ANIMAL HEATH TRUST
BREED SOCIETIES OPEN DAY
31ST OCTOBER 2001

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INTRODUCTION & OVERVIEW

The Animal Health Trust (AHT) was set up about 50 years ago to monitor commercial livestock production and introduce breeding policies that would lead to improved quality animals. The Trust thus originally had a much wider remit than currently, but did not cover the breeding of companion animals. However, there was a gradual evolution of interest away from sheep and cattle and towards horses and dogs (and cats).

This change of focus has gradually led to the AHT becoming a veterinary 'Tertiary Referral Centre', with the main interest being dogs. There are now specialists in Canine ophthalmology, neurology, dermatology and oncology, with a new hospital and MRI scanner. There is also a 'Centre for Equine Studies' which deals mostly with equine physiology. The third major part of the Trust's work is the Canine Molecular Genetics Laboratory where DNA technology is used to improve understanding of the genetics and inherited diseases of the dog. The AHT laboratories also research canine immunology.

The Kennel Club sees the AHT as the provider for DNA technology to develop simple genetics tests to enable dog breeders to eradicate genetic diseases and therefore encourages collaboration.

CANINE GENETICS – THE BIG PICTURE

In 1856, Mendel published his findings on inheritance in pea plants. He stated:

1. Some characteristics are inherited from an individual's parents. These characteristics are determined by 'Units of Inheritance' i.e. Genes.
2. Characteristics of offspring are determined by *both* parents.

The chemical 'identity' of Genes, DNA (Deoxyribonucleic Acid) was discovered in 1920 and the chemical structure of DNA was discovered in 1953. Since this slow start, genetic research has progressed at a tremendously fast rate so that we now have a 'map' of the location of every gene on every chromosome for Humans (the so-called 'Human Genome'); we also know that the genetic 'map' for dogs is very similar to the one for humans. Humans have approximately 40000 genes and dogs have a very similar number, indeed the genes themselves are mostly similar, and in many cases identical to the human genes. There is only a relatively small number of 'Canine Specific' genes.

The fundamental basis behind genetics is that each gene leads to the production of a specific protein and it is the protein that is responsible for the effect that a gene has on the body (gene 'expression') e.g. in golden retrievers, the gene for coat colour causes a yellow pigment to be produced in the hair.

A gene is a section of a DNA molecule; genes are separated from each other by sections of DNA, some of which carry 'instructions' for the activation of the gene nearest to it and other sections which have no known function. Each Chromosome consists of a very long molecule of DNA, twisted and folded upon itself, containing many hundreds of genes. Each cell of the body with a nucleus contains the full complement of chromosomes (and genes), and as a copy of each chromosome comes from both the mother and the father, there are two copies of every gene in every (nucleated) cell. But, as a fertilised ovum develops certain cells differentiate into specific cell types e.g. a brain cell or a liver cell. This happens because some genes are 'switched on' in, for example, liver cells that are never switched on in other cells (limited genetic expression). A gene

coding for any particular characteristic will always be in the same place on the same chromosome in all individuals of a species, this is called the gene 'locus'. However, there may be more than one version of a gene available e.g. a gene at the coat colour locus may be a version leading to black hair or a version leading to brown hair. Different versions of the same gene are called alleles (pronounced al-eels)

No other mammalian species has a many different genetic types as the dog. The domestic dog originated as the wolf one hundred thousand years ago and early on in canine development there were only three or four different types, each specialising in guarding, herding, or hunting. Over the past 500 years, man has manipulated the dog in many ways and there are now over 300 distinct genetic types. This was only possible because there was a great deal of genetic variation in the original wolves i.e. there were many different versions of each gene available, and as a result of selective mating man achieved new combinations of the many different versions (alleles) of each gene. Because there are two copies of each gene present in a cell (one from the mother and one from the father) an individual may have two identical alleles, in which case it is described as 'homozygous' or it may have two different alleles of the gene in which case it is described as 'heterozygous'. (*e.g. Landseer Newfoundlands are homozygous for the 'Landseer' gene, Landseer recessive black Newfoundlands are heterozygous for the 'Landseer' gene).*

GENETIC DISEASES

Sometimes, the information contained within the DNA of a gene becomes 'corrupted' and the genetic structure is altered. This is called a genetic 'mutation' and can be brought about by a variety of agents such as radiation or certain chemicals or may occur when the DNA is replicated in preparation for cell division; the resulting gene is thus not an identical copy of the original gene and may cause a different protein to be produced than the one intended, or may not even 'work' at all. Many mutated genes are identified and repaired but some are not and the structure of the gene will be permanently altered.
(If the mutation occurs in the DNA of a sperm or ovum, the resulting offspring will have a different genetic make-up to either of its parents. A mutated gene may be of benefit to an individual (the basis of evolution) or it may be detrimental and cause either death or an inherited genetic disease.)

There are 370 known different inherited diseases affecting dogs and in 60% of these the mode of inheritance is known; in the other 40% the disease is known to be inherited as it runs in families or is breed specific.

Three-quarters of the 60% are single gene disorders. e.g. PRA (Progressive Retinal Atrophy) where there is a mutation in a gene involved in the normal development of the eye. Von Willebrand's disease is also a single gene disorder.

There are two types of single gene disorders – dominant or recessive. Most single gene disorders in the dog are recessive (in excess of 130) where a carrier cannot be recognised visually; this is crucial because the key to controlling hereditary disorders is the identification of carriers. There are only 10 to 12 known diseases where the abnormal gene is dominant, but the defect is more obvious and affected individuals can be more easily identified before mating. In the remaining quarter (of the 60%) inheritance is more complex, with mutations in more than 1 gene and are known as 'polygenic'. Such diseases are epilepsy, hip dysplasia and autoimmune disease.

Dr. Cathryn Mellersh
Molecular Geneticist, AHT

CANINE GENETICS AND DNA TESTING – THE FUTURE

ULTIMATE AIM

The *ultimate* aim is to identify the disease causing mutation of each genetic disorder in order to develop a Gene-Based Diagnostic Test.

In practice this is very difficult as there are 3 000 000 000 nucleotides in the canine genome (*a nucleotide is a 'building block' of DNA)*, making up 40 000 separate genes and there may only be one single incorrect nucleotide – this is the equivalent of looking for a single letter spelling mistake in 1 000 000 pages of text.

In order to speed up the search, it is possible to sub-divide the DNA in such a way that a whole section of chromosome can be examined at a time, rather than each individual gene. This technique requires the uses of a 'Genetic Map'.

GENETIC MAPS

On each chromosome exist naturally occurring areas of DNA with known nucleotide sequences called 'Microsatellites'. These are not genes, but are dotted around the DNA and have an unknown function. However, it is now possible to positively identify a large number of individual microsatellites spread throughout the chromosomal DNA and these are called 'markers'. Following three to four years of concentrated research, the sequential order of each marker along each of the 38 canine chromosomes is now known and the distance between each marker is also known – this forms a 'Genetic Map'.

As microsatellites are part of the chromosomal DNA, they are inherited in exactly the same manner as genes and each individual has 2 alleles of each microsatellite, which may not be identical i.e. the individual may be homozygous or heterozygous. Because many microsatellites can be positively identified (markers) their inheritance can be followed and this is what makes genetic maps useful in the identification of disease-causing genes.

IDENTIFICATION OF DISEASE-CAUSING GENES

In order to identify a disease-causing gene it is initially necessary to study the pattern of inheritance of the disease and the pattern of inheritance of various microsatellite alleles, with the aim of finding a microsatellite that is inherited in exactly the same way (linked inheritance). The closer a microsatellite is on a chromosome to the disease-causing gene, the more closely its inheritance will follow the inheritance of the disease. A microsatellite allele that is inherited in all individuals with the disease is very close to the disease-causing gene and therefore, by using the canine genetic map, the location of the culprit gene can be estimated i.e. the microsatellite 'marks' the presence of the disease causing gene – a 'Genetic Marker' for that particular disease.

The technique used to identify inheritance of microsatellite alleles in individuals with a genetic disease is called 'Genetic Linkage Analysis', and once identified, the microsatellite ('Genetic Marker') forms the basis of a 'Linkage Based Diagnostic Test' for the disease.

INTERMEDIATE AIM

The *'intermediate'* aim of the Animal Health Trust is to identify a marker(s) linked to each inherited canine disease and hence develop a linkage-based diagnostic test for them all.

STRATAGEM FOR FINDING A LINKED MARKER

1. To be successful in developing a linkage-based diagnostic test the following criteria must be met:
    a. There must be a large pedigree of dogs with the disease.
    b. Diagnosis must be accurate.
    c. DNA must be available from most dogs in the pedigree, particularly affected dogs and their siblings, parents and grandparents, even if they are not affected.
2. Collect and store DNA from affected dogs and those with affected individuals in their pedigrees.
3. Use a computer simulation to calculate how many dogs must provide DNA for the Genetic Linage Analysis to work, the 'Sample Size'. The number of dogs that must be studied depends strongly on the number of generations available for study, the number of individuals in each generation available for study and the prevalence of the disease in the breed – three generations or more is ideal.
4. When DNA is available from sufficient dogs, carry out genetic screening systematically examining each marker along the length of each chromosome for linkage to the disease.

Initial screens use 200 – 300 different microsatellite markers. If a disease gene is at the very end of chromosome 38 it will take the AHT approximately one year to find it. If the geneticists 'get lucky' and the gene is at the start of chromosome 19, it will take approximately half the time.

USE OF A LINKED MARKER TO DIAGNOSE CARRIER STATUS

When a disease is inherited in a 'recessive' manner, affected individuals will have 2 copies of the defective gene (homozygous) and carriers will have 1 normal and 1 defective copy of the gene (heterozygous). An affected individual *must* have inherited its defective gene from *both* parents - they were either carriers or themselves affected.

If two carriers are mated, approximately 25% of their offspring will be normal, 50% will be carriers and 25% will be affected; if a carrier and an affected dog are mated, 50% of the offspring will be carriers and 50% will be affected; if an affected dog and a normal dog are mated, 100% of the offspring will be carriers; if a carrier and a normal dog are mated, 50% of the offspring will be carriers and 50% will be normal.

If a dog is identified as having the marker microsatellite allele (Genetic Marker) then it will *probably* be a carrier, but the accuracy is not 100%. The reason for this lies in the fact that during formation of sperm and ova there is sometimes an exchange of DNA between the chromosomes (one of which will have come from that animal's mother and one from its father); if the break occurs *between* the disease causing gene and the microsatellite allele then the two will no longer be on the same chromosome and will be inherited separately – this process is called 'Genetic Recombination' or 'Crossing Over'. Recombination is quite rare and in this instance will lead to 'false positive' and 'false negative' results; a marker is only used as the basis for a linkage-based diagnostic test if there is recombination in less than 3% of cases.

SUMMARY OF LINKAGE-BASED DIAGNOSTIC TESTS

1. Allows fairly accurate assessment of disease status.
2. Never 100% accurate (due to recombination).
3. Usually more accurate if a pedigree is available.
4. Relatively quick to develop (less than 1 year).

STRATAGEM FOR IDENTIFYING A DISEASE CAUSING GENE

1. Use a linked marker to identify the corresponding region of the Human Genome (Comparative Mapping). This is possible because the relationship between canine and human chromosomes is known e.g. the genes on canine chromosome 1 are found on parts of human chromosomes 18, 6, 9 and 19.
2. Candidate genes are identified and the role of each in the disease is investigated. E.g. for any given disease, once a linked marker has been identified and its position on the human chromosome is determined, since many more genes have been identified in the human it is often possible to work out which genes are in the surrounding areas of DNA e.g. in PRA the marker is in an area of DNA involved in the development of the retina and it was possible to investigate whether the surrounding genes had any role in the canine disease.
3. By definition, the nucleotide sequence in the disease causing gene must be different to that in the normal gene, so the next step is to look for discrepancies in the nucleotide sequences that mirror inheritance of the disease e.g. recessive inheritance. Identification of the allele with 'abnormal' nucleotides (disease-causing gene) is the basis of a 'Gene Based Diagnostic Test' for the disease.
4. If the culprit gene cannot be identified in this way, it is necessary to construct a very high-resolution map of the candidate region of DNA and identify any 'novel' genes (genes not yet identified in the human genome). The time it takes to do this depends on how many genes have to be examined and so a time-frame cannot be estimated – with luck there will be an obvious candidate gene with obvious nucleotide discrepancies that can be identified in under 1 year, otherwise it may take anything up to 7 years.

SUMMARY OF GENE-BASED DIAGNOSTIC TESTS

1. Allows accurate assessment of disease status.
2. 100% accurate.
3. Can be used to diagnose individual dogs – no pedigree is needed.
4. Takes much longer to develop than a Linkage-Based Diagnostic Test.

<u>DISCUSSION</u>

The cost of this type of research is £20 000 per researcher per year at the AHT. Therefore it costs anything up to this amount to develop a Linkage-Based Diagnostic Test, given that sufficient DNA is available from sufficient numbers of inter-related dogs.

Once a 'Linked Marker' has been identified, it will take a further £20 – 140 000 (at today's rates) to develop a 'Gene-Based Diagnostic Test'.

The AHT has the facility to store DNA and encourages breeders to take advantage of this for their breeding stock. The rationale behind this is, should it become necessary to investigate a breed for an inherited disease in the future, hopefully DNA from several generations of inter-related dogs will already be available for study, thus reducing the time taken to amass enough for a test to be developed (it takes 1 year *after* DNA from enough dogs has been collected to develop a Linkage-Based Diagnostic Test, and 1 – 7 years after that to develop a Gene-Based Diagnostic Test).

The AHT does not freeze the 'whole-blood' but only the DNA once it has been extracted.

Due to the very strict laws on animal experimentation in this country, in order to request a sample of biological material from your dog to extract the DNA any researcher MUST have the necessary Home Office Licence. However, the *owner* may request a blood (or other) sample to be taken from a dog, by their own vet, which the *owner* then passes to a third person for the purpose of research into a diagnostic test.

Elaine Mountford, February 2002

(The passages in italics were not part of the original talks, but I have added them to clarify certain points)