, these rely on identification and availability of representative markers. This study provides the first in-depth analysis on sequence diversity of *ABCA4* in the dog, providing

a total of 41 high frequency (>10%) markers in exons and intronic flanking region, resulting in 17 tag SNPs. In addition, several breed-specific variants and rare alleles were identified that are suitable for follow-up investigations in individual breeds. The current data set establishes a baseline for the expected variation of *ABCA4* in the dog and, therefore, enables targeted association studies in the future.

Methods

Animal samples

Blood from dogs with autosomal recessive retinal degeneration, collectively referred to as progressive retinal atrophy (PRA), was collected previously and stored after consent of the owners. All animals were examined either by one of the two authors (GDA, GMA) or collaborating board certified veterinary ophthalmologists, and diagnosis was verified in all cases based on the breed-specific age of onset for retinal disease after exclusion of known mutations [progressive rod-cone degeneration (Zangerl et al. 2006), rod-cone dysplasias (Suber et al. 1993; Kukekova et al. 2009). These include breeds for which (a) the mode of inheritance is known, but insufficient cases for genome wide association studies are available (AKI, BUM, ESS, GOS, GSH, GSP, PAP, TIS, TT; see Table 1 for details on breed abbreviations); (b) only isolated cases were reported, and no literature is available or the mode of inheritance is not yet defined (e.g. EMD: not defined, SCW: no literature); (c) other retinal disorders have been noted as segregating in the breed, but have been excluded as causative in the respective individuals (BRI, CSNB; MSH, Type A-PRA); (d) other mutations are known that may interact with or modify a different disease gene (MLD). In total, 26 affected animals representing 23 known PRA breeds were entered into the current study (Table 1). Due to selection criteria (animals born to unaffected parents) and/or based on previous studies, mode of inheritance was considered autosomal recessive for all cases enrolled in the study (Aguirre and Acland 2006). Unrelated control animals for PRA breeds were included, if available, as part of our archived sample resource (ADT, AKI, BAS, BMD, GIT, IGH, PAP, TT). In addition, three unrelated breeds (BOT, HUS, RRB) were added to address allele frequencies of sequence changes in a wider spectrum of dog breeds, and potentially correct for association by breed rather than disease. Thus, a total of 12 control animals from 11 different breeds were used (Table 1).

Exon screening

DNA was extracted from 200 μl total blood using QIAmp DNA Mini Kit (Qiagen) according to the manufacturer's protocol. PCR primers were designed using Vector NTI™ v.10.3.0 software package based on comparison of the canine *ABCA4* cDNA sequence (NM_001003360) to the dog genome draft (http://www.genome.gov/12511476, version May 2005) to primarily amplify exonic sequences, including small introns in selected cases (Suppl. Table 1). PCR products resulting from standard amplification were sequenced in both directions using PCR primers on an ABI capillary sequencer at the University of Pennsylvania core facility after purification with either QIAquick Gel Extraction Kit (Qiagen) or QIAquick PCR Purification Kit (Qiagen).

Data analysis

Sequences were analyzed with the Sequencher® v.4.7 software; observed polymorphisms were placed on the canine genome draft sequence (http://genome.ucsc.edu/, version May 2005), and compared to the Broad SNP set (Lindblad-Toh et al. 2005). Allele frequencies were calculated manually over the complete data set, haplotype analysis and identification of tag SNPs were performed with the HapBlock software (Zhang et al. 2005). Haplotype structure was evaluated based on common haplotypes (frequency > 5%) to explain the majority of observed variation (80 and 85%, respectively). Population pair-wise F_{ST}'s were computed using Arlequin v.3.1 (Excoffier et al. 2005), and further analyzed for statistical significance between affected and

control animals and different breeds on a global and locus-by-locus basis using analysis of molecular variance (AMOVA). Breed and disease status was taken into account for analysis of polymorphisms and enabled identification of dog specific *ABCA4* haplotypes.

Results

ABCA4 population structure

Genomic variation—A total of 38 dogs from 26 different breeds (Table 1) were sequenced each for 16,484 bp of the complete *ABCA4* coding region and flanking introns. Overall, these data present 13% coverage of the roughly 128,000 bp genomic *ABCA4* region. The analyzed sequence harbored 88 sequence changes (Suppl. Table 2) in comparison to the canine genome draft sequence (http://genome.ucsc.edu/, version May 2005), only 18 of which had previously been reported; novel variants were submitted to the dbSNP data base (NCBI ss# 244242169–244242238). This predicts an average variation of one polymorphism/400 bp within the *ABCA4* coding region (total 17 polymorphisms; 14 transitions, 3 transversions), and an almost threefold increase in the variation for adjacent non-coding areas of the gene. In detail, these changes consist of 60 transitions, 24 transversions, 2 deletions, and 2 insertions. Nonetheless, only 34 polymorphisms are shared in more than three different breeds (Suppl. Table 2, multiple breeds), and almost half (40) of the polymorphisms have a rare allele frequency of <5% in the data set. While these are potentially unfavorable markers for large population screens, rare alleles are commonly associated with disorders and/or specific to certain dog breeds.

Haplotype analysis—Due to the size of the *ABCA4* gene, the most robust haplotype structure, explaining the majority, >85% (>80%), in the diversity of common haplotypes (frequency > 5%), was obtained by dividing the complete data set into 9 (6) blocks (Suppl. figure 1). In general, two haplotypes were observed at high frequency (>10%) for individual blocks, with higher variability predicting up to four common haplotypes for 5′ exons (Suppl. figure 1, block 1 and 2). These data identified 17 (15) tag polymorphisms (Table 2, Suppl. figure 1, Suppl. Table 2), which enable prediction of *ABCA4* haplotypes with the same degree of certainty obtained from a complete genomic exon scan and are the recommended markers for future *ABCA4* population screens.

AMOVA was applied to test for genetic distance among breeds from the total data set resulting in only marginal significance ($p = 0.049 \pm 0.006$). Accordingly, only two pairs of breeds could be distinguished applying a pair-wise comparison between breeds; BAS from GIT, and HUS from MSH. Particular attention was given to 17 polymorphisms located in the coding region (Suppl. Table 3). Haplotype prediction using Arlequin v3.1 estimated up to 31 possible chromosomes in the data set. A single haplotype (Suppl. Table 3, haplotype 14), however, is observed at high frequency (>10%), and over 20% of the chromosomes are only observed once (frequency 0.01) in the data set.

ABCA4 and PRA

Sequence variation—While the main focus of this study aimed to identify haplotype structure and develop screening sets for association studies in dog breeds affected with PRA, five sequence variations identified in affected animals were considered for association with disease. Three heterozygous nucleotide changes (Suppl. Table 2, Suppl. figure 1; 58129706, 58152042, and 58174454) were observed in only one affected dog of the ADT, EMD, and MLD breeds, respectively. The polymorphism specific to the ADT was not present in the non-affected control animal; no control animals were available for the other two breeds. The MLD, on the other hand, is homozygous affected for the *RPGRIP1* mutation (Mellersh et al. 2006), suggesting that the observed polymorphism, at best, may be a modifier for disease. These *ABCA4* sequence variations cannot be excluded with certainty from association with disease.

Another heterozygous polymorphism in the 5′UTR region of an affected BMD (Suppl. Table 2, Suppl. figure 1; 58112910) was examined further in 12 non PRA-affected BMD, and showed an allele frequency of ~7% in this population (data not shown). The PRA-affected GSP was homozygous for a single polymorphism (Suppl. Table 2, Suppl. figure 1; 58124361) in intron 4. This polymorphism was subsequently typed in nine non-affected animals from the same breed, and shown to be breed specific (70% allele frequency), but no linkage with disease could be established (data not shown).

Only five of the identified polymorphisms cause changes in the amino acid (aa) sequence (Table 3; 58168353, 58180284, 58198327, 58207091, 58216455). Three of these have previously been excluded from causative association with PRA (Kijas et al. 2004; 58168353, 58198327, 58216455). Although the remaining two changes are conserved in published *ABCA4* sequences of other species (Table 3), distribution in affected and non-affected animals within the present data set excludes them from association with retinal degeneration (Suppl. figure 1, Suppl. Table 3).

Disease haplotypes—Low frequency SNPs observed in one or a few individuals only contributed to rare haplotypes predicted by HapBlock (Suppl. figure 1). Thus, the affected GOS is the only individual heterozygous at four different polymorphic positions (58167425, 58182988, 58190633, 58231826) not present in normal dogs, but disease association can be excluded based on the presumed recessive nature of the trait in the breed. The same applies to another polymorphism shared between this breed and a PRA-affected BRI (58231826). Focusing on the *ABCA4* coding region only, 16 of the 31 observed haplotypes were present exclusively in affected dogs (Suppl. Table 3; haplotypes 1, 6–9, 16, 17, 19–23, 28–31); the most common one having a frequency of 7.9% (16). To this end, no significant difference was found between affected and non-affected individuals ($p = 0.918 \pm 0.009$) in a global AMOVA analysis, nor did any single polymorphism show significant difference between these two groups in the locus-by-locus analysis. However, two PRA-affected dogs, GIT and GSP, were homozygous for one of those haplotypes, while another 5 individuals, two BAS, BRI, SCW, and SSD, were compound heterozygotes for different haplotype combinations. Although these results could result from a bias of PRA-affected animals and breeds, haplotypes specific to diseased animals could potentially contribute to modifying and/or complex inherited disease traits.

Discussion

Rapid advancements in identification and screening for sequence variations has contributed greatly to the definition of population structure and increased our ability to start teasing out genetic interaction and modification determining phenotypic expression of variable traits. While the *ABCA4* mutation database (http://www.retina-international.org/sci-news/abcrmut.htm) lists several hundred observed changes, only few have been reproducibly associated with distinct disease phenotypes in patients. In fact, many times the development of clinical disorders is ascribed to a combination of gene modifications (Klevering et al. 2002). Despite all efforts to develop suitable model systems addressing genotype–phenotype correlations, as well as offering insights into disease mechanisms, the current limitations of small sample sizes for human cohorts, small animal models, and in vitro studies have yet to be overcome.

Attempts to identify a large animal model are often based on linkage analysis in well characterized pedigrees (Kijas et al. 2004; Wiik et al. 2008). In recent years, these elaborate methods are being replaced by time and cost-effective screening methods. A study investigating *ABCA4* in 25 dog breeds affected with PRA identified 18 sequence variants, including a single aa change, by SSCP (Lippmann et al. 2006). *ABCA4* was excluded as

causative gene for PRA in 17 breeds, but could not be assessed in the remainder. Lack of in-depth sequence variation, however, not only compromises exclusion of some breeds, but also negates deductions of prospective markers and/or test sets. To overcome these limitations, we significantly increased our knowledge of variability and potential haplotype structure of the canine *ABCA4* through DNA sequencing of 38 individual dogs from 26 breeds (Table 1), which resulted in the overall identification of 88 polymorphisms in exons and surrounding intronic sequence (Suppl. Table 2). A threefold increased mutation rate in the *ABCA4* non-coding region, as well as the 2.5 times bias towards transitions than transversions, rejects predisposition to a particular mutation mechanism or hotspot throughout the genomic *ABCA4* region. Thus, a reasonably predictive tag marker set could be developed utilizing 17 or 15 SNPs sufficient to recognize >85% and >80%, respectively, of the observed genomic diversity at this locus (Table 2, Suppl. figure 1), and will facilitate future population and/or association studies of *ABCA4*.

Most variability is observed within the 5′ region of the gene (Suppl. figure 1), without indication that this is related to regulatory function. Despite the lower number of haplotypes in and around 3′ exons, the total amount of polymorphisms is higher due to accumulation of multiple rare variants in few breeds (e.g. AKI, BAS, ADT). One could propose that this is a result of stronger influence of negative selection and genetic drift to eliminate potentially harmful haplotypes, or differences in local recombination rates, irrespective of the location of predicted transmembrane and nucleotide binding domains. It should be pointed out that these rare haplotypes appear more commonly in presumed “ancient” dog breeds, e.g. BAS, AKI, or HUS (Vonholdt et al. 2010). Variations shared between breeds that typically do not cluster based on large genomic data sets (Parker et al. 2004) may hint at more recent breed admixture (BAS and BMD), or result from geographic proximity of the breed origin (SSD and ADT). The inability of the genetic variation to differentiate breeds or affected animals in the current data set is likely due to the small number of animals/breeds sequenced. However, rare, but breed-specific alleles, can now be utilized to address focused questions in the respective breeds.

Non-coding sequence variants were excluded from simple Mendelian disease association based on segregation in the affected animals or allele frequency in the breed (7% BMD; 70% GSP) under the assumption of autosomal recessive disease models. Comparable to few frequent haplotypes (>10%) observed within the complete data set (2–4 in Suppl. figure 1), 17 polymorphisms located within the ABCA4 coding region account for a single common chromosome (Suppl. Table 3, haplotype 14). The vast majority of minor frequency haplotypes is private to specific breeds and/or individuals; therefore, their relevance to gene function cannot be deduced at this time. These haplotypes encompass five missense mutations (Suppl. Table 3, alternative alleles underlined), three of which (58168353, 58198327, 58216455) were excluded from disease association in the current and previous models (Kijas et al. 2004). Despite the potential molecular consequences of 58168353 and 58198327, they are not located within structural or functional domains, such as predicted ABC transmembrane domain (TD), nucleotide binding domains (NBD) or glycosylation sites. On the other hand, 58216455, located within a putative TD, is a conservative change with the mutant allele common in other species (Table 3). One of the remaining mutations, 58180284 (BICF2P1361455), was not identified within known domains or a functional site, nor did the segregation within our sample set (26% allele frequency) or previous studies (Lindblad-Toh et al. 2005) support pathogenic potential. The non-conservative Arg1539Gly change (58207091), however, is correlated with a putative topological domain, and appears to affect a highly conserved site (Table 3, human, rhesus). Although the mutant allele has a higher frequency in normal versus affected animals, we cannot satisfactorily conclude form the current data that the variant by itself, or in combination with other nucleotide changes in *ABCA4* may contribute to a genetic load that serves as negative or protective modifier of retinal diseases, which could particularly benefit such breeds as MLD with other known molecular defects. Our work should provide a

foundation to further evaluate such effects. Interestingly, contrary to recent suggestions from comparative data (Gibbs et al. 2007), we did not find a bias of the human ABCA4 protein towards potentially disease-causing variants based on our data set, but this could simply indicate that none of the sequence changes found have an impact on retinal disorders.

Given the high incidence of *ABCA4*-associated disease in humans, the lack of association between sequence variations and retinal disorders in canines is rather surprising, particularly considering the high conservation of both the gene and the polymorphic coding sites (Table 3). Our results raise the question as to whether the association between retinal disease and sequence variations observed in only one or a few human patients might be associated with rare alleles rather than disease (Stone 2007). Alternatively, disadvantageous polymorphisms might not readily lead to a disease phenotype in the dog. It also has to be pointed out that *ABCA4* disease involving the macula in human patients (Cideciyan et al. 2005) may manifest differently in non-primate species missing this characteristic formation of rod/cone arrangements. Recent studies suggest that such structural aspects could significantly contribute to species variation and even alter the mode of inheritance for some disease models (Guziewicz et al. 2007).

We anticipate that our study will lead the way to an efficient and in-depth screening of the *ABCA4* gene in a variety of canine populations. This will enable the rapid identification of individuals with potential mutations in this gene, and also provide insight into the influence of *ABCA4* haplotypes on retinal disease. Such steps are essential for the advancement of comparative genomic approaches, and the suitable study of pedigrees to address phenotype–genotype correlations at this highly variable locus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Sue Pearce-Kelling for support and coordination of research samples, and James Kijas for methodical and inspirational comments. The work was supported by The Foundation Fighting Blindness Center and Individual Investigator grants; Morris Animal Foundation; NEI/NIH grants EY-06855, -017549 (The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Eye Institute or the National Institutes of Health); the Van Sloun Fund for Canine Genetic Research; Vision Research Center grant P30 EY-001583; Hope for Vision Foundation; The ONCE International Prize for R&D in Biomedicine and New Technologies for the Blind.

References

Aguirre, GD.; Acland, GM. Models, mutants and man: searching for unique phenotypes and genes in the dog model of inherited retinal degeneration. In: Ostrander, EA.; Giger, U.; Lindblad-Toh, K., editors. The Dog and its genome. Cold Spring Harbor Laboratory Press; Cold Spring Harbor: 2006. p. 291-325.

Albrecht C, Viturro E. The ABCA subfamily-gene and protein structures, functions and associated hereditary diseases. Pflugers Arch 2007;453(5):581–589. [PubMed: 16586097]

Allikmets R, Shroyer NF, Singh N, Seddon JM, Lewis RA, Bernstein PS, Peiffer A, Zabriskie NA, Li Y, Huchinson A, Dean M, Lupske JR, Lappert M. Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. Science 1997;277(5333):1805–1807. [PubMed: 9295268]

Cideciyan AV, Swider M, Aleman TS, Sumaroka A, Schwartz SB, Roman MI, Milam AH, Bennett J, Stone EM, Jacobson SG. ABCA4-associated retinal degenerations spare structure and function of the human parapapillary retina. Invest Ophthalmol Vis 2005;46(12):4739–4746.

Cideciyan AV, Swider M, Aleman TS, Tsybovsky Y, Schwartz SB, Windsor E, Roman AJ, Sumaroka A, Steinberg JD, Jacobson SG, Stone EM, Palczewski K. ABCA4 disease progression and a proposed strategy for gene therapy. Hum Mol Genet 2009;18(5):931–941. [PubMed: 19074458]

Cremers FP, van de Pol DJ, van Driel M, den Hollander AI, van Haren FJ, Knoers NV, Tijmes N, Bergen AA, Rohrschneider K, Blankenagel A, Pinckers AJ, Deutman AF, Hoyng CB. Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene ABCR. Hum Mol Genet 1998;7(3):355–362. [PubMed: 9466990]

Ducroq D, Rozet JM, Gerber S, Perrault I, Barbet D, Hanein S, Hakiki S, Dufier JL, Munnich A, Hamel C, Kaplan J. The ABCA4 gene in autosomal recessive cone-rod dystrophies. Am J Hum Genet 2002;71 (6):1480–1482. [PubMed: 12515255]

Excoffier L, Laval G, Schneider S. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 2005;1:47–50. [PubMed: 19325852]

Gibbs RA, Rogers J, Katze MG, Bumgarner R, Weinstock GM, Mardis ER, Remington KA, Strausberg RL, Venter JC, Wilson RK, et al. Evolutionary and biomedical insights from the rhesus macaque genome. Science 2007;316(5822):222–234. [PubMed: 17431167]

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. Invest Ophthalmol Vis Sci 2007;48(5):1959–1967. [PubMed: 17460247]

Holland, IB.; Cole, SPC.; Kuchler, K.; Higgins, CF. ABC Proteins. Elsevier Science Ltd.; London: 2003.

Kijas JW, Zangerl B, Miller B, Nelson J, Kirkness EF, Aguirre GD, Acland GM. Cloning of the canine ABCA4 gene and evaluation in canine cone-rod dystrophies and progressive retinal atrophies. Mol Vis 2004;10:223–232. [PubMed: 15064680]

Kitiratschky VB, Grau T, Bernd A, Zrenner E, Jagle H, Renner AB, Kellner U, Rudolph G, Jacobson SG, Cideciyan AV, Schaich S, Kohl S, Wissinger B. ABCA4 gene analysis in patients with autosomal recessive cone and cone rod dystrophies. Eur J Hum Genet 2008;16(7):812–819. [PubMed: 18285826]

Klevering BJ, Blankenagel A, Maugeri A, Cremers FP, Hoyng CB, Rohrschneider K. Phenotypic spectrum of autosomal recessive cone-rod dystrophies caused by mutations in the ABCA4 (ABCR) gene. Invest Ophthalmol Vis Sci 2002;43(6):1980–1985. [PubMed: 12037008]

Klevering BJ, Deutman AF, Maugeri A, Cremers FP, Hoyng CB. The spectrum of retinal phenotypes caused by mutations in the ABCA4 gene. Graefes Arch Clin Exp Ophthalmol 2005;243(2):90–100. [PubMed: 15614537]

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. Mamm Genome 2009;20(2):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, et al. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature 2005;438(7069):803–819. [PubMed: 16341006]

Lippmann T, Pasternack SM, Kraczyk B, Dudek SE, Dekomien G. Indirect exclusion of four candidate genes for generalized progressive retinal atrophy in several breeds of dogs. J Negat Results Biomed 2006;5:19. [PubMed: 17134500]

Liu J, Lu W, Reigada D, Nguyen J, Laties AM, Mitchell CH. Restoration of lysosomal pH in RPE cells from cultured human and ABCA4$^{-/-}$ mice: pharmacologic approaches and functional recovery. Invest Ophthalmol Vis Sci 2008;49(2):772–780. [PubMed: 18235027]

Mellersh CS, Boursnell ME, Pettitt L, Ryder EJ, Homes 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(3):293–301. [PubMed: 16806805]

Molday RS, Zhong M, Quazi F. The role of the photoreceptor ABC transporter ABCA4 in lipid transport and Stargardt macular degeneration. Biochim Biophys Acta 2009;1791(7):573–583. [PubMed: 19230850]

Parker HG, Kim LV, Sutter NB, Carlson S, Lorentzen TD, Malek TB, Johnson GS, DeFrance HB, Ostrander EA, Kruglyak L. Genetic structure of the purebred domestic dog. Science 2004;304(5674): 1160–1164. [PubMed: 15155949]

Quignon P, Herbin L, Cadieu E, Kirkness EF, Hedan B, Mosher DS, Galibert F, Andre C, Ostrander EA, Hitte C. Canine population structure: assessment and impact of intra-breed stratification on SNP-based association studies. PLoS ONE 2007;2(12):e1324. [PubMed: 18091995]

Radu RA, Mata NL, Nusinowitz S, Liu X, Sieving PA, Travis GH. Treatment with isotretinoin inhibits lipofuscin accumulation in a mouse model of recessive Stargardt's macular degeneration. Proc Natl Acad Sci USA 2003;100(8):4742–4747. [PubMed: 12671074]

Radu RA, Yuan Q, Hu J, Peng JH, Lloyd M, Nusinowitz S, Bok D, Travis GH. Accelerated accumulation of lipofuscin pigments in the RPE of a mouse model for ABCA4-mediated retinal dystrophies following Vitamin A supplementation. Invest Ophthalmol Vis Sci 2008;49(9):3821–3829. [PubMed: 18515570]

Shroyer NF, Lewis RA, Yatsenko AN, Wensel TG, Lupski JR. Cosegregation and functional analysis of mutant ABCR (ABCA4) alleles in families that manifest both Stargardt disease and age-related macular degeneration. Hum Mol Genet 2001;10(23):2671–2678. [PubMed: 11726554]

Stone EM. Finding and interpreting genetic variations that are important to ophthalmologists. Trans Am Ophthalmol Soc 2003;101:437–484. [PubMed: 14971589]

Stone EM. Genetic testing for inherited eye disease. Arch Ophthalmol 2007;125(2):205–212. [PubMed: 17296896]

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 beta-subunit gene. Proc Natl Acad Sci USA 1993;90:3968–3972. [PubMed: 8387203]

Sun H, Nathans J. Stargardt's ABCR is localized to the disc membrane of retinal rod outer segments. Nat Genet 1997;17(1):15–16. [PubMed: 9288089]

Sun H, Nathans J. Mechanistic studies of ABCR, the ABC transporter in photoreceptor outer segments responsible for autosomal recessive Stargardt disease. J Bioenerg Biomembr 2001;33(6):523–530. [PubMed: 11804194]

Sun H, Molday RS, Nathans J. Retinal stimulates ATP hydrolysis by purified and reconstituted ABCR, the photoreceptor-specific ATP-binding cassette transporter responsible for Stargardt disease. J Biol Chem 1999;274(12):8269–8281. [PubMed: 10075733]

Sun H, Smallwood PM, Nathans J. Biochemical defects in ABCR protein variants associated with human retinopathies. Nat Genet 2000;26(2):242–246. [PubMed: 11017087]

Vonholdt BM, Pollinger JP, Lohmueller KE, Han E, Parker HG, Quignon P, Degenhardt JD, Boyko AR, Earl DA, Auton A, et al. Genome-wide SNP and haplotype analyses reveal a rich history underlying dog domestication. Nature 2010;464(7290):898–902. [PubMed: 20237475]

Webster AR, Heon E, Lotery AJ, Vandenburth K, Casavant TL, Oh KT, Beck G, Fishman GA, Lam BL, Levin A, et al. An analysis of allelic variation in the ABCA4 gene. Invest Ophthalmol Vis Sci 2001;42(6):1179–1189. [PubMed: 11328725]

Weng J, Mata NL, Azarian SM, Tzekov RT, Birch DG, Travis GH. Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice. Cell 1999;98(1):13–23. [PubMed: 10412977]

Wiik AC, Ropstad EO, Bjerkas E, Lingaas F. A study of candidate genes for day blindness in the standard wire haired dachshund. BMC Vet Res 2008;4:23. [PubMed: 18593457]

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(5):551–563. [PubMed: 16938425]

Zhang K, Qin Z, Chen T, Liu JS, Waterman MS, Sun F. HapBlock: haplotype block partitioning and tag SNP selection software using a set of dynamic programming algorithms. Bioinformatics 2005;21(1):131–134. [PubMed: 15333454]

Table 1

PRA-affected and control dogs by breed used in present study

Breed data		Dogs affected		Dogs not affected	
Breed	**Mode of inheritance**	**Age (years)**	**Sex**	**Age (years)**	**Sex**
ADT: Airedale terrier	No literature	2.5	F	2.2	F
AKI: Akita	Autosomal recessive	N/A	M	N/A	M
BAS: Basenji	Not defined	6.8	F	11.7	M
		N/A	M		
BMD: Bernese mountain dog	Not defined	8	F	8.7	M
BOT: Boston terrier	No literature	–	–	4.3	F
BRI: Briard	Autosomal recessive	12	F	–	–
BUM: Bullmastiff	Autosomal recessive	2.6	F	–	–
CCR: Curly coated retriever	Not defined	5.3	M	–	–
EMD: Estella mountain dog	Not defined	0.4	F	–	–
ESS: English springer spaniel	Autosomal recessive	7.8	M	–	–
GIT: Glen of Imaal terrier	Not defined	8	M	N/A	F
GOS: Gordon setter	Autosomal recessive	7.1	M	–	–
GSH: German shepherd	Autosomal recessive	7.5	M	–	–
GSP: German shorthaired pointer	Autosomal recessive	2.5	F	–	–
HUS: Siberian husky	X-linked	–	–	2.7	M
				3.5	M
IGH: Italian greyhound	Not defined	3	M	10	F
MLD: Dachshund, miniature longhaired[a]	Autosomal recessive	11	F	–	–
MSH: Schnauzer, miniature	Autosomal recessive	0.8	M	–	–
		3.1	F		
		N/A	F		
OES: Old English sheepdog	Not defined	6.1	M	–	–
PAP: Papillon	Autosomal recessive	5	F	7	F
ROT: Rottweiler	Not defined	7.5	M	–	–
RRB: Rhodesian ridgeback	No literature	–	–	N/A	N/A
SCW: Soft coated wheaten terrier	No literature	5	M	–	–
SSD: Shetland sheepdog	Not defined	8	M	–	–

Breed data		Dogs affected		Dogs not affected	
Breed	**Mode of inheritance**	**Age (years)**	**Sex**	**Age (years)**	**Sex**
TIS: Tibetan spaniel	Autosomal recessive	4.2	M	–	–
TT: Tibetan terrier	Autosomal recessive	4.8	M	11.8	F

Note that animals for which exact age is not available exceeded the breed typical age of onset for disease

[a]This animal is homozygous affected for a deletion in *RPGRIP1* (Mellersh et al. 2006)

Table 2

Based on observed allele frequencies in the presented data set, a minimum recommended screening set of 17 polymorphic markers is suggested, which allows identification of over 85% of all haplotypes present at more than 5% frequency in the overall dog population

Location		SNP	Alleles/Frequency		
CGD	Gene		CGD	Alternative	
58112873	5′UTR	BICF2P667777	G	A	0.71
58112955	5′UTR	BICF2P667778	G	A	0.37
58120873	Intron 1	BICF2P279470	T	C	0.20
58124046	Intron 3	NCBI_ss# 244242173	T	–	0.20
58124194	Exon 4	BICF2P814239	T	C	0.54
58134085	Exon 6	NCBI_ss# 244242177	T	A	0.43
58134379	Intron 6	NCBI_ss# 244242179	A	G	0.13
58148138	Intron 7	NCBI_ss# 244242181	T	A	0.75
58151928	Intron 9	BICF2P662491	C	T	0.08
58168353	Exon 13	BICF2P530460	A	C	0.47
58168436	Intron 13	NCBI_ss# 244242189	T	A	0.16
58180284	Exon 17	BICF2P1361455	G	C	0.74
58183106	Intron 18	BICF2P1361464	A	G	0.59
58194254	Intron 25	**BICF2P808832**	C	G	0.58
58202694	Exon 30	**BICF2P64836**	G	A	0.59
58207091	Exon 31	NCBI_ss# 244242214	G	A	0.17
58223870	Intron 41	BICF2P1380019	A	G	0.71

The minimum screening set can be reduced to 15 markers for reliable recognition of 80% of all haplotypes by eliminating BICF2P808832 and BICF2P64836 (bold). Position of the respective markers is indicated by the genomic location on CFA6 [canine genome draft (CGD), version May 2005] and respective location within the gene structure (Gene). Nomenclature reflects either inclusion in the previously published canine SNP set (Lindblad-Toh et al. 2005) or dbSNP accession number

Table 3

Nucleotide changes observed in canine *ABCA4* coding region and comparison to the human (NM_000350) and rhesus macaque (XR_012919.1) gene

Canine *ABCA4*				Human *ABCA4*		Rhesus *ABCA4*	
Location[a]	**Exon**	**Nucleotide change**	**aa**	**Nucleotide**	**aa**	**Nucleotide**	**aa**
58124194	4	c.303 T > C	Thr101Thr	C	Thr101	C	Thr101
58124332	4	c.441 G > A	Ala147Ala	A	Ala147	A	Ala147
58134085	6	c.588 T > A	Pro196Pro	G	Pro196	G	Pro196
58150351	8	c.867 T > C	His289His	T	His289	T	**Arg289**
58167425	12	c.1581 C > T	Ser527Ser	C	Ser527	C	Ser527
58168353	13	c.1880 A > C	**Glu627Ala**	A	Glu627	A	Glu627
58180284	17	c.2602 C > G	**Pro868Ala**	C	Pro868	C	Pro868
58198327	27	c.3958 G > A	**Ala1320Thr**	C	**Pro1322**	C	**Pro1322**
58201657	29	c.4254 A > G	Pro1418Pro	A	Pro1422	G	Pro1422
58201666	29	c.4263 G > A	Glu1421Glu	G	Glu1425	G	Glu1425
58201675	29	c.4272 A > G	Ala1424Ala	G	**Thr1428**	G	**Thr1428**
58202589	30	c.4368 T > C	Pro1456Pro	C	Pro1460	C	Pro1460
58202694	30	c.4473 G > A	Lys1491Lys	G	Lys1495	G	Lys1495
58202718	30	c.4497 T > C	Pro1499Pro	C	Pro1503	C	Pro1503
58207023	31	c.4548 A > G	Glu1516Glu	A	Glu1520	A	Glu1520
58207091	31	c.4616 G > A	**Arg1539Gln**	G	Arg1543	G	Arg1543
58216455	37	c.5227 G > A	**Val1743Ile**	A	Ile1747	A	Ile1747

Changes leading to substitutions in the amino acid (aa) sequence are highlighted

[a]Based on CFA6 of UCSC server canine genomic sequence v. May 2005 (http://genome.ucsc.edu/)