UNIVERSITY OF VETERINARY MEDICINE BUDAPEST

Institute for Animal Breeding, Nutrition and Laboratory

Animal Science.

Department for Animal Breeding and Genetics.

The Genetic and Clinical aspects of Common Canine Retinopathies, Progressive Retinal Atrophy.

Review of Literature by Kathleen McAlary

Diploma work

Supervisor; Professor. Zöldág László

Department for Animal Breeding and Genetics

University of Veterinary Medicine

Budapest, Hungary 2019

Table of Contents

1 Introduction

1.1 Background

Dogs, like humans, are affected by multiple hereditary disorders. In the dog, there are numerous inherited eye disorders both discovered and characterised. This is due to the eye being of accessible nature and by using non-invasive diagnostics, much of it can be examined in detail making the detection of abnormalities relatively easy. One group of these abnormalities comprises of retinal disorders or retinopathies, which creates a problem in canine breeding. Retinal diseases in dogs can be classified as stationary, developmental or progressive.

My literature review will be on the topic of common canine retinopathies, in particular, Progressive Retinal Atrophy (PRA) in dogs. It will be an overview of PRA as a disease, the genetic background of the disease, inheritance in different breeds, the clinical presentation, diagnostic tools, treatment options and Retinitis Pigmentosa in humans. I will also discuss briefly another similar retinal disorder in dogs, Cone Rod Degeneration.

PRA is a group of genetic diseases often referred to as an 'umbrella disease' seen in certain breeds of dogs. I will later discuss in more detail what PRA is, but, briefly, PRA is an inherited condition which causes deterioration of the retina. The extent of retinal involvement and time of onset in dogs determines the degree of visual impairment.

I chose this ophthalmology topic for my thesis due to my interest in lectures and practical's held by Dr. Szentgali at the University of Veterinary Medicine Budapest.

My own dog, a 14-year-old English Springer Spaniel called Oscar, developed a deep, traumatic corneal ulcer last year. He was treated by the veterinary ophthalmologist specialist Isabel Buehler. It was amazing to see the intricate work Isabel implemented when treating Oscar. Dr. Buehler has won the Northern Irish VSSCo bursary award in the field of ophthalmology and is a member of the British Association of Veterinary Ophthalmologists.

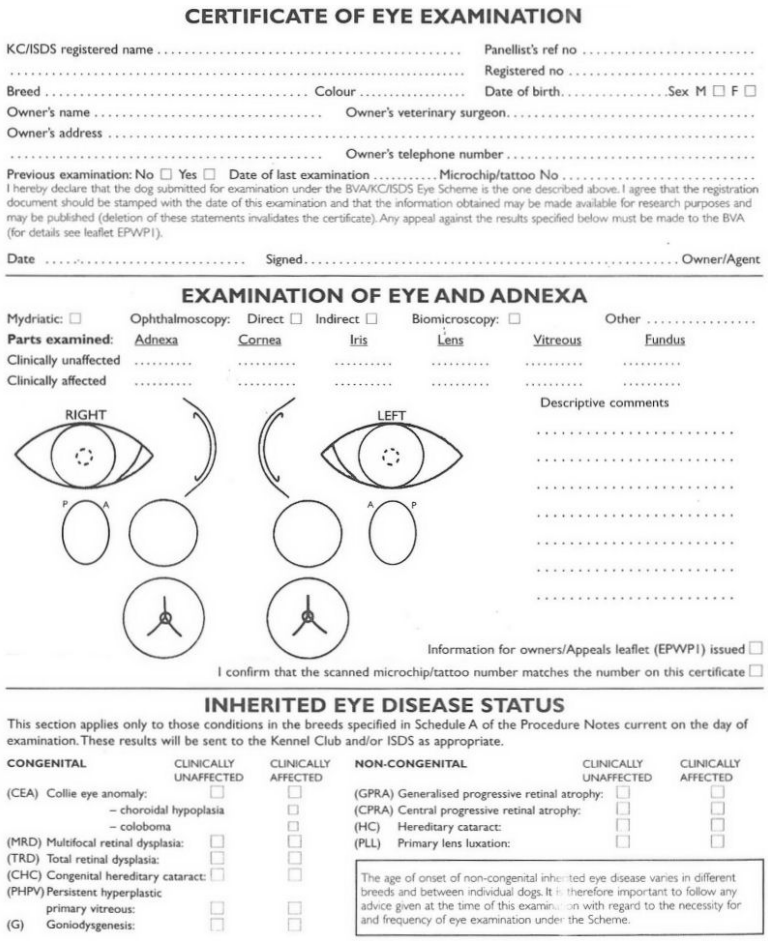
My dog, Oscar now has developing cataracts which also enhances my enthusiasm in the topic of ophthalmology. The English Springer Spaniel as a breed has quite significant inherited eye problems including RPED = Retinal Pigment Epithelial Dystrophy (formerly Central Progressive Retinal Atrophy, CPRA) and GPRA, Generalised Progressive Atrophy.

To further strengthen my knowledge in ophthalmology, I participated in a placement at Earlswood Veterinary Hospital, Belfast with their ophthalmologist expert Dr. Ian Millar. Dr. Millar has gained considerable clinical knowledge and surgical technique in complex cases. When working with Dr. Millar he undertook cataract removal surgery using the phacoemulsification method *(Figure 15 and 16).* Dr. Millar is an eye panelist for the British Veterinary Association (BVA) he believes that identification of inherited eye disease through The Eye Scheme in breeding dogs to be of outstanding importance.

The Eye Scheme is done in association with the BVA, the Kennel Club (KC) and the International Sheep Dog Society (ISDS). The scheme is based on a clinical eye examination and is a means of identifying inherited and non-inherited eye conditions in dogs. The results of the examinations should then be used to inform breeding programs. Puppies aged from 5 to 12 weeks old in a litter can be examined by the Canine Health Schemes for congenital inherited disorders such as collie eye anomaly and multifocal retinal dysplasia. Under the eye scheme, all dogs of 8 years and older that have been bred from should also be re-examined this allows for the identification of later onset inherited eye disease and gathers longitudinal information for further investigations. (BVA, 2019)

I was with Dr. Millar when he carried out eye examinations under the BVA eye scheme. Once the examination was complete, Dr. Millar issued a CHS (Canine Health Scheme) Eye Examination Certificate *(Figure 1)* which records the inherited eye disease status relevant to the dog being examined. The results are recorded as Clinically Unaffected (does not have the condition) or Clinically Affected (has the condition).

The results of registered Kennel Club dogs if a specific condition is known to be inherited and certified as such are published on a website, the Kennel Club Mate Select.



CERTIFICATE OF EYE EXAMINATION

KC/ISDS registered name ..... Panellist's ref no .....
..... Registered no .....
Breed ..... Colour ..... Date of birth..... Sex M ☐ F ☐
Owner's name ..... Owner's veterinary surgeon.....
Owner's address .....
..... Owner's telephone number .....
Previous examination: No ☐ Yes ☐ Date of last examination ..... Microchip/tattoo No .....
I hereby declare that the dog submitted for examination under the BVA/KC/ISDS Eye Scheme is the one described above. I agree that the registration document should be stamped with the date of this examination and that the information obtained may be made available for research purposes and may be published (deletion of these statements invalidates the certificate). Any appeal against the results specified below must be made to the BVA (for details see leaflet EPWP1).

Date ..... Signed..... Owner/Agent

EXAMINATION OF EYE AND ADNEXA

Mydriatic: ☐ Ophthalmoscopy: Direct ☐ Indirect ☐ Biomicroscopy: ☐ Other .....

Parts examined:	Adnexa	Cornea	Iris	Lens	Vitreous	Fundus
Clinically unaffected	.....	.....	.....	.....	.....	.....
Clinically affected	.....	.....	.....	.....	.....	.....

RIGHT LEFT

Descriptive comments
.....
.....
.....
.....
.....
.....
.....

Information for owners/Appeals leaflet (EPWP1) issued ☐
I confirm that the scanned microchip/tattoo number matches the number on this certificate ☐

INHERITED EYE DISEASE STATUS

This section applies only to those conditions in the breeds specified in Schedule A of the Procedure Notes current on the day of examination. These results will be sent to the Kennel Club and/or ISDS as appropriate.

CONGENITAL	CLINICALLY UNAFFECTED	CLINICALLY AFFECTED
(CEA) Collie eye anomaly:	☐	☐
– choroidal hypoplasia		☐
– coloboma		☐
(MRD) Multifocal retinal dysplasia:	☐	☐
(TRD) Total retinal dysplasia:	☐	☐
(CHC) Congenital hereditary cataract:	☐	☐
(PHPV) Persistent hyperplastic primary vitreous:	☐	☐
(G) Goniodysgenesis:	☐	☐

NON-CONGENITAL	CLINICALLY UNAFFECTED	CLINICALLY AFFECTED
(GPRA) Generalised progressive retinal atrophy:	☐	☐
(CPRA) Central progressive retinal atrophy:	☐	☐
(HC) Hereditary cataract:	☐	☐
(PLL) Primary lens luxation:	☐	☐

The age of onset of non-congenital inherited eye disease varies in different breeds and between individual dogs. It is therefore important to follow any advice given at the time of this examination with regard to the necessity for and frequency of eye examination under the Scheme.

Figure 1: BVA eye scheme certificate. (BVA, 2019)

I am writing my thesis under the supervision and mentorship of Professor. László Zöldág. His influence and knowledge in veterinary education on teaching of hereditary disorders and genetic diseases of domestic animals makes him a great mentor for my thesis.

1.2 The general objectives of the study

Areas I would like to focus on in particular with the topic of my thesis are;

- The genetics behind PRA, the identification of causative mutations of the disease and DNA tests.
- The prevalence of PRA in selected breeds. I will focus on in particular eight breeds and discuss which form of PRA they are predisposed to.

- The occurrence of the disorder today. With the further identification of inherited eye disease through for example the BVA eye scheme or through the use of genetic testing, their impact on breeding programs, is PRA becoming less frequent?
- Clinical aspects, in particular, late onset and early onset PRA, how these different forms present in the dog and which breeds they occur in most frequently.
- Different diagnostic approaches, the most common differentials of PRA, and potential treatment options for PRA. And;
- The condition of Retinitis Pigmentosa in humans, the bilateral degeneration of the retina. Can the dog act as an important animal model to further study this condition?

2 List of abbreviations.

AR:	Autosomal-recessive trait
BDNF:	Derived neurotropic factor
CNTF:	Ciliary Neurotropic Factor
CRD/CORD:	Cone-Rod Dystrophy
CPRA:	Central progressive retinal atrophy
DOK	Dortmunder Kreis, German Eye Panel
ERG:	Electroretinography
NTF:	Non tapetal fundus
NGF:	Neurotropic growth factor
NTF:	Non tapetal fundus
OE:	Ophthalmological examination
ONH:	Optic nerve head
PLR:	Pupillary light reflex
Prcd:	Progressive rod/cone degeneration
RCD:	Rod-Cone Dysplasia
RP:	Retinopathia Pigmentosa
PRA:	Progressive retinal atrophy
RPE:	Retinal pigment epithelium
RPED:	Retinal pigment epithelial dystrophy
TF:	Tapetal fundus

3 Progressive Retinal Atrophy, a Literature Review.

3.1 What is Progressive Retinal Atrophy, PRA?

PRA is defined as the degeneration of the retina this causes progressive loss of vision and eventually blindness.

In the condition of PRA, rod photoreceptor responses are lost first, followed by cone photoreceptor responses. Observed in PRA are fundus changes which are bilateral and symmetrical. This includes tapetal hyper-reflectivity in the early stages, followed by vascular attenuation and pigmentary changes. In the later stages of disease atrophy of the optic nerve head is observed. (Mellersh et al , 2013)

3.2 The first discovery of PRA

In Sweden, Magnusson in 1911 supplied the first detailed description of a canine inherited retinal degeneration affecting the Gordon Setter breed. He had the foresight to recognise a correlation in the condition and retinitis pigmentosa (RP) in man. Since then, PRA has been described in many breeds worldwide. (Petersen-Jones S. M., 1998)

3.3 Anatomy of the eye, the sensation of vision.

To interpret the perplexing nature and presentations of PRA we first must fathom the complex organ that is the eye and understand the clarity of canine vision by investigating the visual acuity.

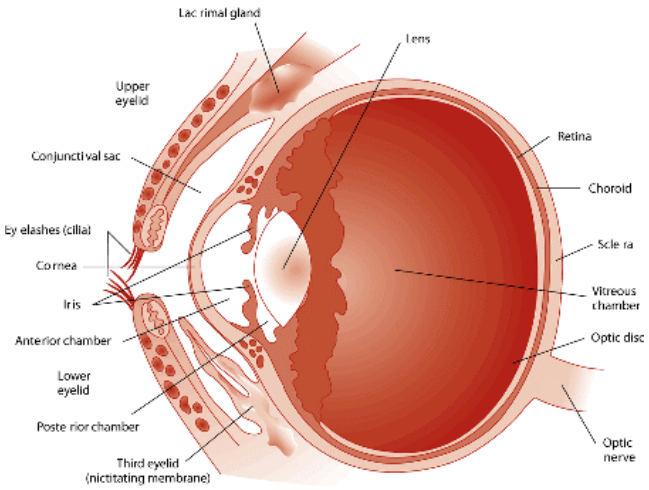


Figure 2: The eye (Gelatt, Eye Structure and Function in Dogs, 2014)

3.3.1 The sensation of vision

There are multiple factors involved in how well dogs see, the sensation of vision is thus of complex nature. How dogs view the world can be ascertained by describing their visual acuity, their abilities to detect light or colour, or the features of other individual visual parameters. (C.J. Murphy and P. E. Miller, 1995)

Visual Acuity; dogs have advanced night vision and their sight is developed well to detect movement. There is some compensation between visual acuity and the ability to see in the dark. In comparison to the human eye, the dog has a larger lens and a correspondingly larger corneal surface, enhancing its ability to capture light and therefore see in reduced lighting conditions. In addition, further enhancing low-light vision, behind the dog's retina is a reflective layer of tissue, the tapetum lucidum. However, the dog's visual acuity has been estimated at six times weaker than an average human. (Kidd, 2004)

3.3.2 The formation and function of the Retina.

It is important to understand the formation and function of the retina as the inherited disorder of PRA leads to its dysplasia and degeneration. In particular, the retinal layers, the photoreceptor cells and the blood supply.

From the neural ectoderm, neural crest, secondary mesenchyme and surface ectoderm the eye develops, with only minor contributions from the mesoderm. After birth, the retina of the canine matures between the ages, three to six weeks.

From the optic cup's posterior part, the retina originates and is made up of two layers of epithelium of neuroectodermal origin. Facing the imminent sclera, the outer epithelial layer will differentiate into the sensory retina. The subretinal space is the area in between the inner and outer epithelial layer. (David J. Maggs, 2013)

The Retinal pigment epithelium (RPE) is the outermost layer and forms from the outer-pigmented layer of the optic cup. The neurosensory layer forms from the inner, non-pigmented layer. (Simon M Petersen-Jones and Sheila Crispin, 2002)

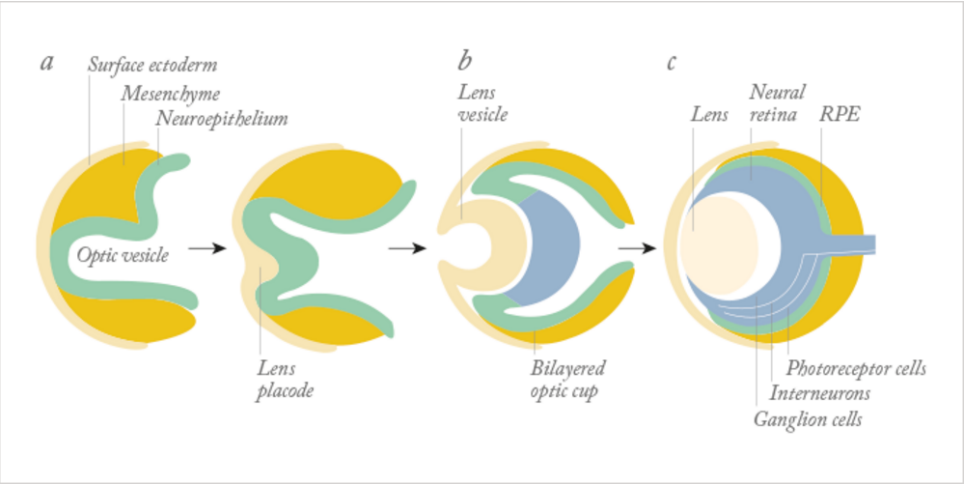


Figure 3: Formation of the Retina (Hillen, 2016)

The role of the retina; the retina's main functional responsibility is to transfer light into neuronal signals, which are processed and recognised as a visual image.

The signals travel from the retina, via the optic nerve, which crosses at the optic chiasm. Signals move from there to the lateral geniculate nucleus, via the optic radiation to the visual cortex. (David J. Maggs, 2013)

3.3.2.1 *Histology of the retina*

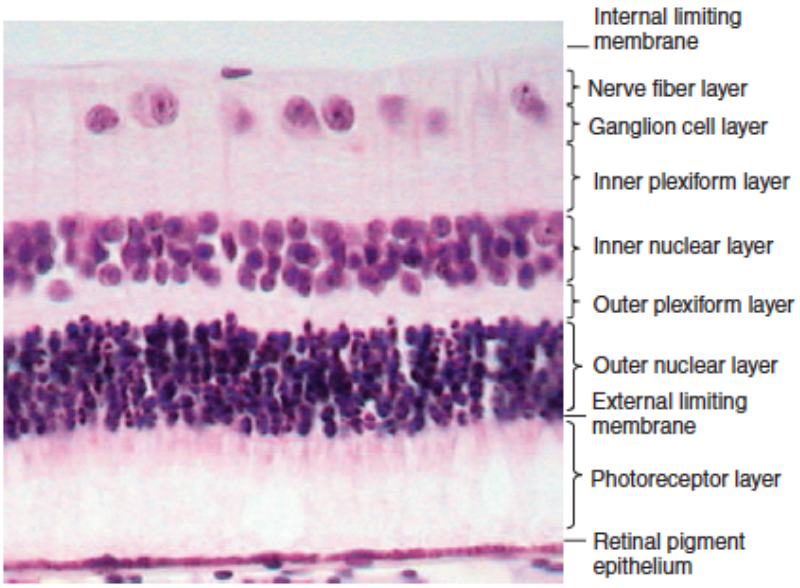


Figure 4: Retinal layers (David J. Maggs, 2013)

The retina has ten layers in total. The retinal pigment epithelium (RPE) is the outermost layer, strongly adherent to the choroid. The RPE's main functional responsibility is to transport nutrients from the choroid to the outer retinal layers and the phagocytosis of outer segments of photoreceptors as shedding is continuous.

The nine remaining layers of the retina form the neuroretina. These nine layers are considered from outside inward:

-The photoreceptor layer, containing the outer and the inner parts of the photoreceptors. This is where the light absorbing pigmented molecules are located.

-The outer limiting membrane.

-The outer nuclear layer.

-The outer plexiform layer.

-The inner nuclear layer.

-The inner plexiform layer.

-The ganglion cell layer.

-The nerve fibre layer and

-the inner limiting membrane containing muller cells are glial cells. These cells are vertically oriented in the neuroretina providing support and nutrition for the retina. (Liapis, 2004)

3.3.2.2 *Inner and outer segment of rods and cones, the photoreceptor layer*

A photoreceptor cell is a neuroepithelial cell found in the retina. Two classic photoreceptor cells are the rods and cones.

Rod and cone photoreceptors are specialised retinal neurons that have a fundamental role in visual perception. This involves the transferal of neuronal signals by capturing light. (Leonardo Murgiano et al, 2019)

Vision can be classified as scoptic vision, which is active in low level light, has no colour vision and is mediated by rod cells. The alternative, photopic vision, which is active with high light levels, capable of colour vision and high visual acuity mediated by cone cells.

Two to three weeks after birth, the development of photoreceptor cells occurs. After that, the organisation of the retinal layers is completed eight weeks postpartum. (Simon M Petersen-Jones and Sheila Crispin, 2002)

Cones are responsible for the perception of colour and visual acuity. They function in high light intensity, but are insensitive to small changes in brightness. Cones are sensitive to contrast. There are two types of cones in the dog, each containing a different protein, which provides partial colour vision. (David J. Maggs, 2013)

Rods are sensitive to small changes in brightness, but are less accurate than cones. They are able to detect motion and only contain one photo pigment. (David J. Maggs, 2013) Both dogs and humans use rod photoreceptors to function in dim light. The difference being, the central twenty-five degrees of the retinae in dogs consists predominantly of rods thus effectively improving vision in dim light. (C.J. Murphy and P. E. Miller, 1995)

3.3.2.3 *Blood supply of the retina*

Radiating from the optic disc and from three to four major veins, the canine retina has twenty cilioretinal arterioles. In the canine eye, a very short central retinal vein exists and retinal vessels tend to be more tortuous than in other animals. (Kristen Wrycha, n.d.) The retina has a high oxygen demand to attend its function and is a highly metabolically active organ. (David J. Maggs, 2013)

3.3.3 The Tapetum Lucidum

The Tapetum is situated immediately behind the retina. Anatomically, the tapetum lucidum is composed of multiple layers of cells arranged in a brick-like structure, rich in zinc and cysteine. (Yamaue, 2014) It is between 9 and 20 layers thick at its centre. The variety of colours seen in the region of the tapetum lucidum during ophthalmoscopy as represented in *figure 5*, result from the differential interaction of light with the tapetum's physical structure rather than from the inherent spectral composition, or colour, of its pigments. (C.J. Murphy and P. E. Miller, 1995)

The phenomenon of eye-shine in a number of vertebrates including carnivores is due to light reflection from the tapetum lucidum of the eyes. By reflecting light, the tapetum can increase vision in low level lighting. The tapetum is thought to reflect light back to the retina, thus enhancing scotopic sensitivity. It is positioned between the choroid capillary layer beneath

the RPE and the choroidal proper substance. In the tapetal area, the RPE lacks pigment so that the tapetum can be clearly seen through the retina. (Yamaue, 2014)

The tapetum lucidum enhances the dog's ability to detect objects in dim light, therefore enhancing scoptic vision. Presumably, it does so by reflecting light that has already passed through the retina back through it a second time, thus providing the photoreceptors at least two chances at capturing each quantum of light. This reflection has the unfavorable impact of scattering light during this process resulting in a reduced ability of the eye to precisely resolve the details of an image. (C.J. Murphy and P. E. Miller, 1995)

The canine tapetum is most likely less efficient at reflecting light than is that of the feline because of anatomic differences, but its light-reflecting properties are still undoubtedly substantial. (C.J. Murphy and P. E. Miller, 1995)

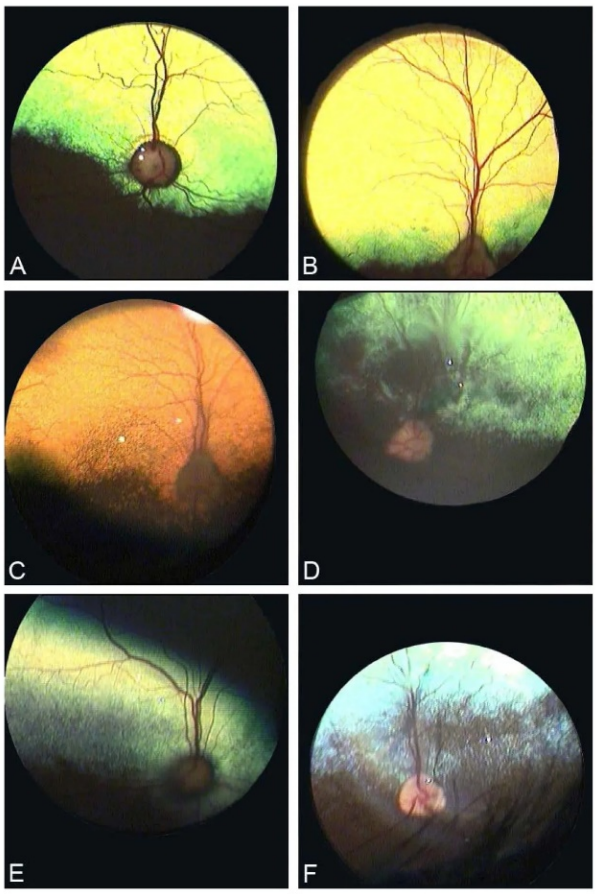


Figure 5; Examples of different colours of the tapetal area in the dog A: Yellow-green, B: Yellow, C: Orange, D: Green, E: Green, F: Blue-green. (Marie IKS Granar et al , 2011)

3.4 The Effects of PRA

The main effects of PRA are exerted upon the photoreceptor cells, the blood vessels, the tapetum lucidum and the optic nerve. I will later discuss how these changes appear with an ophthalmoscopic examination as represented in *figure 11* and *figure 13*.

The photoreceptor cells: PRA involves the retinal photoreceptors and two major types are recognised, developmental (dystrophies) and degenerative.

The developmental disorders are of early onset and involve the rod or cone photoreceptors, or both. The affected photoreceptors fail to differentiate normally thus the loss of photoreceptors and rate of progression is usually rapid. (Crispin, 2016) The photoreceptors appear to develop normally for the first 13 postnatal days, after which rod development ceases and rod degeneration has commenced by postnatal day 25. Although initially spared, cones have also begun to degenerate by about day 128, with total photoreceptor loss by approximately one year. (Peter J.M.Clement et al, 2013)

By contrast, the degenerative disorders usually involve photoreceptors that have differentiated normally, therefore less rapid progression and the age of onset is later. (Crispin, 2016)

The blood vessels: Concurrent with retinal death and thinning, the superficial retinal blood vessels become attenuated. Initially the smaller arterioles become more difficult to visualise, and then, as the condition progresses, larger vessels are obviously thinned. (Petersen-Jones S. M., 1998)

The Tapetum: Early signs observed are an initial granular appearance which then progresses to a generalized tapetal hyperreflectivity. At the periphery, there is often the appearance of radial bands of variable reflectivity due to grooving from underlying choroidal blood vessels. (Petersen-Jones S. M., 1998)

The optic nerve: In later stages of the disease, the optic nerve head becomes pale and is atrophied.

4 Genomics

The concept and origin of species along with the variation of each species was generated after the research work of Charles Darwin and Alfred Wallace, genetics therefore originated in 1858, with the theory of evolution.

Genetics is the study of heredity. Heredity is a biological process where a parent passes certain genes onto their offspring. From both of their biological parents every offspring inherits genes which express specific traits. Some genes may also carry the risk of certain diseases and disorders. (Mandal, 2019) In this case, genes that carry risk of canine retinal disorders and as consequence result in PRA.

Dog domestication has occurred over several thousand years. The artificial selection for desired traits during breed development in certain dog populations have inadvertently retained deleterious disease-associated mutations. The frequency of specific mutations and their association with disease vary among the different dog breeds depending on when the mutation occurred during breed development. (Shaffer, 2019)

4.1 The Genetics of PRA

It is valuable to study the genetics behind PRA in the aim to identify causative mutations of the disease and further develop DNA tests as a useful device for breeders to eliminate known PRA mutations. This is a necessity as the disease PRA causes a great deal of discomfort in the breeding industry as well as to the owners as the disease is incurable. (Millichamp, 1990)

The association between the gene mutation and the development of PRA is typically straightforward, with most being fully penetrant. However, the situation occasionally may appear to be more complex. (Miyadera et al, 2012) To date, in over one hundred breeds, thirty-two mutations have been associated with stationary and progressive forms. Twenty-five of these are associated specifically with PRA. (Rebekkah J. Hitti et al, 2019) It has become increasingly apparent that more than one form of PRA segregates in many of the breeds affected and that some forms of PRA affect more than one breed. (Mellersh et al , 2013)

Most of the mutations identified thus far, cause PRA to be inherited in an autosomal recessive manner (males and females are equally affected and that two copies of the mutation are needed to cause PRA) although some have been associated with X-linked and one case of autosomal dominant.

X-linked PRA has been found in mixed breed dogs like, the Siberian Husky and the Samoyed. The Siberian Husky; they commonly have night blindness by two to four years old. (Gelatt , 2014) In hemizygous male Siberian Husky's, the outer segments of rods are affected initially. With time, the rod outer segments almost completely disappear and cone outer segment degeneration becomes apparent. At the age of sexual maturity or young adulthood, affected males begin to show clinical signs. Female carriers demonstrate the rod specificity of disease. (Palanova, 2016) The Samoyed differs as it suffers a more severe X-linked PRA disease than the Husky. (Petersen-Jones S. M., 2003)

One breed has been found to have autosomal dominant PRA, the Mastiff (Old English- and Bull Mastiff). Examination of pedigrees demonstrated that the majority of affected individuals had an affected parent, indicating dominance. Controlled outcross matings were performed to confirm this dominant mode of inheritance. The appearance of affected offspring from the mating indicates the presence of a dominant allele. (Palanova, 2016)

Forms of PRA have been documented in numerous (more than one hundred dog breeds) and while they exhibit similar clinical signs, the aetiology, age of onset, and rate of progression vary between and within breeds. (Mellersh et al , 2013)

4.2 Genotypes

Three genotypes exist; clear, carrier and affected dogs.

Clear dogs will have no copies of the mutant gene and thus will neither develop the condition nor pass the gene on to their offspring. Carrier dogs have one copy of the normal gene and one copy of the mutant gene; they will not develop the condition, but will pass a mutant gene on to approximately half of their offspring. Affected dogs have two copies of the mutant gene that causes the condition and will therefore develop the disease. (TheKennelClub, n.d.) This is demonstrated in *figure 6* a Punnett square expressing the expected status of a dog when the parent possesses a certain genotype.

Parent 1 Status	Parent 2 Status		
	Normal/Clear	Carrier	Affected
Normal/Clear	All = Normal/Clear	1/2 = Normal/Clear 1/2 = Carrier	All = Carrier
Carrier	1/2 = Normal/Clear 1/2 = Carrier	1/4 = Normal/Clear 1/2 = Carrier 1/4 = Affected	1/2 = Carrier 1/2 = Affected
Affected	All = Carrier	1/2 = Carrier 1/2 = Affected	All = Affected

Figure 6: Expected status for breeding strategies (through use of genetic testing).

(Optigen, 2017)

4.3 Testing for PRA, Genetic testing

Currently, there is no treatment or cure for PRA; therefore, with the aim to reduce the frequency of PRA-associated variants in dog breeds, the use of genetic technologies to identify PRA-causing variants is crucial to facilitate diagnostic DNA test development for dog breeders and owners. (Rebekkah J. Hitti et al, 2019)

DNA tests are of great importance as they allow us to determine whether any dog is PRA affected, a carrier or clear of the disease. Testing can be done form an early age and predict a disease before it shows clinical signs.

Eye schemes enable breeders and owners to screen for a list of inherited eye conditions in certain breeds to reduce the prevalence of eye diseases. DNA tests can be used alongside clinical eye screening scheme. Eye screening schemes include the British Veterinary Association/ Kennel Club/ International Sheep Dog Society eye scheme in the UK and the ECVO. (European College of Veterinary Ophthalmologists). (Rebekkah J. Hitti et al, 2019) As represented by the Schedule A BVA scheme, *(Figure 7)* an alphabetical list of breeds and their eye conditions for certification under the Inherited Eye Disease Status section of the Certificate of Examination *(Figure 1)*. PRA (General PRA) along with other diseases is a problem in multiple breeds and therefore the prevalence should be reduced with an aim to ensure that in dogs used for breeding there is no clinical evidence of hereditary eye disease.

A better genotype–phenotype correlation among the affected breeds is achieved by having a diagnostic test in more than one clinical laboratory allowing the testing of a large number of dogs. (Shaffer, 2019)

Breeders can avoid affected puppies by selective breeding. Genes can be eliminated from a line. (Zöldág, 2008)

As these laboratories are direct-to-consumer, the canine genetic testing laboratory serves as an important resource for breeders and veterinarians. They should therefore be transparent and make sure to clearly communicate testing information on their website, reports and provide genetic counselling as needed. (Shaffer, 2019) Genetic testing is important for dogs that cannot be diagnosed through routine ophthalmology eye examinations or to catch early onset PRA. The test is easily executed by DNA extraction from blood samples and from buccal mouth swabs. (Mellersh et al , 2013)

Studies of canine PRA offer a source for novel candidate gene identification and target gene discovery for retinal disease across species. This includes humans, where a large proportion of patients still have an unknown molecular diagnosis. (Rebekkah J. Hitti et al, 2019)

4.3.1 Disadvantages of genetic testing

Genetic tests have their limitations because they identify only one specific mutation or one specific marker, or set of markers. But there may be multiple different marker combinations in a breed, which first of all, must be discovered and then be individually tested.

Currently, there is no uniformity regarding quality assurance within the testing community and no regulatory oversight for canine clinical genetic testing. The industry as a whole has to commit to improving processes with the aim to provide accurate results to the consumer. The individual laboratories have to implement protocols that help ensure high-quality testing and for providing critical resources to the customer. (Shaffer, 2019) A minimum baseline of high quality is thus required for all testing companies and laboratories to have reached before being certified to provide DNA tests.

It has to be deliberated that some breeds are affected by more than one genetic form of PRA. In many breeds, DNA tests fail to work as they have an acquired form of PRA that cannot be detected. (Optigen, 2017)

In 2014, a study performed demonstrated that PRA within a breed and among different breeds only represents genetic heterogeneity. Even though the dogs tested have PRA, out of a total of 231, 71 dogs did not have a positive blood test for PRA. (Mellersh et al , 2013)

4.3.2 The Tests

The Kennel Club acknowledges DNA tests and schemes in conjunction with Breed Clubs and laboratories. Results of such tests, in breeds of particular interest to the Kennel Club are recorded on the registration database and are available to view via the online tool Health Test Results Finder. (KC, 2019) If any abnormalities are found, they are registered so there is a clear transparency between veterinarians, breeders and owners.

There is a large number of laboratories which implement DNA tests across the UK. An example of such is OptiGen through Laboklin and Animal Genetics UK.

OptiGen, The OptiGen tests can be done on multiple dogs including young puppies. They own the patent for the prcd test in the UK, USA and Canada. OptiGen states that with genetic testing, there is no longer a need for test matings combined with expensive and potentially inconclusive ERG testing for PRA.

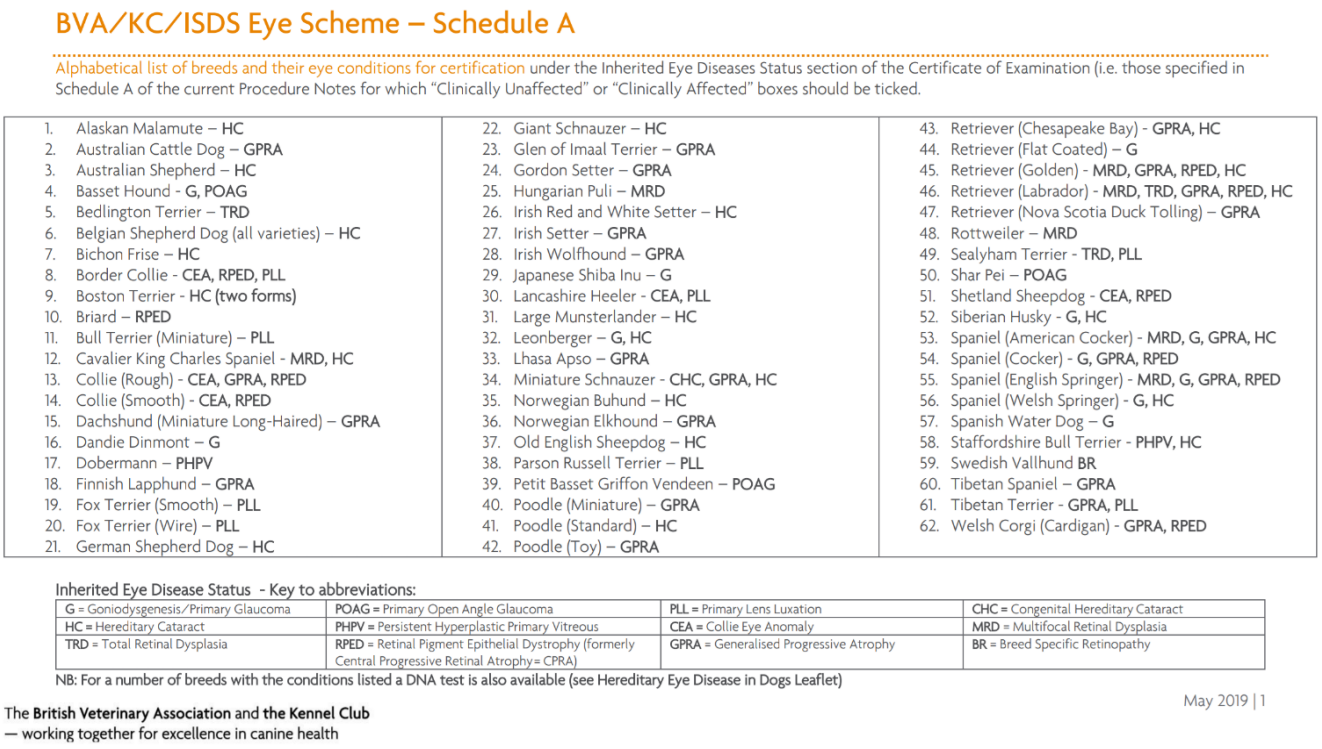
Because carriers and affected dogs having other desirable traits can be bred to normal/clear dogs, the increased value of the genetically tested dog is very high. OptiGen states that they are committed to an active program of continued research and development of faster, simpler, less-expensive testing procedures for all forms of retinal disease as well as for dozens of other genetic diseases that affect dogs. (Optigen, 2017)

Samples requires either whole blood in EDTA tube (0.5 - 1 ml) or Buccal Swabs. DNA is isolated from EDTA-stabilized blood using a salting out procedure. (Miller SA et al., 1988) The salting out method obtains high quality genomic DNA. DNA degradation and contamination are monitored on 1% agarose gels. Using a nanophotometer spectrophotometer, DNA purity is checked. DNA concentration is measured using a fluorometer. (P. Karlskov-Mortensen et al, 2018).

The main provider of the OptiGen test in the UK is, Laboklin. Since 1989, Laboklin has been providing wide array of diagnostic services in the areas of genetics, haematology, serology, microbiology, pathology, allergy, hygiene and molecular biology. (Laboklin, 2019)

***Animal Genetics UK*,** Animal Genetics UK can send to owners, breeders and veterinarians, a canine sample collection kit, this includes a canine buccal brush sample. Simplistic methods such as a buccal sample make the diagnosis of PRA a lot more accessible.

5 Prevalence of PRA in breeds



BVA/KC/ISDS Eye Scheme – Schedule A

Alphabetical list of breeds and their eye conditions for certification under the Inherited Eye Diseases Status section of the Certificate of Examination (i.e. those specified in Schedule A of the current Procedure Notes for which "Clinically Unaffected" or "Clinically Affected" boxes should be ticked.

1. Alaskan Malamute – HC
2. Australian Cattle Dog – GPRA
3. Australian Shepherd – HC
4. Basset Hound - G, POAG
5. Bedlington Terrier – TRD
6. Belgian Shepherd Dog (all varieties) – HC
7. Bichon Frise – HC
8. Border Collie - CEA, RPED, PLL
9. Boston Terrier - HC (two forms)
10. Briard – RPED
11. Bull Terrier (Miniature) – PLL
12. Cavalier King Charles Spaniel - MRD, HC
13. Collie (Rough) - CEA, GPRA, RPED
14. Collie (Smooth) - CEA, RPED
15. Dachshund (Miniature Long-Haired) – GPRA
16. Dandie Dinmont – G
17. Dobermann – PHPV
18. Finnish Lapphund – GPRA
19. Fox Terrier (Smooth) – PLL
20. Fox Terrier (Wire) – PLL
21. German Shepherd Dog – HC
22. Giant Schnauzer – HC
23. Glen of Imaal Terrier – GPRA
24. Gordon Setter – GPRA
25. Hungarian Puli – MRD
26. Irish Red and White Setter – HC
27. Irish Setter – GPRA
28. Irish Wolfhound – GPRA
29. Japanese Shiba Inu – G
30. Lancashire Heeler - CEA, PLL
31. Large Munsterlander – HC
32. Leonberger – G, HC
33. Lhasa Apso – GPRA
34. Miniature Schnauzer - CHC, GPRA, HC
35. Norwegian Buhund – HC
36. Norwegian Elkhound – GPRA
37. Old English Sheepdog – HC
38. Parson Russell Terrier – PLL
39. Petit Basset Griffon Vendeen – POAG
40. Poodle (Miniature) – GPRA
41. Poodle (Standard) – HC
42. Poodle (Toy) – GPRA
43. Retriever (Chesapeake Bay) - GPRA, HC
44. Retriever (Flat Coated) – G
45. Retriever (Golden) - MRD, GPRA, RPED, HC
46. Retriever (Labrador) - MRD, TRD, GPRA, RPED, HC
47. Retriever (Nova Scotia Duck Tolling) – GPRA
48. Rottweiler – MRD
49. Sealyham Terrier - TRD, PLL
50. Shar Pei – POAG
51. Shetland Sheepdog - CEA, RPED
52. Siberian Husky - G, HC
53. Spaniel (American Cocker) - MRD, G, GPRA, HC
54. Spaniel (Cocker) - G, GPRA, RPED
55. Spaniel (English Springer) - MRD, G, GPRA, RPED
56. Spaniel (Welsh Springer) - G, HC
57. Spanish Water Dog – G
58. Staffordshire Bull Terrier - PHPV, HC
59. Swedish Vallhund BR
60. Tibetan Spaniel – GPRA
61. Tibetan Terrier - GPRA, PLL
62. Welsh Corgi (Cardigan) - GPRA, RPED

Inherited Eye Disease Status - Key to abbreviations:

G = Goniodysgenesis/Primary Glaucoma	POAG = Primary Open Angle Glaucoma	PLL = Primary Lens Luxation	CHC = Congenital Hereditary Cataract
HC = Hereditary Cataract	PHPV = Persistent Hyperplastic Primary Vitreous	CEA = Collie Eye Anomaly	MRD = Multifocal Retinal Dysplasia
TRD = Total Retinal Dysplasia	RPED = Retinal Pigment Epithelial Dystrophy (formerly Central Progressive Retinal Atrophy= CPRA)	GPRA = Generalised Progressive Atrophy	BR = Breed Specific Retinopathy

NB: For a number of breeds with the conditions listed a DNA test is also available (see Hereditary Eye Disease in Dogs Leaflet)

May 2019 | 1

The **British Veterinary Association** and **the Kennel Club**
— working together for excellence in canine health

Figure 7: BVA Schedule A (BVA, 2019)

Today, PRA is reported in over 100 dog breeds and considered a significant health concern in purebred dogs. (Miyadera et al, 2012)

I will refer to in particular a selection of purebred dogs. Due to the high occurrence of PRA and literature available I will evaluate the Gordon and Irish Setter, the Miniature Poodle, the Cocker Spaniel, the Golden Retriever, the Labrador, the Basenji and the Dachshund.

The Gordon and Irish Setter

The Gordon Setter is in particular a valuable breed for the topic of PRA as it is the breed where PRA was first described. (Gelatt , 2014)

Progressive Retinal Atrophy in the Gordon Setter is clinically indistinguishable from PRA in other breeds. The mode of inheritance is consistent with an autosomal recessive trait and the age of diagnosis is typically around 10 years, resulting in the disease in this breed being referred to as late-onset PRA or rod–cone degeneration 4 (rcd4). Rod–cone degeneration is indicated from evidence from owners. The evidence suggests that affected Gordon Setters often present with nyctalopia (night blindness) initially, prior to more extensive visual impairment. (Mellersh et al., 2012)

In the closely related Irish Setter breed, the only form of PRA for which a causal mutation has been identified is rod–cone dysplasia (rcd1). The time of onset in the Red Setter is early. Photoreceptors are disturbed in their normal development and never manage to develop properly. Rcd1 is a recessive trait in which photoreceptors are rapidly lost. As already mentioned, it is an early-onset form of PRA that is caused by a mutation in PDE6B and that affects dogs before 1 year of age. (Suber et al., 1993) The Irish Setter has 4.5 times higher risk to be affected as a female.

The Miniature Poodle

In Miniature and Toy Poodles, PRA presents as Progressive rod-cone degeneration. Prcd-PRA is frequent and is the predominant form, causing 75% or more of all PRA cases in Miniature and Toy Poodles. (Optigen, 2017)

Progressive rod-cone degeneration (prcd) is an inherited as an autosomal recessive disease in the miniature poodle. Prcd is a degenerative disorder in which, after normal postnatal development, rods and cones degenerate both structurally and functionally it is therefore classified as a late-onset disorder. (Palanova, 2016) The disease manifests initially at 12–14 weeks, with apparent structural abnormalities in the outer segment part of the photoreceptors. With the use of an ophthalmoscopic, the disease cannot be diagnosed until 3–4 years of age, with blindness following after 5 years. (Aguirre et al, 1982). During the early stages (4–8 months), both rod and cone electroretinograms (ERGs) are normal. Over the next 12–26 months, rod ERG amplitudes begin to decline, whereas cone responses

remain sufficiently intact. (Nicholas J Willmott and Ali A.Hussain, 1996) In Bangladesh, the same mutation as the one in the Miniature Poodle was found in a patient with autosomal recessive retinitis pigmentosa. (arRP) This provides insight into the link between PRA and human RP. (Palanova, 2016)

The Cocker Spaniel

In the English Cocker Spaniel, PRA presents as Progressive rod-cone degeneration (prcd). However, the English Cocker Spaniel demonstrates a slower rate of photoreceptor degeneration histologically, compared to the Miniature Poodle (MP) and American Cocker Spaniel. (Koll-Hampp, 2019) The causative mutation in the newly identified gene *PRCD* (progressive rod–cone degeneration) was found; it is a single G to A transition at nucleotide 5 of the coding sequence, which causes a cysteine to tyrosine change (C2Y) at the second amino acid of the protein in the affected animals. (Palanova, 2016)

The Golden Retriever

In the Golden Retriever, there is a late onset PRA. The breed is affected by more than one form of PRA with mutations in three distinct genes. Both mutations are autosomal recessive. Two of such mutations are known as PRA1 and PRA2. PRA1 results from a mutation in the SLC4A3 gene and accounts for over 60% of diagnosed Golden Retrievers. PRA2 results from a mutation in the TTC8 gene and accounts for 30% of Golden Retrievers diagnosed with PRA. Clinical signs of GR-PRA1 appear around 6 years of age. Clinical symptoms of GR-PRA2 appear around 4 years of age. (UCDavis, 2019)

The Labrador Retriever

The most common eye disease in Labrador Retrievers is a late-onset form of Progressive Retinal Atrophy known as progressive rod-cone degeneration (prcd-PRA). (UCDavis, 2019) When on placement with Dr. Millar, a Labrador Retriever bitch was diagnosed with PRA through the use of both direct and indirect ophthalmoscopy. This was then documented on the BVA eye scheme certificate (*figure 1*) which states the bitch is clinically affected and shouldn't be bred.

The Basenji

The Basenji as a breed suffers from late onset PRA. The phenotype is similar to progressive rod-cone degeneration, prcd disease, but the prcd mutation was excluded as causative in the Basenji breed. (Palanova , 2016) Similar to almost every identified cause of PRA, Bas-PRA1 is inherited in an autosomal recessive manner.

The form of PRA that is caused by the Basenji PRA1 mutation is typically first diagnosed when dogs are, on average, five years of age. The initial symptoms consist of retinal thinning marked by vascular attenuation. Retinal degeneration progresses gradually and ultimately results in complete blindness. (Optigen, 2017) Many affected Basenjis retain adequate daylight vision for many years, sometimes for their entire life. In the S-antigen (SAG) gene transition mutation (T-C), which changes a normal stop codon to a code for the amino acid arginine, which would result in a deduced addition of 25 amino acids, was identified. (Palanova , 2016)

The Dachshund

The different sizes and coat varieties of the Dachshund complicate determining the forms and causes of PRA in Dachshunds. PRA has been diagnosed in the Rabbit, Miniature and Standard Dachshunds of Smooth, Longhaired and Wirehaired varieties.

Two forms of PRA are known to affect Dachshunds: cone-rod dystrophy 1-PRA (cord1-PRA) and cone-rod dystrophy PRA (crd-PRA). (Purina, 2010)

In a study of the dachshunds *(figure 8*), a total of 187 dogs were affected by PRA. This was 98 of all the males (2.5%) and 88 of all the females (1.1%). This is interesting as males are examined far less often than females. This highlights the importance of testing both sexes as the male was diagnosed with PRA more than twice as often.

Long-haired dachshunds were affected more frequently by PRA than the rest and from the information provided, the mean age of the first diagnosis was 5.5 ± 3.19 years.

A total of 67 dogs diagnosed with PRA were also diagnosed with Hereditary Cataracts, HC. The relative risk of being affected by a cataract, if diagnosed with PRA, was ten times higher than without PRA. Due to the limitation as to what is written in the eye certificates, it is possible that these cataracts might have been secondary and not primary. (Sarah Koll. et al, 2016)

Dachshund varieties		*n*–total	Progressive Retinal Atrophy		Hereditary cataract	
			n–affected	%	*n*–affected	%
Rabbit	Smooth haired	74	0	0	1	1.4
	Long haired	133	3	2.3	7	5.3
	Wire haired	282	3	1.1	7	2.5
	Total	489	6	1.2	15	3.1
Miniature	Smooth haired	211	2	0.9	3	1.4
	Long haired	636	14	2.2	26	4.1
	Wire haired	1284	6	0.5	38	3.0
	Total	2131	22	1.0	67	3.1
Standard	Smooth haired	1247	11	0.9	28	2.2
	Long haired	1403	32	2.3	69	4.9
	Wire haired	6970	116	1.7	302	4.3
	Total	9620	159	1.7	399	4.1
Total	Smooth haired	1532	13	0.8	32	2.1
	Long haired	2172	49	2.3	102	4.7
	Wire haired	8536	125	1.5	347	4.1
	Total	12240	187	1.5	481	3.9

Figure 8: Absolute and percent prevalence of HC and PRA in the various Dachshund varieties*, examined by the DOK, Germany in the years 1998–2011* (Sarah Koll. et al , 2016)

5.1 The prevalence of PRA today

DNA testing for autosomal recessive disease mutations in many dog breeds is now a common occurrence. As a result of probable selection based on the results of DNA testing, the 'hereditary status' of a dog and the result of DNA tests are reported to the UK Kennel Club and are used to determine changes in the frequency of disease causing mutations. (T. W. Lewis and C. S. Mellersh, 2019)

Statistics as of 09/1/2019

	1991-2014			2015-2019		
Breed	# Aff.	Total Exam.	% Aff.	# Aff.	Total Exam.	% Aff.
Gordon Setter	17	2,125	0.80%	N/a		
Irish Setter	18	1,995	0.90%	N/a		
Miniature Poodle	579	46,049	1.30%	13	8,378	0.20%
English Cocker Spaniel	423	10,746	3.90%	1	843	0.10%
American Cocker Spaniel	457	55,651	0.80%	12	5,596	0.20%
Golden Retriever	164	148,558	0.10%	17	36,182	0.00%
Labrador Retriever	978	221,401	0.40%	17	33,822	0.10%
Basenji	377	10,149	3.70%	4	1,300	0.30%
Dachshund	115	5,900	1.90%	12	1,019	1.20%

Figure 9: Statistics of PRA in breeds over a 28-year period (OFA, 2019)

These results demonstrate that in the time period between 1991 to 2014 and then from 2015 to 2019, there is a decline in the number and percentage of dogs affected by PRA. For example, the Labrador Retriever in the period between 1991-2014 had a percentage affected of 0.4%, this fell to 0.1% between 2015 and 2019.

This table does not include data on the breeds, Gordon (GDN) and Irish Setters between 2015 and 2019. In another study as represented by T. W Lewis and C. S. Mellersh below in *figure 10,* the number of affected Gordon Setters and Irish Setters have declined from the year 2000. The number of carriers remains moderately high, highlighting the importance of DNA testing.

As expressed by these results, dog breeders appear to be incorporating the results of DNA testing and ophthalmology examination into their selection strategies to successfully decrease the frequency of the mutation.

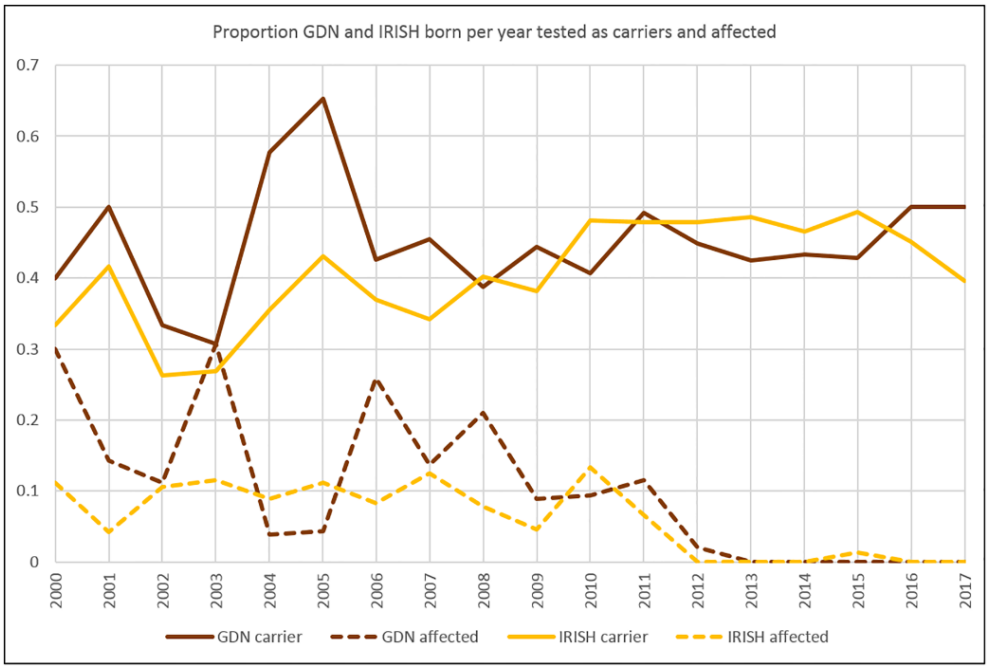


Figure 10: Proportion tested affected and carriers born per year. (T. W. Lewis and C. S. Mellersh, 2019)

6 Clinical aspects

Figure 11: Normal eyes vs. Eye with PRA. (ASTC, 2019)

6.1 Progressive Retinal Disorders

In animals, inherited and progressive retinal diseases are commonly referred to as Progressive Retinal Atrophy (PRA). They are characterised by progressive retinal degeneration resulting in loss of vision. (Mellersh et al , 2013) Progressive Retinal Disorders can be divided into two categories as represented in *figure 12*. Progressive Retinal Atrophy (PRA) and cone-rod dystrophy (CRD) are examples of two broad terms used to describe degenerative forms of progressive disease that both affect the retinal photoreceptor cells.

Distinct from PRA is a cone-led condition, Cone-rod dystrophy. However, due to similar ophthalmoscopic findings, the two conditions are often mistaken, and the umbrella term 'PRA' is often used ambiguously to describe both. (Mellersh et al , 2013)

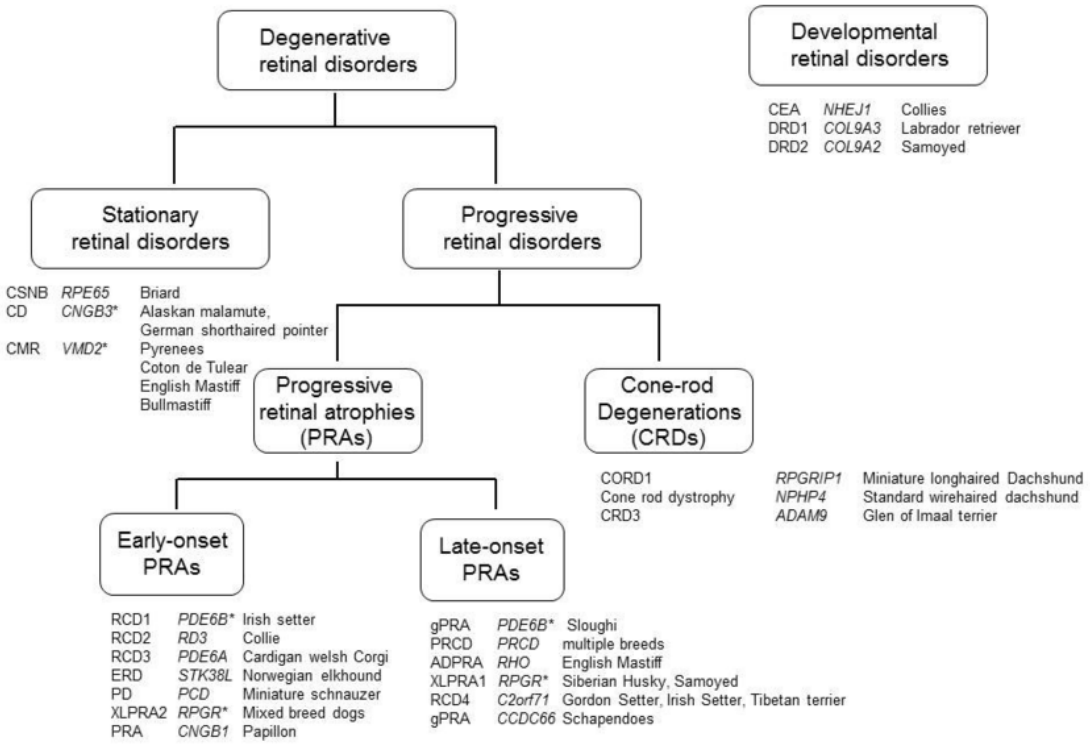


Figure 12: Degenerative Retinal Disorders (Mellersh, 2014)

6.1.1 Cone Rod Dystrophy

In Cone-Rod Dystrophy, a group of diseases, CRD1, CRD2, CRD3 and CORD1 have been identified.

In Cone–Rod Dystrophies, the cone system is primarily affected. The consequences of this as seen in affected dogs are the severe loss of central vision, colour vision and photophobia.

Cone-Rod Dystrophy as previously discussed occurs in Dachshunds. The Miniature Long-haired Dachshunds were found to have CORD1. With CORD1 first ophthalmoscopically observable signs were found in affected puppies at the age of 25 weeks. In the Standard Wire-haired Dachshund, CRD was identified. Initial onset of changes was observed between 10 months and 3 years, and changes were always bilateral and symmetrical. A complete retinal atrophy was evident at the age of 5–6 years. (Palanova , 2016)

In the Glen of Imaal Terrier, CRD3 was identified. It is a late onset disease, with the first signs presenting at 3 years of age in affected dogs and progressing to end-stage retinal degeneration over several years. (Palanova , 2016)

Two early onset retinal degenerations both in the American Staffordshire Terrier (CRD1) and American Pit Bull Terrier (CRD2) were identified. At the age of less than one year in both breeds, impairment of vision is observed. At early adulthood, the progression to severe blindness occurs.

6.1.2 Early Onset PRA

Early onset PRA is categorised under photoreceptor dysplasias and dystrophies. With early onset PRA, morphological and electrophysiological abnormalities are detectable prior to maturation of the retina, this is estimated around eight weeks in dogs. The consequences of early onset PRA are evident in early life and result in significant visual deficits. (Richard R. Dubielzig et al, 2010)

Early-Onset PRA includes Photoreceptor Dysplasias (pd) for example in Miniature Schnauzers (Optigen, 2017). Photoreceptor Dysplasia is an early-onset retinal disease that affects both rod and cone photoreceptors. It is transmitted in an autosomal recessive manner. In pd-affected dogs, a missense mutation was found (codon 82, CGA to GGA) in the phosducin (PDC) gene. (Palanova , 2016)

Another example of early onset PRA as represented in *figure 12*, is Rod-Cone Dysplasia (RCD) type 1,2 and 3. RCD1 occurs both in the Red Setter and the Sloughi, it is due to mutations (a different mutation in each breed) in the gene encoding beta subunit of cyclic GMP phosphodiesterase (PDE6B). (Palanova, 2016)

The Rough Collie, RCD2. The Rough Collie has a similar disease although the causal gene mutation has remained elusive. (Petersen-Jones S. M., 2003)

In the Cardigan Welsh Corgi, RCD3. It is due to a mutation in the alpha subunit of cyclic GMP phosphodiesterase (PDE6A) (Petersen-Jones S. M., 2003).

Early Retinal Degeneration and Rod Dysplasia are other forms of early onset photoreceptor degeneration and both occur in the Norwegian Elkhound. (Crispin, 2016)

Morphologic features of early onset PRA include: shortening, disorganisation and distortion of photoreceptor outer segments. These morphological changes result in the loss of photoreceptor outer and inner segments and the loss of photoreceptor nuclei with thinning of the outer nuclear layer. Although retinal disease is diffuse, often photoreceptor loss is not uniform across the fundus. The age of onset and relative extent of involvement of rods and cones is dependent on the underlying disease process. (Richard R. Dubielzig et al, 2010)

6.1.3 Late onset PRA

Late onset, photoreceptor degenerations or progressive rod cone degeneration (prcd). This retinal degeneration is inherited as an autosomal recessive trait and was originally described in the Miniature Poodle. (Palanova , 2016)

Although the photoreceptors appear morphologically normal in young animals, rod photoreceptor outer segments are often affected initially. They become shortened, disorganised and then degenerate. Ultimately, cone photoreceptors are also affected. Blindness ensues in middle-aged adults. (Richard R. Dubielzig et al, 2010)

Prcd is a late-onset form of PRA but the age of onset and rate of progression varies significantly between the breeds affected and also within the breeds, notably the English Cocker Spaniel. It is also suggested that genetically affected dogs in certain breeds may never develop clinical signs of PRA, this is also known as incomplete penetrance, examples of such is the Nova Scotia Duck Tolling Retrievers. (Petersen-Jones S. M., 2003)

Initially, many of the well described forms of PRA affect the rod photoreceptors and the damage is more considerable. Cone dystrophies and degenerations are a lot less common than disorders involving the rod photoreceptors, and many forms do not cause night blindness, or do not demonstrate rod photoreceptor abnormalities until late in the course of disease. (Richard R. Dubielzig et al, 2010) An example of cone degeneration is the Alaskan Malamute, a diseased dog at eight to ten weeks old has temporary loss of vision in daylight (hemeralopia). There is a purely rod cell retina by four years old. (Gelatt , 2014)

Morphologic features of longstanding PRA, in globes removed for other reasons, include: retinal atrophy, with the outer retina most profoundly affected and the presence of phagocytic cells within the neurosensory retina. Photoreceptor atrophy progresses to end-stage retinal atrophy with gliosis. (Richard R. Dubielzig et al, 2010)

Chronic PRA may lead to complications that include retinal detachment and cataract development, both of which may lead to lens luxation or lens-induced uveitis (Richard R. Dubielzig et al, 2010) and to glaucoma. (Labelle, 2017)

6.2 Clinical Features

The clinical features in PRA are notably similar whatever the underlying pathogenesis. Owners usually notice a loss of night vision, especially when the dog is in unfamiliar surroundings. The condition advances to generate a loss of vision under all lighting conditions. On examination, there is a poor pupillary light reflex with dilated pupils. When the disease progresses, a secondary cataract formation is common. (Crispin, 2016)

The diagnosis is based on clinical history (as represented above, walking into obvious objects such as table and chair legs), ophthalmologic and complementary examination such as ophthalmoscopy, electroretinography (ERG) and genetic testing. (Gomes et al., 2013)

Common clinical features include: Reduced pupillary light reflexes, Diffuse hyper-reflectivity of the tapetum, attenuation of the retinal blood vessels and pigment clumping visible in the non-tapetal fundus. (Richard R. Dubielzig et al, 2010)

7 Diagnostics

The canine eye can be examined in detail using non-invasive diagnostic methods. Its accessibility is thus, of great ease making it relatively easy to detect abnormalities.

7.1 Ophthalmologic Examination

To determine the clarity of vision and health of eyes, veterinarians use an ophthalmic examination. This examination includes a thorough evaluation of the eye structures externally, including the tissues surrounding, the eyelids, the tear ducts, also potential signs of cataracts in the lens and damage of the cranial nerves that affect the eyes. They will also use the pupillary light reflex (PLR), Menace Reflex and the falling cotton ball reflex. However, these tests have their limitations, they are not very reliable because the PLR and dazzle reflex are subcortical reflexes that do not need conscious visual perception of a stimuli. (Petersen-Jones S. M., 2003)

The maze test was described to me by Dr. Millar. Dr. Millar believes dogs are very intelligent animals and even if nearly completely blind they can orientate their way in common surroundings due to memory. However, if an obstacle is put in the way of a common path in their home surroundings it may be a different story and determine whether vision is present. The limitation of this test may be that the dog might not be obedient enough to perform the test or the dog may be too intimidated.

Visual placing response. This test lets the veterinarian assess the proprioceptive abilities of the dog. The animal is held by the examiner and moved towards a table. If the dog can see the table, it lifts the limb, on the table's surface, touching it with the palmar surface, before it touches the side of the table dorsally with the foot. (David J. Maggs, 2013)

7.2 The Ophthalmoscope

Both the indirect and direct ophthalmoscopy are used to evaluate the eye. Indirect technique is used to get a survey using the binocular view. The direct technique to examine specific areas under higher magnification with great detail using the monocular view. (David J. Maggs, 2013)

As previously mentioned, the main effects of PRA are evident in the tapetum lucidum, retinal photoreceptor cells, blood vessels and optic nerve head. The extent of damage or progression is determined through ophthalmoscopic examination. Ophthalmoscopic examination of PRA indicates a generalised, bilaterally symmetrical increase in tapetal reflectivity (a

consequence of retinal atrophy). There is attenuation of the retinal vessels, particularly the small peripapillary arterioles, which may become barely visible and are often referred to as 'ghost vessels' or disappear completely concurrent with retinal death and thinning. In dogs with a poorly developed tapetum or an atapetal fundus, the attenuation of the retinal vessels may be the only obvious ophthalmoscopic sign of early progressive retinal atrophy, careful observation is therefore required. (Crispin, 2016) This illustrates the importance of an extensive, detailed examination.

As the disease progresses, a pale optic disc is observed due to atrophy of its capillaries and nerve fibres and the non-tapetal fundus also shows extensive areas of depigmentation.

Cataracts, which form late on in the disease, may manifest as opacities in the posterior cortex, or as radial opacities, before advancing to total cataract. (Crispin, 2016)

The ophthalmoscope is an easy and fast way to examine an eye which may potentially have PRA however in temperamental and nervous animals, this may pose problems. Other methods such as electroretinography where by the animal is sedated may be of more diagnostic use for the diagnosis of PRA.

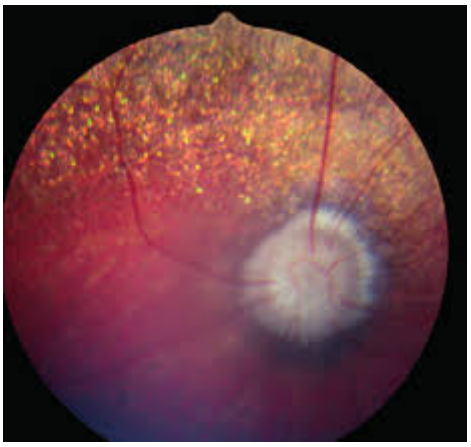


Figure 13: Generalised progressive retinal atrophy in a Cocker Spaniel.

An ophthalmoscopic examination, as represented in *figure 13*. The most obvious features observed in this dog a Cocker Spaniel include, an attenuation of the retinal vessels and pallor of the optic nerve head representing a progressed form of the disease. Tapetal islets (a normal variant) do not produce the striking hyperreflectivity seen with a more extensive tapetum. Vision was seriously damaged in this dog. (Crispin, 2016)

7.3 Electroretinography (ERG)

Electroretinography is used to evaluate the progression of retinal disorders; it is particularly useful as it can confirm early onset PRA. ERG is indicated when retinal disease is suspected and it also can help to identify the cause of blindness. (Liapis, 2004)

ERG helps to prevent an increasing number of animals being carriers or affected by PRA. It does so as it can confirm PRA in early stages (months or years) before the appearance of the ophthalmoscopic signs. The affected animals, confirmed to have PRA through ERG are excluded from reproduction. Indications of PRA include decreased b-wave amplitude, decreased flicker-fusion frequency and changed implicit times. (Liapis, 2004)

Dogs are exposed to dark adaption for a minimum of 20 minutes. Later the rod function is tested (scotopic ERG) initially for the right eye followed by left eye. Following scotopic ERG of both eyes, the dogs were subjected to light adaptation for a minimum of 10 minutes. Cone function was tested (photopic ERG) in a similar pattern. (D. N. Kelawala et al , 2017)

Two primary waves of the typical canine flash ERG can be recognised, the a and b wave. The a-wave, which is the first negative peak, refers to the hyperpolarisation of photoreceptors, the b-wave is the first positive peak, which follows the a-wave, and is principally generated by Muller cells. In addition, the c-wave it is a late positive potential after the b-wave. The c-wave refers to the function of RPE; however, it is unusual and is not examined in most cases.

This is represented below, in *Figure 14* when comparing the early onset of PRA with that of a normal dog

Implicit time is the time from the light flash (0 msec) to the a-wave trough and b-wave peak respectively. The implicit time and the amplitude of the a- and b-waves are used for interpretation of ERG. The a-wave amplitude is measured from the pre stimulus baseline to the a-wave trough whereas the b-wave amplitude is measured from the a-wave trough to the peak of b-wave. (Liapis, 2004)

ERG is a useful, objective tool used to indicated PRA, however it cannot be solely relied on as stated by Sarah Koll-Hampp.

ERGs are useful in supporting the suspicion of PRA but no conclusion on visual outcome associated with ERG results can be drawn. This is due to the fact the ERGs are not standardised. (Koll-Hampp, 2019)

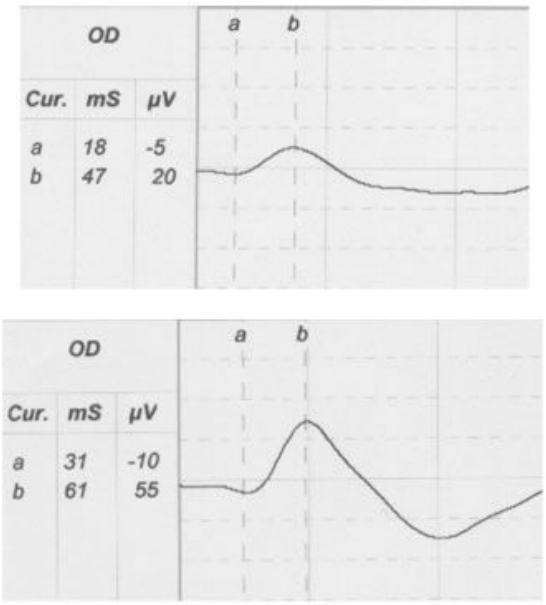


Figure 14: An ERG image comparing early onset PRA and a normal dog.

A scotopic ERG after 20min dark adaptation of a dog with early stage PRA, ***(top)*** in comparison with an ERG of a normal dog, ***(bottom).*** (Liapis, 2004)

7.4 Other Diagnostic methods

Here I will discuss potential and promising new diagnostic methods that have been implemented already in either the human or the dog and hold a promising future in the diagnosis of PRA.

Fluorescein angiography (FA), is a form of diagnostic imaging mainly used to determine the integrity of blood ocular barriers. FA provides direct visualisation of retinal vasculature and thus, allows the evaluation of posterior segment neoplasm, hypertension, retinal detachment, inflammatory processes, diabetic retinopathy and retinal degenerative processes. FA in veterinary medicine is performed under deep sedation or anaesthesia to avoid eye movement. (Aleksandra Tomkowicz et al, 2015)

Today, FA is currently limited in veterinary. This is due to the cost of the procedure, necessary equipment and differences of anatomy such as persistence of tapetum lucidum or degree of pigmentation. However, it is important to understand, fluorescein angiography is a new field in ophthalmology, the number of animal reports on this subject increasing. As a result, it can be stated that it is the future of human ophthalmology as well as veterinary medicine. (Aleksandra Tomkowicz et al, 2015)

Confocal scanning laser ophthalmoscopy (CSLO), is a video technique that aids ocular fundus image recording and retinal dynamic angiography to be performed. The ocular fundus image is acquired successively, point by point, and is formed on a video monitor at an impressive rate of 25 images per second. (Serge G. Rosolen et al , 2001)

Optical Coherence Tomography (OCT), is a noninvasive, noncontact imaging technique. OCT is capable of producing high-resolution images of the retina and optic nerve. The progression of the disease can be followed by the information provided in these accurate images. With rapid advances in OCT technology images are becoming increasingly more detailed. (Gillian J. McLellan et al, 2012)

The principle operation of OCT is largely comparable with ultrasonography, except that it uses light rather than sound. OCT, unlike ultrasonography, allows measurement of structures and distances and does not require any contact with the eye (therefore avoiding the potential for tissue compression and/or distortion). Ultrasound differs as it requires direct contact between probe and tissues or between probe, coupling medium and tissues.

However, adapting instrumentation designed for use in human patients for use in animals can be challenging. (Gillian J. McLellan et al, 2012)

7.5 Differential Diagnostics

It is important to consider the differentials of PRA as many diseases' presentations are very similar and comparable to PRA. The difference however in some cases, these diseases can be treated and cured unlike that of PRA.

Differential diagnoses for retinal degeneration include, sudden acquired retinal degeneration syndrome (SARDS) in dogs, Toxic retinopathies, Choroidal perfusion problems/vascular disease, Nutritional retinopathies such as Vitamin E deficiency in dogs, diseases of the RPE such as lipofuscinosis. (Richard R. Dubielzig et al, 2010) Retinal detachment (neoplasia, retinal dysplasia, hereditary/congenital, exudative/transudative disorders such as systemic hypertension or infection-induced inflammatory disease. (Thompson, 2018) Radiation and light-exposure can cause retinopathies that damage the vulnerable photoreceptors. (Gelatt , 2014)

8 Treatment

There is currently no treatment available for dogs with PRA. It is a complex disease where by with damage of these extremely vulnerable neural cells, the photoreceptor cells, there is a low chance of repair. Therefore, salvation of these cells proves very difficult. Veterinarians can treat the disease symptomatically to salvage vision and reduce anxiety.

8.1 Simplistic measures

Simplistic measures, as recommended by (DDC Veterinary , 2018), owners should make their dog more comfortable by keeping furniture in its current familiar location and being sure to keep the floor clear of obstacles such as toys. Also, other measures such as taking their dog on a well-known route during walks to help prevent anxiety.

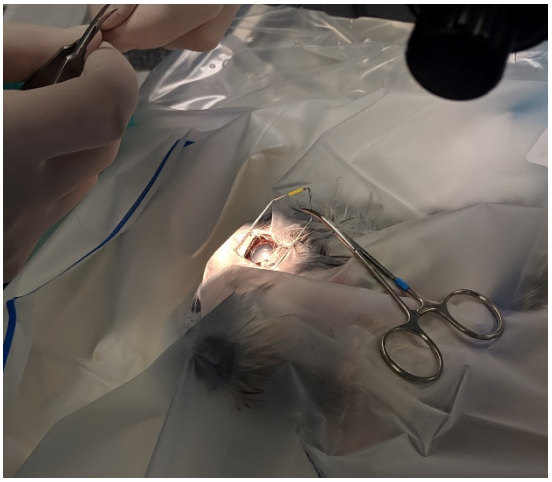
8.2 Antioxidants

Antioxidants supplementation was trialed in dogs in a study lasting six months and the pre- to post-treatment levels with ERG were compared. The dogs who received antioxidant supplementation demonstrated an increase in a-wave and b-wave amplitudes, and a decrease in the implicit time for the a-wave, and a decrease in implicit time for the b-wave when compared with those in the control group of dogs. Antioxidants can therefore improve retinal responses as measured by ERG and also slow down the refractive error myopic shifting. (Wang, 2016) Affected dogs diagnosed with PRA before complete vision loss occurs may be helped by antioxidant support, to save some day vision (cone function). (Terri L McCalla, n.d.)

8.3 Phacoemulsification

Phacoemulsification is surgical method of cataract removal in which the eye's internal lens is emulsified by an ultrasonic hand piece and then from the eye it is aspirated.

Phacoemulsification was carried out in a study on an English Cocker Spaniel with suspected PRA and secondary cataract development. It was found that visual improvement can be obtained for some time, but as expected the day vision will be lost eventually. No predictor for a successful visual outcome following surgery could be identified. However, vision was present for up to two years in this study population, and this was noteworthy when comparing to vision before surgery. (Koll-Hampp, 2019)



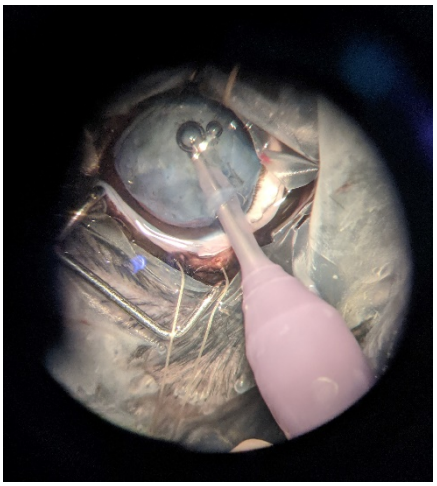


Figure 15 and Figure 16: *Phacoemulsification Cataract removal by Dr. Ian Millar. I took these photos while on placement at Earlswood Clinic.*

9 Link with humans

As suggested by Magnusson in 1911, canine PRA can serve as a natural model for human Retinitis pigmentosa (RP).

From investigating and studying the disease PRA, a lot may be learned regarding retinal biology. PRA represents an increasing clinical spectrum and it is important to acknowledge and describe new forms since. (D. N. Kelawala et al , 2017) Dog breeds with inherited PRA are natural animal models. They can be used to investigate the genetics, disease progression and therapies in dogs. This will in turn provide benefit of both dogs and humans. (Morgane Bunel et al , 2019)

In a recent study from the general population, it was indicated that approximately one in four to five individuals may carry a null mutation responsible for human retinal disease. (Nishiguchi KM et al , 2012) Among inherited human retinal diseases, 50% are pigmentary retinopathies called Retinitis pigmentosa (RP). (S P Daiger et al, 2013) Almost 200 genes have been identified to be involved in human retinal disorders, and the number of genes known to be involved in canine retinal disorders is likely to increase. (A.C Wiiki et al., 2015)

Recent studies now effectively allow the identification of mutations involved in canine genetic diseases. This is done by investigating the canine genome and through the accessible nature of new genotyping and sequencing technologies. To date, PRA genes identified in

dog breeds correspond to the same genes in humans and represent relevant retinitis pigmentosa (RP) models, and new genes found in dogs represent good candidate for still unknown human RP. (Morgane Bunel et al , 2019)

Early retinal degeneration (erd) was identified in Norwegian Elkhound dogs as an early-onset autosomal recessive disease corresponding to human Leber congenital amaurosis a family of congenital retinal dystrophies (LCA), through its gene, STK38L/NDR2. The Norwegian Elkhound dog can act as a naturally occurring erd animal model which will provide new insights into PR development and role of STK38L protein in other, especially neuronal tissues. (Palanova , 2016)

Identifying the dog as a naturally occurring animal model for human diseases is an excellent opportunity for the development of future treatments/therapeutic strategies in PRA or RP. Importantly, the dog as a homologous model of human RP is fully compliant with the 3R (Reduce, Refine, Replace) rule for managing animal models, replacing experimental models such as the rodent in some instances. (Morgane Bunel et al , 2019)

The research into the link of human and dog retinal disorders is important as it helps with the development of diagnostic tests and also better predictions. It allows veterinarians to investigate potential treatments and better management of the disease.

9.1 New methods

I will list some methods that have been investigated as potential treatment for RP in humans and consequently represent a promising future for the treatment of PRA in dogs.

9.1.1 Nerve Growth Factor, NGF eye drops.

One of the first indications of NGF's ability to protect photoreceptors was published in 1996 using the mouse strain C3H, characterised by the progressive photoreceptor degeneration during early postnatal life. The authors demonstrated that intravitreal NGF administration was able to delay photoreceptor degeneration. (Lambiase A , 1996)

In a more recent study, the short-term administration of NGF eye-drops caused neither significant adverse effects nor visual function losses in the tested RP patients. A minority of patients experienced an improvement of visual performance as shown by a visual field test and ERG. This study therefore supports the safety and possible efficacy of NGF eye-drops administration in RP patients. (Benedetto Falsini, 2016)

9.1.2 Microfluidic chip

The microfluidic chip mimics the human blood-retinal barrier (BRB) in the eye. The device mimics the layered structure of the retina by its composure of several parallel compartments. In each compartment, a specific cell type is grown to replicate the different layers as closely as possible. (Dale, 2018)

The multi-compartment microfluidic device with integrated electrophysiological monitoring electrodes will allow scientists to make more accurate measurements of cell behaviour. It can therefore be anticipated as a promising new approach for recapitulating multi-barrier models. In particular, it will facilitate the study of retinal diseases affecting blood-retinal barrier integrity and neurovascular coupling. (Jose Yeste et al, 2018)

9.1.3 Calcium-blockers

Calcium blockers can be administered to prevent retinal degeneration. In a study of the administration of four different calcium antagonists (D-cis-diltiazem, nifedipine, nicardipine, and nilvadipine) and their vehicle solutions to 9-day-old mouse retina daily for 7 days, the thickness of each retinal layer was compared to study the effects of Calcium-2-þeta antagonists on the retinal morphology of the mouse. To study the effects of these Calcium-2-þeta antagonists on retinal function, ERG responses were measured. In the study, nicardipine and nilvadipine protected against degeneration of retinal layers of mice. Most importantly, nilvadipine showed significant preservation of retinal layer. (Yoshiko Takano et al, 2003) However in other reports such as that by (Miren Agurtzane, 2009) , calcium blockers have shown limited success and provide no beneficial effect.

9.1.4 Valproic acid (VPA)

VPA can exert its effects through a wide variety of mechanisms, such as indirectly enhancing GABA signalling, influencing cell signalling and glycogen synthase kinase, reducing Endoplasmic Reticulum stress and inhibiting histone deacetylases (HDACs). It was found that VPA, potentially acting through HDAC inhibition and autophagy, can promote the clearance of misfolded rhodopsin, slow rates of photoreceptor death and preserve visual activity. This data supports VPA or inhibition of HDACs in general, as a potential treatment for some forms of RP. However, it has been found that VPA treatment was not beneficial across genotypes and accelerated degeneration. (Levi Todd, 2017)

9.1.5 Ciliary neurotropic factor (CNTF) implants

Ten participants received CNTF implants in one eye. After 6 months, when the implants were removed, they contained viable cells with minimal cell loss and gave CNTF output, at levels previously shown to be therapeutic for retinal degeneration in rcd1 dogs. The results raise the intriguing possibility that CNTF may improve visual acuity in some eyes with advanced RP and atrophic macular degeneration. (Paul A. Sieving et al., 2006)

9.1.6 Gene Therapy

In general, gene therapy is best when performed at early stages of RP (the early dysfunctional phase), the therapeutic success is narrowed as the disease progresses and more photoreceptors are lost. A Study in dog models was performed in 2013, it indicated that gene therapy is highly protective in the early dysfunctional phase, but is not able to save photoreceptors once the disease progressed to the degenerative stage. (Viviana Guadagni et al , 2015)

Gene Therapy is currently a new area of RP treatment. A recent study performed by a team lead by the University of Pennsylvania vision scientists is proving successful in treating a form of retinitis pigmentosa. The scientists could disrupt the mutant rhodopsin gene using a knockdown reagent called short-hairpin RNA (shRNA) engineered to target an area away from the mutated sections of the gene. They then added back a healthy copy of the rhodopsin gene that is resistant to this shRNA compensated for the knockdown. In dogs with the rhodopsin mutation, they used the same strategy, finding the best success when both the shRNA and the healthy copy of rhodopsin were co-delivered in the same vector, as opposed to using two different vectors. The team restored roughly 30 percent of the normal level of rhodopsin, enough to prevent deterioration of rod cells in the retina. (University of Pennsylvania, 2018)

9.1.7 Retinal Replacement Therapy, Neuronal Transplantation

Neuronal transplantation aims to replace photoreceptors that have been lost. Either embryonic cells or retinal tissue is implanted into the subretinal space. In one study where rod rich retinal sheets were implanted in mice, the implant formed neuronal connections to the endogenous retina. The remaining cones survived longer. (Miren Agurtzane, 2009) However, due to ethical considerations limited availability of human tissue, it is important to find an alternative cell source for retinal replacement therapy.

Transplantation of stem cells and/or progenitor cells, including retinal progenitor cells, bone marrow-derived cells, and induced pluripotent cells, may eventually provide an alternative approach to restore vision. (Shibo Tang et al, 2013)

9.1.8 Stem Cell Therapy

Seventeen patients with bilateral visual loss due to Retinitis Pigmentosa underwent autologous bone marrow derived stem cell treatment within the Stem Cell Ophthalmology Treatment Study (SCOTS). The bone marrow derived of the SCOTS achieved meaningful visual acuity improvements or stability in RP patients. Duration of disease did not appear to affect the ability of eyes to respond. Safety was confirmed. Given the successful outcome in this otherwise progressive condition, consideration should be given to providing this treatment option. (Levy, 2018) The impact of stem cells can be improved by combining them with growth factors. (Miren Agurtzane, 2009)

9.1.9 Retinal prostheses

Retinal prostheses are devices that electrically stimulate the retina. Restoration of vision may be achieved by this stimulation. Retinal prostheses receive and process incoming light and then transmit the information in the form of electrical impulses to the remaining inner retinal layers for visual function. Five companies have performed chronic implants of retinal prosthetic devices in humans. Three of the groups have used an epiretinal approach, the other two a subretinal approach. (Rizzo, 2010)

It has been demonstrated that retinal network architecture undergoes significant remodelling as a consequence of photoreceptor degeneration. The future remains uncertain how an improvement in vision may be achieved by retinal prosthesis devices throughout the time course of these remodelling processes however, with at least five retinal prosthesis systems either currently undergoing or nearing human clinical trials for outer retinal degenerative diseases including one for advanced macular degeneration, it seems the future of retinal prosthesis devices holds promise. (Leo A. Kim, 2019)

10 Conclusion

The aim of my thesis was to review literature on the present state of genetics and clinical aspects of Progressive Retinal Atrophy. From my findings and data presented by the OFA this year, over a twenty-eight-year period, the incidence of PRA has declined. This is due to the increased education of breeders, owners and veterinarians. Schemes such as that of the BVA and ECVO allow breeders, owners and veterinarians to screen for inherited disease and it allows for transparency within the breeding industry.

Alongside ophthalmologic tests by an experienced specialist, ERG and newly developed diagnostics such as fluorescein angiography, genetic testing is the key to reducing the incidence of PRA. Although the tests are not fully reliable and cannot detect unknown mutations, tests have many benefits including their use at a young age.

PRA has proven to be a popular subject among scientists and ophthalmologists both in human and veterinary medicine. Expanding research has been carried out in the area of PRA as new genes found in dogs represent good candidates for still unknown human Retinitis Pigmentosa.

Due to the perplexing nature of the disease and sensitive neural cells that have a low chance of repair, treatment proves at this time, impossible. The disease can only be treated symptomatically through for example supplementation of antioxidants and removal of secondary cataracts through phacoemulsification.

From researching new treatment methods such as the exciting retinal prostheses, the future looks promising for human RP. However, a lot of these recent and advanced methods come with complexity, expense or technical issues that cannot be implemented when treating a dog today. With more owners willing to insure and pay for expensive treatments for their pets hopefully in the future these promising methods can be achieved with the dog.

In the present day, the best way to manage PRA is for everyone, breeder, owners and veterinarians alike is to be under the impression that “prevention is better than cure.”

11 Summary

PRA is an autosomal inherited condition that causes deterioration of the retina affecting photoreceptor cells the extent of retinal involvement and time of onset in dogs determines the degree of vision impairment. The aim of my thesis was to give a comparative literature review of common canine retinopathies, in particular Progressive Retinal Atrophy today.

I focused on genetic testing, screening, the different forms of the disease, how it presents in different breeds, the prevalence of the disease today and new methods and studies to help find a treatment. Currently there is a no treatment available. Only symptomatic treatment is feasible through antioxidant supplements and simplistic measures.

The dog is an excellent model to naturally investigate Retinopathia pigmentosa, a disease in humans with similar clinical signs and disease progression like in PRA of the dog. The fact the dog is a natural animal model is an extraordinary opportunity for the development of future treatments/therapeutic strategies in PRA or RP.

As summarised by Anna Palanova, “the dog, man’s best friend, represents not only our assistant in many facets of our lives, it is our pet, friend, and a suitable animal model of many diseases too. Dogs are dependent on our care and protection, and that is why we must take care of their health and welfare.”

12 Materials and Methods

12.1 Textbooks

When completing my thesis, I used many textbooks both online and borrowed. For greater insight into the genetics I used a book written by my thesis supervisor, László Zöldág – Veterinary genetics and animal breeding. It proved very useful in getting to grips with basic genetic terminology and also to help build the foundations of my knowledge of inherited ophthalmology disorders. For a more in depth understanding of ophthalmology I used:

BSAVA Manual of small animal Ophthalmology, Kirk N. Gelatt, Caryn E. Plummer ;Colour Atlas of Veterinary Ophthalmology, Kirk N. Gelatt ;Veterinary Ophthalmology and Slatter’s; Fundamentals of Veterinary Ophthalmology.

12.2 Online research

I found very useful, the BVA website in particular their article “Hereditary eye disease in dogs” and the Kennel Club website for information on particular breeds.

I used Ebsco Discovery Service, NCBI, Wiley Online Library and Research Gate to analyse articles. I used the following research criteria to obtain my literature; (Progressive Retinal Atrophy), (Progressive Retinal Atrophy) AND (dog), (Retinitis Pigmentosa) AND (dog) AND (human) and (Retinopathia Pigmentosa).

Three authors I found particularly insightful and interesting were Anna Palanova, David J Maggs and Simon M Petersen-Jones.

12.3 Placement

My placement at Earlswood Veterinary clinic in Belfast with the ophthalmologist Ian Millar was very insightful and helpful. Dr. Millar described to me the steps of an ophthalmological exam, I was with him in consultations when he implemented these exams and diagnosed different eye disorders including PRA. As a BVA specialist he highlights the importance of diagnosing inherited eye disorders in dogs.

13 Bibliography

A.C Wiiki et al. (2015). Progressive retinal atrophy in Shetland sheepdog is associated with a mutation in the CNGA1 gene. *Animal Genetics*. 515-521

Aguirre et al. (1982). Non-allelism of three genes (rcd1, rcd2 and erd) for early-onset hereditary retinal deneration. *Experimental Eye Research*, 610-30.

Aleksandra Tomkowicz et al. (2015). Use of fluorescein in Veterinary Ophthalmology. Angiography. *EEJVO*.

ASTC. (2019). *American Staffordshire Terrier Club of New South Wales.* Retrieved from https://amstaffnsw.weebly.com/eye-conditions.html. (Accessed in August 2019)

Benedetto Falsini. (2016). NGF eye-drops topical administration in patients with retinitis pigmentosa, a pilot study. *Journal of Translational Medicine* , 1-7.

BVA. (2019). Retrieved from https://www.bva.co.uk/Canine-Health-Schemes/Eye-scheme/(Accessed in August 2019)

C.J. Murphy and P. E. Miller (1995). Vision in dogs. *leading edge in medicine- a review*. JAVMA, Vol 207, No. 12, 1623-1634

Crispin, S. (2016). *Hereditary eye disease in dogs.* Retrieved from BVA: https://www.bva.co.uk/uploadedFiles/Content/Canine_Health_Schemes/20171102%20CHS%20Eye%20disease%20guide%202017%20v4%20PRINT.PDF (Accessed in August 2019)

D. N. Kelawala et al . (2017). Clinical studies on progressive retinal atrophy in 31 dogs. *IJVR*, 119-123.

Dale, A. (2018, 01 24). *New Retinitis Pigmentosa Treatments? This Blood-Retina Chip Could Help!* Retrieved from Lab Bio Tech: https://www.labiotech.eu/medical/eye-disease-chip-retinitis-pigmentosa/(Accessed in August 2019)

DDC Veterinary . (2018, October 11). *VetDNACenter*. Retrieved from https://vetdnacenter.com/pra-prcd-in-dogs/ (Accessed in August 2019)

David J. Maggs. (2013). Slatter's Fundamentals of Veterinary Ophthalmology (5th edition ed.). St. Louis, Missouri, USA: ELSEVIER SAUNDERS.

Gelatt, K. N. (2014). *Eye Structure and Function in Dogs*. Retrieved from MSD Vet manual: https://www.msdvetmanual.com/dog-owners/eye-disorders-of-dogs/eye-structure-and-function-in-dogs. (Accessed in August 2019)

Gelatt, K. N. (2014). *Essentials of Veterinary Ophthalmology* (third edition ed.). Wiley Blackwell.

Gillian J. McLellan et al. (2012). Optical coherence tomography for the evaluation of retinal and optic nerve morphology. *Veterinary Ophthamology*, 13-28.

Gomes et al. (2013). Generalized progressive retinal atrophy in Cocker Spaniel dogs. *Cienc. Rural*, 1405-1414.

Hillen, M. (2016). *the opthomologist.com.* (Accessed in September 2019)

Jose Yeste et al. (2018). A compartmentalized microfluidic chip with crisscross microgrooves and electrophysiological. *Lab on a chip*. 95-105.

KC. (2019). *GPRA*. Retrieved from Kennel Club: https://www.thekennelclub.org.uk/search-results?q=GPRA. (Accessed in September 2019)

Kidd, R. (2004). Structure of the Canine Eye. *Whole Dog Journal*. https://www.whole-dog-journal.com/health/structure-of-the-canine-eye/ , 2nd of August.(Accessed in October 2019)

Koll-Hampp, S. (2019). Visual outcome following phacoemulsification in English Cocker Spaniels with suspected progressive retinal atrophy: A retrospective multicenter study of 54 cases (2002-2017). *Veterinary Ophthalmology* . 591-599.

Kristen Wrycha. (n.d.). *www.vetmed.wisc.edu*. (Accessed in September 2019)

Labelle, P. (2017). Canine Progressive Retinal Atrophy. *Pathologic Basis of Veterinary Disease (Sixth Edition)*. (Accessed in September 2019)

Laboklin. (n.d.). Retrieved from http://www.laboklin.co.uk (Accessed in September 2019)

Lambiase A . (1996). Nerve growth factor delays retinal degeneration in C3H mice. *Europe PMC*. 1455 - 1465.

Leo A. Kim, M. (2019, May 13). *Retina Prosthesis*. Retrieved from EyeWiki: https://eyewiki.aao.org/Retina_Prosthesis#Results_6. (Accessed in September 2019)

Leonardo Murgiano et al. (2019). Complex Structural PPT1 Variant Associated with Non-syndromic Canine Retinal Degeneration. *G3: GENES, GENOMES, GENETICS*, 425-437.

Levi Todd. (2017). Valproic Acid for a Treatment of Retinitis Pigmentosa: Reasons for Optimism and Caution. *The Journal of Neuroscience* , 5215-5217.

Levy, J. N. (2018). Stem Cell Ophthalmology Treatment Study: bone marrow derived stem cells in the treatment of Retinitis Pigmentosa. *Stem Cell Investigation*, 1-9.

Liapis, I. C. (2004). Electroretinography in Small Animal Practice. *World Small Animal Veterinary Association World Congress Proceedings.* Halandri, Greece.

Mandal, D. A. (2019, September). *What is Genetics*. Retrieved from news-medical: https://www.news-medical.net/life-sciences/What-is-Genetics.aspx (Accessed in September 2019)

Marie IKS Granar et al . (2011). Normal color variations of the canine ocular fundus, a retrospective study in Swedish dogs. *Acta Veterinaria Scandinavica*. 1-9.

Mellersh et al . (2013). Genetic screening for PRA-associated mutations in multiple dog breeds shows that PRA is heterogeneous within and between breeds. *Veterinary opthomology Volume 17, Issue 4*, 309-310.

Mellersh et al. (2012). Late-onset progressive retinal atrophy in the Gordon and Irish Setter breeds is associated with a frameshift mutation in C2orf71. *Animal Genetics*. 169-177

Mellersh, C. S. (2014). The genetics of eye disorders in the dog. *Canine genetics and epidemiology* , 1-14.

Miller SA et al. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Research*, 1215.

Millichamp. (1990). Retinal degeneration in the dog and cat. *Vet Clin North Am Small Anim Pract*, 799-835.

Miren Agurtzane, E. V. (2009). Animal models and different therapies for treatment of retinitis pigementosa, Review. *Histology and Histopathology*, 1295-1322.

Miyadera et al. (2012). Genetic and phenotypic variations of inherited retinal diseases in dogs: the power of within- and across-breed studies. *Mammalian. Genome*, 40-61.

Morgane Bunel et al . (2019). Natural models for retinitis pigmentosa: progressive retinal atrophy. *Human Genetics* , 1-13.

Nicholas J Willmott, et al. (1996). Non-retinal Abnormalities Associated with Progressive Retinal. *Experimental Eye Research*, 527-533.

Nishiguchi KM et al . (2012). Genes associated with retinitis pigmentosa and allied diseases are frequently mutated in the general population. *PLOS One* .7.

OFA. (2019, September 1st). Generalized Progressive Atrophy (PRA*)*. Retrieved from Orthopaedic Foundation for Animals : https://www.ofa.org/diseases/eye-certification/blue-book. (Accessed August 2019)

Optigen. (2017). Retrieved from Optigen: https://www.optigen.com/opt9_test_prcdprabs.html (Accessed August 2019)

P. Karlskov-Mortensen et al. (2018). Identification of the mutation causing progressive retinal atrophy in Old Danish Pointing Dog. *Animal Genetics*. 237-241

Palanova, A. (2016). The genetics of inherited retinal disorders in dogs: implications for diagnosis and management. *Dove Press*, Volume 2016:7 , 41-51.

Paul A. Sieving et al. (2006). Ciliary neurotrophic factor (CNTF) for human retinal degeneration: Phase I trial of CNTF delivered by encapsulated cell intraocular implants. *PNAS*, 3896-3901.

Pennsylvania, U. o. (2018, August 20). *Knockdown and replace: A gene therapy twofer to treat blindness.* Retrieved from Sciene Daily : https://www.sciencedaily.com/releases/2018/08/180820155107.htm. (Accessed August 2019)

Peter J.M.Clement et al. (2013). Progressive retinal atrophy: a model for retinitis pigmentosa in companion animals. Gene Therapy, 1 Suppl 1: S89

Petersen-Jones, S. M. (1998). A review of research to elucidate the causes of the generalized progressive retinal atrophies. The Veterinary Journal, Volume 155, Issue 1, 5-18.

Petersen-Jones, S. M. (2003). Progressive Retinal Atrophy: An Overview. *World Small Animal Veterinary Association World Congress Proceedings.* Bangkok.

Purina. (2010). Cord1-PRA Genetic Testing Recommended for Miniature Dachshunds. *Dachshund Update, Vol. 3*, 1-2.

Rebekkah J. Hitti et al. (2019). Whole Genome Sequencing of Giant Schnauzer Dogs with Progressive Retinal Atrophy Establishes NECAP1 as a Novel Candidate Gene for Retinal Degeneration. *Genes 2019*, (10)5, 385.

Richard R. Dubielzig et al. (2010). *Veterinary Ocular Pathology: A Comparative Review*. Saunders, Elsevier. 355.

Rizzo, M. A. (2010). Visual prostheses and other devices. *Ocular Disease*, 590-598.

S P Daiger et al. (2013). Genes and mutations causing retinitis pigmentosa. *Clinical Genetics*, 132-141.

Sarah Koll. et al . (2016). The effect of repeated eye examinations and breeding advice on the prevalence and incidence of cataracts and progressive retinal atrophy in German dachshunds over a 13-year period. *Veterinary Ophthalmology.1-9*

Serge G. Rosolen et al . (2001). Ocular fundus images with confocal scanning laser ophthalmoscopy in the dog, monkey and minipig. *Vetrainary Ophthalmology*,Volume 4, Issue 1, 41-45

Shaffer, L. G. (2019). Special issue on canine genetics: animal models for human disease and gene therapies, new discoveries for canine inherited diseases, and standards and guidelines for clinical genetic testing for domestic dogs. *Human Genetics*, Volume 138, Issue 5, 437–440.

Shibo Tang et al. (2013). Hereditary Vitreoretinal Degenerations. *Retina (Fifth Edition)*, 836-851.

Simon Peterson-Jones, S. C. (2002). *BSAVA Manual of Small Animal Ophthalmology* (second edition ed.). British Small Animal Veterinary Association.

Suber et al. (1993). Irish setter dogs affected with rod/cone dysplasia contain a nonsense mutation in the rod cGMP phosphodiesterase beta-subunit gene. *Proceedings of the National Academy of Sciences*,(9) 3968-72.

T. W. Lewis and C. S. Mellersh, (2019). Changes in mutation frequency of eight Mendelian inherited disorders in eight pedigree do,g populations following introduction of a commercial DNA test. *PLOS One (1)*, 1-21.

Terri L McCalla. (n.d.). *PRA in dogs*. Retrieved from Animal Eye Care: http://animaleyecare.net/diseases/pra/ (Accessed August 2019)

TheKennelClub. (n.d.). *DNA screening*. Retrieved from The Kennel Club. (Accessed September 2019)

Thompson, M. S. (2018). Systemic Approach to Differential Diagnosis. *Small Animal Medical Differential Diagnosis (Third Edition),* Publication Date: 11 Jan 2018, Saunders. St Louis, Missouri, USA.

UCDavis. (n.d.). *Golden Retriever Progressive Retinal Atrophy*. Retrieved from Veterinary Genetics Laboratory: https://www.vgl.ucdavis.edu/services/dog/GoldenRetrieverProgressiveRetinalAtrophy.php (Accessed September 2019)

Viviana Guadagni et al . (2015). Pharmacological approaches to Retinitis Pigmentosa: a laboratory perspective. *Progress in Retinal and Eye Research*, volume 48, September 2015, Pages 62-81

Wang, W. (2016). Antioxidant supplementation increases retinal responses and decreases refractive error changes in dogs. Journal of Nutritional Science (2016), vol. 5, e18,1-7

Yamaue, Y. (2014). Macroscopic and Histological Variations in the Cellular Tapetum in Dogs. *Journal of Veterinary Medical Science*, Volume: 76 Issue 8, 1099-1103

Yoshiko Takano et al. (2003). Study of drug effects of calcium channel blockers. *Science Direct* , volume 2011, Article ID 292040, (7 pages) 1-8.

Zöldág, P. L. (2008). *Veterinary Genetics and animal breeding.* (P. L. Zöldág, Ed.) Budapest, Hungary: Peter Mohai.

14 Acknowledgements

I would like to thank my thesis supervisor Professor Zöldág László for approving my thesis topic and through his vast knowledge of genetics assisting me when needed.

I would like to acknowledge Dr. Millar of Earlswood Veterinary clinic for sharing his extensive knowledge in the field of veterinary ophthalmology. His ongoing work in Ophthalmology is the building blocks for the future of understanding, managing and treating inherited eye disorders.

I would like to thank my parents, for their proof reading and constructive criticism which were of great help during the process. My parents have been there for me since day one and without them and their constant support and love I wouldn't be where I am today.

I would like to dedicate my thesis to the inspiration of my topic of ophthalmology, Oscar my Springer Spaniel.

15 Electronic License Agreement and Copyright Declaration

HuVetA

ELECTRONIC LICENSE AGREEMENT AND COPYRIGHT DECLARATION*

Name: Kathleen Anne McAlary

Contact information (e-mail): kathleenmcalary@gmail.com

Title of document (to be uploaded:

The Genetic and Clinical aspects of Common Canine Retinopathies, Progressive Retinal Atrophy.

Publication data of document: 2019

Number of files submitted: x1 (One File)

By accepting the present agreement, the author or copyright owner grants non-exclusive license to HuVetA over the above-mentioned document (including its abstract) to be converted to copy protected PDF format without changing its content, in order to archive, reproduce, and make accessible under the conditions specified below.

The author agrees that HuVetA may store more than one copy (accessible only to HuVetA administrators) of the licensed document exclusively for purposes of secure storage and backup, if necessary.

You state that the submission is your original work, and that you have the right to grant the rights contained in this license. You also state that your submission does not, to the best of your knowledge, infringe upon anyone's copyright. If the document has parts which you are not the copyright owner of, you have to indicate that you have obtained unrestricted permission from the copyright owner to grant the rights required by this Agreement, and that any such third-party owned material is clearly identified and acknowledged within the text of the licensed document.

The copyright owner defines the scope of access to the document stored in HuVetA as follows (**mark the appropriate box with an X)**:

[X] I grant unlimited online access,

[ ] I grant access only through the intranet (IP range) of the University of Veterinary Medicine,

[ ] I grant access only on one dedicated computer at the Ferenc Hutÿra Library,

[ ] I grant unlimited online access only to the bibliographic data and abstract of the document.

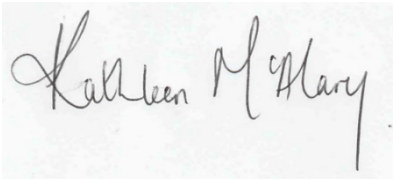
Please, define the **in-house accessibility of the document** by marking the below box with an **X**:

[X] I grant in-house access (namely, reading the hard copy version of the document) at the Library.

If the preparation of the document to be uploaded was supported or sponsored by a firm or an organization, you also declare that you are entitled to sign the present Agreement concerning the document.

The operators of HuVetA do not assume any legal liability or responsibility towards the author/copyright holder/organizations in case somebody uses the material legally uploaded to HuVetA in a way that is unlawful.

Date: 22nd of November 2019, Budapest.



Author/copyright owner signature

HuVetA Magyar Állatorvos-tudományi Archívum – Hungarian Veterinary Archive *is an online veterinary repository operated by the Ferenc Hutÿra Library, Archives and Museum. It is an electronic knowledge base which aims to collect, organize, store documents regarding Hungarian veterinary science and history, and make them searchable and accessible in line with current legal requirements and regulations.*

HuVetA relies on the latest technology in order to provide easy searchability (by search engines, as well) and access to the full text document, whenever possible.

Based on the above, HuVetA aims to:

- *increase awareness of Hungarian veterinary science not only in Hungary, but also internationally;*
- *increase citation numbers of publications authored by Hungarian veterinarians, thus improve the impact factor of Hungarian veterinary journals;*
    - *present the knowledge base of the University of Veterinary Medicine Budapest and its partners in a focused way in order to improve the prestige of the Hungarian veterinary profession, and the competitiveness of the organizations in question;*
- *facilitate professional relations and collaboration;*
- *support open access.*