

# Diagnosis of Hereditary Disease in the Purebred Dog

Dr Roslyn Atyeo BSc (Hons) BVSc BVMS (Hons) PhD  
Springvale Animal Hospital, Melbourne

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## Introduction

Welcome to this presentation of DNA based diagnosis of hereditary disease in purebred dogs. The presentation will commence with a revision of basic genetics as there is a lot of jargon that many might benefit from a quick refresher before we dive into the subject. We will rapidly move into explaining molecular genetics and DNA tests, discuss the availability of techniques such as DNA typing, parentage, direct tests and indirect tests for hereditary disease, and how these tests will allow breeders to plan the future of their kennels as disease free. We will also touch on the importance of continuing physical examinations for some diseases, particularly when the disease gene is widespread in the breeding population. These notes have been provided as a reference to the material that will be discussed today.

## Background Genetics- an explanation of basic genetic inheritance and terminology

All breeders should have a sound understanding of basic genetic concepts in order to make informed breeding choices. Ideally, a family tree of at least four to six generations should be researched prior to carrying out a mating. The next section is a summary of important genetic terminology including inheritance patterns with examples.

### Genotype and phenotype

The **genotype** of an individual animal can be defined as its total genetic profile. This profile is unique to the individual, and is a combination of the genetic material derived from both parents. The outward appearance of an animal is known as its **phenotype**. The phenotype of an animal is largely

determined by the genotype, but is also subject to environmental influences.

### **Where and how is genetic material stored?**

The tissues of every animal are composed of innumerable microscopic cells. There are many different types of cells within the body, for example, the cells which make up liver tissue are quite different to those that comprise the skin. However, all cells in the body contain a complete set of identical genetic information in structures known as **chromosomes** contained within the cell's **nucleus**. The number of chromosomes is consistent within a species, for example dogs have 78 chromosomes. The chromosomes contain information for specific **genes** along their length. It is variable expression of the genes on the chromosomes that allow different types of cells to develop into tissues. Genes code for proteins that have specific roles in cellular function. Some genes are expressed continually within the cell, others may be switched on and off at different times.

Genes that are carried on the same chromosome in reasonably close proximity are described as linked. The closer the genes are situated to each other, the more strongly they are linked and inherited with each other.

### **Cell types and chromosome number**

There are two main types of cells within the body: somatic cells (regular tissue cells), and sex cells (sperm or eggs). The number of chromosomes within somatic cells is species-specific, and the chromosomes inside the nucleus are paired in morphology (termed **homologous**). For example, a dog somatic cell has a total of 39 pairs of chromosomes, comprising of 38 homologous pairs of **autosomal chromosomes**, and one pair of **sex chromosomes**. Sex chromosomes are responsible for sex differentiation, with females having the genotype XX and males the genotype XY.

Sex cells (eggs and sperm) contain a total of 38 single autosomal chromosomes ie. one of every homologous pair, and only one sex chromosome. This totals exactly half the genetic material of somatic cells. When the egg is fertilised by a sperm in the process of sexual reproduction, the full genetic complement is restored to the embryo that is formed. Thus each parent contributes exactly half of the genetic makeup to the offspring.

### Genes, alleles and loci

Every chromosome has a number of **genes** situated along their length. The specific location of a gene on a chromosome is termed its **locus**. Each pair of homologous chromosomes subsequently has paired genes for every locus, one each of paternal and maternal origin. Each gene plays some role in the phenotype or function of the animal, however there may be variations in the gene so that they have a different effect on the animal. These possible variations of the gene that may occupy specific loci are referred to as **alleles**. Alleles of a gene are commonly denoted by a letter or shortened word. For example the locus for coat colour may contain one of two alleles: black coat or chocolate coat. It is possible to predict the ratios of genotypes of offspring if the genotype of both parents is known, and the relationship between the alleles has been established.

### Autosomal dominance and recessiveness

As has been described, every gene is paired in an individual because there are pairs of homologous chromosomes contributing to the animal's genotype. A **dominant gene** will mask the presence of the other paired allele even if there is only one copy of the dominant gene present. A **recessive gene** is will only be expressed when there are two copies of the gene present.

An example of a dominant/recessive gene relationship is shown below. The dominant gene of the pair is the normal gene producing a healthy animal (suppose for simplicity we denote this gene "D"), whilst the recessive gene causes the disease (which we will denote "d" for our purposes of this example). If we mate two apparently healthy dogs, both of whom have the recessive gene present for the disease (genotype Dd), we have a 25% chance of puppies being affected with disease (genotype dd) in the litter. This is best shown pictorially, shown below.

1. Two normal dogs carrying the disease gene (Dd) mated together:

♂ ♀	D	d
D	DD	Dd
d	Dd	dd

Ratio of offspring

DD (normal dogs - free of disease gene): Dd (normal dogs - carrying the disease gene): dd (dogs affected with disease)

= 1 : 2 : 1

= 25%: 50%:25%

### Dominant genes

Dominant genes only have to be present in an animal's genotype as a single copy to be expressed, ie. if the animal has one copy of the defective gene then disease is present. These diseases therefore can be inherited from one parent, and the disease does not skip generations. A clear cut example of a dominant gene in dogs is the **Merle** gene, whereby a merled dog put to a dog with a solid coat colour will result in 50% of offspring being merled. It all sounds easy but dominant gene diseases can sometimes still be difficult to track. For example von Willebrand's (vWD) is variably expressed in Dobermann pups, with different forms of clinical and genetic expression (Ackermann, 1999). One of the hypotheses put forward is that this gene is incompletely dominant, meaning that the faulty gene may not be continuously expressed. In both of these cases- merle and vWD - when two animals carrying the disease gene are mated it is thought that the proportion of pups which contract two copies of the disease gene (25%) will die during foetal development, or require euthanasia due to severe health defects.

Example 1. Merle dog (Mm) mated to a solid coloured bitch (mm):

♂	M	m
♀	Mm	mm
m	Mm	mm

Ratio of offspring

mm (solid coloured dogs): Mm (merle dogs)

= 1 : 1

= 50%:50%

Example 1. Two merle dogs (Mm) mated:

♂ \ ♀	M	m
M	MM	Mm
m	Mm	mm

Ratio of offspring

mm (solid colour dogs): Mm (merle dogs): MM (homozygous merle - often die in utero).

= 1 : 2 : 1

= 25%: 50%:25%

NB. But what you may actually see in the litter is 33% solid colour dogs and 66% merle pups.

### Polygenic traits

If a trait or problem is likely to be caused by several genes, it is classified as **polygenic**. A polygenic fault is much more complicated than disease caused by a single gene. In these diseases, it becomes important to look two or three generations behind the affected animals, and it involves several generations of careful breeding to reduce the incidence of the fault within a line. However it is extremely difficult to completely eradicate these types of diseases from a breed, especially if the disease only develops later in life, whereby the dog or bitch may have already been bred and passed on disease genes.

### Development of DNA-based technology.

#### Background- Microstructure of chromosomes

Chromosomes and genes are composed of long chains of DNA bases. There are four types of bases that comprise DNA: adenine (A), guanine (G), cytosine (C) and thymine (T). These four bases in different combinations code for the vast majority of the proteins that our cells produce, and are responsible for the growth, structure and function of the living body. An animal's DNA is the blueprint of their entire being, and is unique to every individual. The physical structure of DNA is complex but has been well-characterised.

## **Mutations**

When DNA is replicated prior to cell division during tissue growth or repair it is not uncommon to get errors. There are various cellular procedures of safe-checking and quality control during replication, and these fix the majority of errors, but nevertheless some slip through the system. Sometimes a single base, or even several, may be deleted from the new copy, or extra bases may be inserted. Alterations to the structure of DNA are known as **mutations**. Mutations may have no effect or occasionally improve the potential of the animal to survive, but frequently they are deleterious to the animal and decrease their potential to survive. Many of the known disease genes we encounter as breeders are recessive, such as CEA, many types of PRA, CL. These recessive genes are likely a mutation of the healthy gene which occurred a very long time ago in our dog's ancestors, and became disseminated in the breed because the carrier state could not be distinguished from a genetically healthy animal.

## **Microsatellite DNA- Markers**

The chromosomes are composed of DNA sequences that code for functional genes interspersed between large tracts of non-functional residual DNA. The residual DNA often contains chunks of highly repetitive sequences of genetic material, referred to as **microsatellite DNA**. These microsatellites serve as identification markers. They are also used in DNA profiling of individuals and parentage testing.

## **The Dog Genome Project**

The Dog Genome Project is a collaborative research project currently situated at the National Human Genome Research Institute, Maryland USA (formerly at the Fred Hutchinson Cancer Research Center). The dog genome has already been successfully mapped with several families of microsatellite markers. Researchers are currently working to develop resources necessary to map and clone canine genes in an effort to utilize dogs as a model system for genetics and cancer research (NHGRI, 2008). This project is enabling the identification of individual genes causing disease in dogs, with the aim to develop accurate diagnostic tests for them.

## **DNA testing available to date**

### **DNA typing of individuals**

Apart from being useful markers on genetic maps, microsatellite DNA sequences are quite **polymorphic** (different) between individuals. This makes them extremely useful as unique identifiers of individuals. Subsequently a range of microsatellite DNA markers may be sequenced and used to identify an individual animal, a technique known as DNA typing.

### **Parentage testing**

By comparing a number of different microsatellites DNA markers, the progeny of two parents can be accurately identified. The DNA sequence of the progeny will always be a combination of the specific sequences from the parents, as they receive half their genetic material from each parent.

### **Direct testing for disease genes**

Direct testing is used when the individual gene responsible for disease has been identified and its DNA sequence characterised. The test is targeted at detecting the disease gene itself.

### **Linkage Tests (Indirect test) for disease genes**

Indirect tests are used when the gene responsible for disease has not been identified, but only localised to a chromosome. Linkage tests are used to link DNA markers to unknown genes. Indirect tests require an evaluation of the pedigree to establish a familial link to the gene. These tests are much more difficult, time-consuming and expensive to develop, but may be the only option if the gene causing a disease has not yet been identified.

## **Where do DNA tests become important for registered breeders?**

### **Autosomal recessive diseases**

Autosomal recessive diseases are some of our most sinister to date - they have often played havoc with carefully selected breeding lines, skipping generations to be seen years later, causing breeders and owners alike much heartbreak. Carriers of autosomal recessive disease are completely normal, and no physical examination can distinguish them from dogs that are free of the disease gene. To complicate things further, some of these diseases in affected animals manifest well after the animal is old enough to have been bred from - such as PRA, HC and CL, which has

kept the disease gene well and truly alive in the breeding population. Some breed clubs have imposed age limits on breeding in order to combat the spread of disease genes, such as CL in Border Collies, but this approach was hit and miss because unidentified carrier animals continued to perpetrate the disease gene in the breeding population.

DNA tests have already been developed and are available for a large number of canine diseases. The tests can firstly distinguish between "DNA normal" or "clear" animals and carrier animals. Additionally, they can differentiate between carrier animals (with one copy of the disease gene) and affected animals (with two copies of the disease gene). Ideally all matings should be planned to avoid producing puppies affected with disease. If a mating is performed which risks producing affected puppies, then it becomes the responsibility of the breeder to establish the DNA status of all pups in the litter, and euthanase any puppies that would have developed the disease causing them pain and suffering, rather than selling affected pups to pet homes.

Sometimes a disease gene is widely prevalent in a population. This may be because the disease is not always severe, or in the past it has been difficult to detect the presence of affected animals. An example of this is CEA, and this will be discussed in a later section.

### **Autosomal dominant diseases**

Autosomal dominant traits causing disease are also being identified with DNA tests. Like for recessive disease traits, the tests can distinguish between animals carrying zero, one or two disease genes. This again will solve the problem of incomplete penetrance or late onset of disease.

### **Benefits of DNA testing procedures to breeders**

So, where does this take us with our breeding future? The benefits of DNA testing to registered breeders are enormous.

\*We can now breed away from a disease gene whilst maintaining the diversity of the breed's gene pool - quite crucial in Australia - as we are one of the most isolated countries.

\*Animals that are proven carriers of disease, but are otherwise outstanding examples of the breed no longer have to be culled from the breeding population.

\* Where a DNA test is available for a disease, breeders will no longer bear the risks of producing or selling a puppy that will develop a painful or debilitating disease sooner or later in their life.

\*We can provide health guarantees based on the genetic status or clear by parentage of the puppy to new owners. This will set registered breeders apart from back yard breeders and puppy mills, and protect breeders who are doing the right thing by the breed from litigation.

\*We can eradicate a disease gene from our breeding population over several generations by selecting the best animals that are genetically clear of the disease gene to continue breeding with.

### **Marrying DNA tests and physical examinations to make your breed healthier**

In some instances it may be that a disease gene is very widespread in within a breed. One of the most notable examples of this is CEA. At this stage for some breeds, it may be necessary to carry out matings with known carriers or affected animals, otherwise the breed will become non-existent. Because of this, many pups affected with CEA may still be produced, so it will be important to continue with ophthalmological testing at the approximate five to eight week mark so that affected pups can be graded. Pups with severely affected eyes (certain types of colobomas or retinal detachment or haemorrhage) should not be sold as pets, and should not be bred from. Alternatively pups with mild choroidal hypoplasia may be sold to pet homes and should be able to live normal healthy lives with no vision deficits, or if they are particularly outstanding in conformation (more appropriately so a bitch than a dog) she may be bred to a DNA normal sire line, and the offspring continued with as carriers. By using both ophthalmological examination for grading purposes, and DNA testing to distinguish carriers from those free of the disease gene, suitable matings may be carried out to ensure pups are free of the debilitating forms of the disease, until there are enough DNA normal animals available in the gene pool to go on with.

There are other health problems out there that any dog of any breed may develop. In particular, polygenic diseases - such as Hip Dysplasia, allergic skin disease, heart failure, a whole array of eye diseases or faults - are extremely difficult to predict and control, and all breeding animals should be routinely checked for these. Regardless of what DNA tests are available, it is still going to be important that your breeding animals have thorough physical examinations regularly.

## **Conclusions**

Over the last two decades an enormous amount has been researched and discovered about genetics in dogs. Many DNA tests are becoming available to breeders at a very reasonable cost. Registered breeders now have the power to eliminate a disease gene in their breed without losing the gene pool or having to sacrifice outstanding animals as carriers, and should never unknowingly have to sell a puppy that will develop a debilitating disease where there is a test available. The take home message is simple:

- Wherever a DNA test is available for a disease in your breed establish your breeding stock's DNA status
- plan your matings to breed away from the disease gene wherever possible. Matings that will produce affected animals should be a last resort for any breeding program
- Use DNA tests to improve the health and reputation of your breed
- Remember there is qualified veterinary assistance available for advice on DNA testing and how to use this technology to benefit your breeding program

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