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Relevance of the Canine Genome Project to Veterinary Medical Practice (1-Jun-2001)

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There is an ongoing revolution in medicine that is changing the way that we, as veterinarians, will be diagnosing diseases, applying new therapies, and counseling clients. Of course we expect advances to be made in the genetics of organisms that are pathogenic to dogs, and these should be useful in designing vaccines, antibiotics, and other medications. However, information on the genetics of dogs themselves should be useful to combat inherited disorders and help us to understand the interaction between genetics and the environment in causing disease. Genetic information from the human genome project is already being applied to medicine and veterinary medicine. It is reasonable to expect that applications will emerge rapidly in veterinary medicine within the next few years as we obtain new information from the canine genome project. We can describe the canine genome project by three events:

1. mapping markers on canine chromosomes,
2. mapping gene locations on canine chromosomes, and
3. obtaining the nucleotide sequence of the entire canine genome.

The first two steps have been underway for some time and are already providing diagnostic tools. An integrated canine map of over 724 markers was recently published [1]. This locates the chromosomal positions of polymorphic markers and specific genes. The map can be used to find other genes through linkage analysis and comparative mapping. For example, linkage analysis was used to find gene mutations that cause cystic fibrosis and Huntington's disease in humans and narcolepsy in Doberman pinscher dogs [2].

The last step in the canine genome project, sequencing the canine genome, is underway during 2001 through public and private initiatives. When the entire nucleotide sequence becomes available on an internet database, as the human genome is, researchers will not have to clone genes one by one in the laboratory. Instead, they will be able to search for genes on their computers. They will also be able to compare dog sequences to those of the human, mouse, and other mammals to aid in their search for specific genes and for mutations that cause inherited disorders.

Effects of the revolution in canine genetics today - Presently, we are testing genomic DNA obtained from blood or tissue samples for disorders caused by single gene mutations. These disorders generally fall under one of three categories: autosomal dominant traits, X-linked traits, and autosomal recessive traits.

Autosomal Dominant Traits

In autosomal dominant disorders, a mutation in one allele is sufficient to cause the phenotype, even though the other allele may be normal. For example, the dominant allele at the D gene locus is signified by a capital letter (D) and the allele that is not dominant is signified by a lower case letter (d). The dog inheriting one D allele (D_) will express the mutant/disease phenotype, regardless of whether the second allele is D (genotype DD) or d (genotype Dd). The DD genotype is termed homozygous dominant, and the Dd genotype is termed heterozygous, at the D locus.

In disorders that are inherited as autosomal dominant traits, the affected individual is usually heterozygous (Dd). Autosomal dominant traits can appear spontaneously within a pedigree as the result of a new mutation in one individual. However if it is not a new mutation that arose in the affected individual, then that individual received the mutant allele (D) from at least one parent. Undesirable traits that are inherited as autosomal dominant traits with complete penetrance can be eliminated if the breeder/owner can recognize the mutant phenotype before breeding age. Thus many of these disorders have been eliminated by breeders and owners [3]. However, autosomal dominant disorders with incomplete penetrance, such as oculoskeletal dysplasia in the Labrador Retriever, are more difficult to eliminate and may benefit from molecular tests. There are no DNA tests for autosomal dominant disorders to our knowledge at present.

X-linked Traits

The normal chromosome constitution of male dogs is 78,XY and that of females is 78,XX. Only a few genes on the X chromosome also have a locus on the Y chromosome. Therefore, mutations in genes located on the X are usually expressed in males. Pedigrees of such dogs have affected males much more frequently than affected females, when the trait is recessive. For X-linked mutations in general, unless the mutation has arisen spontaneously in an affected male, the affected males (X*Y) have received the X-linked mutation (X*) from a heterozygous mother (XX*) that is clinically normal. Unaffected male siblings (XY) will not be carriers because they received the normal X chromosome from their mother. Thus unaffected males can be used for breeding. On average, 50 % of the female siblings of affected males will be carriers. Thus a DNA test is most useful to detect female carriers of X-linked recessive traits. Identification of noncarrier females allows them to contribute to the gene pool, which is particularly important in maintaining genetic diversity in breeds having small gene pools. Thus there is some demand for DNA testing for X-linked traits. For example, Severe Combined Immune Deficiency (SCID) and hemophilia due to Factor IX deficiency are X-linked disorders for which DNA tests are available (Table 1).

Table 1. Web addresses of labs that perform canine DNA testing:

- DNA testing for several domestic animal species, including dogs http://www.vgl.ucdavis.edu/
- DNA testing for dogs http://www.vgl.ucdavis.edu/Service/Canine/
- DNA testing for canine inherited diseases http://www.vet.upenn.edu/penngen/ http://www.VetGen.com/index.html http://www.genesearch.net/
- DNA testing for canine inherited eye diseases http://www.optigen.com/
- PRA testing in Cardigan Corgis http://www.cardigancorgis.com/PRAtest.htm
- Information regarding AKC requirements for paternity certification http://www.akc.org (search the site on "DNA testing")
- Registry of genetic disease for purebred dogs in the US and worldwide http://www.vetmed.ucdavis.edu/gdc/gdc.html
- Canine genome mapping (marker and gene locations on dog chromosomes) http://mendel.berkeley.edu/dog.html http://www-recomgen.univ-rennes1.fr/doggy.html http://www.fhrc.org/science/dog_genome/map/map3/mapgroups.html

Autosomal Recessive Traits

Much of the DNA testing for genetic disease in dogs will be for recessive traits. Among the canine diseases due to single gene inheritance where the mode of inheritance is known, the majority are autosomal recessive disorders [3]. For example, these include several types of progressive retinal atrophy (PRA), pyruvate kinase deficiency, and cystinuria (Tables 2 & Table 3).

In autosomal recessive disorders, a mutation in one allele is not sufficient to cause the disease phenotype when the other allele is normal. The mutant/disease phenotype is expressed when both alleles have the same mutation. Affected dogs are homozygous recessive (rr). Therefore, they received one (r) allele from the mother and one from the father, and both parents are carriers. The risk of inheriting the affected genotype is equal for males or females, since the gene is located on an autosome and not on a sex chromosome. Matings between affected dogs (rr) will produce only affected dogs. Matings of affected dogs (rr) to carriers (Rr) will produce 50 % affected (rr) and 50 % carrier (Rr) offspring, on average. Carriers appear clinically normal. In matings between heterozygous carriers (Rr), 25 % of the offspring will be affected, 50 % will be carriers, and 25 % will be normal noncarriers, on average. Notably, in matings of a heterozygous carrier (Rr) and a homozygous normal (RR) animal, none of the offspring will be affected, but approximately 50 % will be carriers. Thus carrier status can remain unknown if:

1) a carrier fails to produce an affected offspring because it was always bred to noncarriers, or 2) a carrier produced few offspring when bred to a carrier, and by chance, none were affected. The inability to detect carriers prior to production of affected offspring is one reason that autosomal recessive traits have been difficult to eliminate from purebred dog populations. For many autosomal recessive diseases, the gene is unknown and no practical biochemical test is available to diagnose carriers. Progeny testing is still used to identify carriers for some of these disorders. For progeny testing to be useful, the mode of inheritance must be known, the dog of unknown genotype must be bred to a proven carrier or affected dog (e.g., genotype is known), and all offspring must be examined for disease by a reliable method after the age of onset of

clinical disease. Through sequencing of the canine genome, we expect to identify these genes. This will allow us to design and implement DNA testing for carrier and affected status so that we will not have to rely on progeny testing.

Table 2. Examples of DNA (Mutation) Tests for Canine Inherited Diseases		
Disease	Breed	Test Laboratory
Canine Leukocyte Adhesion Deficiency	Irish Setter	Optigen LLC
Congenital Stationary Night Blindness	Briard	Optigen LLC GeneSearch LLC
Cystinuria	Newfoundland	PennGen Laboratories
Fucosidosis	English Springer Spaniel	PennGen Laboratories
Mucopolysaccharidosis	German Shepherd	PennGen Laboratories
Myotonia Congenita	Miniature Schnauzer	PennGen Laboratories
Phosphofructokinase Deficiency	American Cocker Spaniel English Springer Spaniel Mixed Breeds	PennGen Laboratories VetGen LLC GeneSearch LLC
Progressive Retinal Atrophy	Irish Setter	OptiGen LLC VetGen LLC
Progressive Retinal Atrophy	Irish Setter Cardigan Welsh Corgi	GeneSearch LLC
Progressive Retinal Atrophy	Cardigan Welsh Corgi	Michigan State U
Pyruvate Kinase Deficiency	Basenji, Dachshund West Highland White Terrier	PennGen Laboratories
Pyruvate Kinase Deficiency	Basenji	VetGen LLC GeneSearch LLC
Severe Combined Immune deficiency	Basset Hound Cardigan Welsh Corgi	PennGen Laboratories
Von Willebrand's Disease	Doberman Pinscher Manchester Terrier, Poodle Shetland Sheepdog	GeneSearch LLC
Von Willebrand's Disease	Doberman Pinscher Manchester Terrier Pembroke Welsh Corgi Poodle, Scottish Terrier Shetland Sheepdog	Vetgen LLC

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Table 3. Examples of DNA (Marker) Tests for Canine Inherited Diseases		
Disease	Breed	Test Laboratory
Copper Toxicosis	Bedlington Terrier	Vetgen LLC
Renal Dysplasia	Lhasa Apso Shih Tzu Soft Coated Wheaten Terrier	Vetgen LLC
PRCD (Progressive Retinal Atrophy, PRA)	Labrador Retriever Chesapeake Bay Retriever Portuguese Water Dog English Cocker Spaniel	Optigen LLC

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DNA Testing Today

Testing for inherited disease has, in the past, relied upon biochemical testing for specific enzymes, sugars, etc. to describe the phenotype and arrive at a diagnosis. Although urine or blood could be used for metabolic screening of many diseases, some tests required specific tissue samples that would contain a particular enzyme. Such metabolic screening tests are still performed for disorders for which molecular tests are not available (see metabolic screening for diseases, PennGen website). Examples of diseases for which metabolic screening, that is not DNA testing, is currently available are methylmalonic aciduria, mitochondrial myopathy, mucopolysaccharidosis III, and Gm1 and 2 gangliosidoses.

For DNA tests that we are discussing, the sample must contain genomic DNA. Any cell of the body that contains a nucleus will do: anticoagulated blood, blood dried on special filter paper (Guthrie card), semen, hair roots, or samples from the buccal mucosa (cheek swabs or bristle brushes). For blood samples, note that RBCs do not have nuclei, and it is the WBCs in a blood sample that provide DNA. If necessary, tissue samples can be taken at necropsy and frozen. However, tests in each lab are usually optimized for certain types of samples, so it is best to first contact the lab to obtain information on sample type, handling, and shipping. Most labs provide the collection systems, such as buccal brushes, with full instructions on their use. (see websites listed in Table 1).

There are a few important points to note regarding sample collection: The veterinarian must be certain that s/he is collecting the sample from the correct animal and properly label the sample with the animal's identity. Note that it is very important to collect samples so that they are free from contamination. Since most tests use very sensitive assays (polymerase chain reaction, PCR), even small amounts of DNA from another animal can cause serious difficulties in test result interpretation. Therefore, one should not use recycled syringes, needles, or scalpel blades to collect samples for DNA testing, even if they are washed and sterilized, since they can contain DNA from other dogs. If samples are contaminated, the results are invalid, and new samples should be collected. If one is collecting samples for the American Kennel Club's (AKC) certification program, the certification tests must be performed by the laboratory specified by the AKC. See their website for information (Click here).

There are now DNA tests for over 20 genetic disorders being offered by various private and university-based laboratories in the U.S.A. (Tables 2 & Table 3). Fortunately, genomic DNA is easily obtained, and a dog needs to be tested only once to determine if there is a mutation in the DNA that causes a particular disease. However, each new generation will require testing. Some of these are direct tests, and others are indirect tests.

Direct DNA Testing: Mutation-based Tests

For these tests, the specific mutation that causes the disorder is known. Samples are tested for the presence of a specific mutation in the DNA sequence; and usually tested for the normal sequence as well, as a control. Polymerase Chain Reaction (PCR) is a method to amplify a small amount of DNA of known sequence from any individual dog. It allows researchers to use small amounts of DNA isolated from a few cells. The PCR reaction can be designed to be specific for a particular sequence of DNA allowing the distinction between normal and mutant alleles of a disease gene. The mutations are typically breed specific and therefore a PCR reaction designed for one breed's disease will likely be unique to that breed.

Direct DNA tests are very valuable when all affected animals in the population have one particular mutation that causes the inherited disorder. Within a breed, the mutation causing the disorder is often identical by descent, that is, the mutation is the same because all affected dogs are descendants of one individual that had the mutation. This is also termed "the founder effect". For example, there is a DNA test for progressive retinal atrophy (PRA) to test prospective Irish setter parents. After the mutation that causes Irish setter PRA was described, a molecular survey of the breed showed that this disorder in this breed was caused by only one mutation. This allowed a single DNA test to be designed to identify all carriers in this breed [4].

Thus, before a DNA test is used widely, it is important that studies are performed to determine that the disorder in that breed is caused by only one mutation. Occasionally there are different mutations in the same gene that can cause a disease, which is termed "allelic heterogeneity". Allelic heterogeneity is a more common problem for DNA testing in human populations because they are more genetically heterogeneous than are purebred dog populations.

Indirect DNA Testing: Linkage-based or Marker-based Tests

Because the genetic mutations for many diseases are presently unknown, we cannot use direct DNA testing to detect them at present. Indirect tests, based upon linkage analysis, have been designed for some of these diseases (Table 3). Linkage analysis determines the location of markers on a chromosome. Then markers that segregate with the affected phenotype are assumed to be close to the gene that causes the disease. When the gene mutation causing a disease is identified, linked marker tests will be replaced by a direct DNA test.

It is important to remember that these indirect tests have an intrinsic error rate that must be taken into account when interpreting test results. A linked marker test can give a misleading result in two ways. If a crossover occurs in the chromosome between the locations of the linked marker and the mutant gene causing the disease, then the marker will no longer be linked to the disease in that individual or its offspring. Markers that are very close to the disease gene are the most useful because it is less likely that a crossover will occur between the location of the marker and the disease gene. A linked marker test can also give a misleading result if a marker allele is erroneously assumed to be carried on the same chromosome as a disease allele. One can not assume that the same disease is caused by the same gene in two different breeds, or even in different families within the same breed. This assumption could result in a false negative or a false positive error.

Interpretation of Test Results

For both direct and indirect tests, errors should be suspected when the test result does not correlate with clinical data. In such cases, tests should be repeated to determine whether the problem is in identification of the dog, sample handling, the nature the test, laboratory quality control, etc...[5].

The Future

The full impact of the genomic revolution on canine medicine is unknown, but we can extrapolate from present events in human medicine. Upon completion of the human genome sequence, the scientific community was given access to all 35,000 human genes estimated to be present in the genome. Microarray and computer technology has increased the efficiency with which these genes are being studied. The methodology is available to describe genetic variation between many individuals at a time, as well as expression of thousands of genes at a time in specific cells, tissues, and organs. These methods are also being applied to disease states to find changes in gene expression that are relevant to the cause and treatment of disease. For example, such research is being used to categorize neoplasias based upon genetic expression profiles rather than histologic characteristics, and to predict which drugs are likely to be successful in causing remission. Further, these studies are likely to identify new metabolic pathways in normal and disease states that will provide new therapies. Similarly, small genetic differences (polymorphisms) between individuals are likely to be useful in predicting disease susceptibility and response to specific therapies [6].

Canine medicine can directly use some advances from human studies. But it will not be long before we are studying canine genes in the same manner. It is likely that some information from canine studies will be useful to human medicine through the comparative genomics approach that will also benefit canine medicine. For example, variation due to polygenic traits, or quantitative trait loci (QTL), are of particular interest. Identification of genes involved in such complex inheritance patterns in humans is expected to be difficult. Considerable variation in polygenic traits between purebred dogs is likely, which should make the dog a good model to determine how groups of genes control phenotypic variability.

Although humans, dogs, and mice are not closely related species, they have many genes in common. Frequently, clusters of genes have remained together during evolution and appear together in the same order on chromosomes of different species. Such conserved synteny is useful in comparative genomics. It is quite possible that the dog will be a better model than the mouse for some human disorders with complex inheritance. For example, the dog may be a better model for behavioral disorders or disease related to large body size, such as disorders of weight bearing structures like bones or joints. The genetic information gained from such studies can be used in therapy and preventive measures for humans and dogs.

DNA Testing in the Future

First of all, information from the canine genomics project can be used to solve nonmedical problems, such as providing a genetic fingerprint of an individual dog for the purposes of identification or paternity certification. This could be important to the veterinary profession, to certify that semen or tissue was obtained from a specific animal, for example. It may also be possible to identify breeds by polymorphisms in genes that are specific to each breed, such that the genealogy, or breed heritage, of individuals could be identified. Eventually, all the genes controlling coat color, eye color, hair length, etc... should be identified, and these may be of indirect use to our profession as well. However, veterinarians are most interested in the information directly relevant to the genetic basis of disease.

Eventually we should expect that all canine genes, and mutations as they are identified, would be accessible on an internet database that is continually updated. Today we have DNA tests that examine one gene at a time for a single mutation in one individual. In future, we can expect that thousands of genes will be examined at once, through the use of microarray technology. This will allow us to examine all genes of one individual at once, for one or more mutations, providing the DNA profile that is unique to that individual. We could then compare the genetic profile of any dog to the canine genetic database. These tools will also allow us to look at one gene or a group of genes in many individuals at once. They will also make it possible to apply genetic selection on a large scale to dog populations, and faster than we have ever done before. We will be able to perform DNA testing for diseases caused by groups of genes acting in concert; those due to polygenic inheritance. The goal will be to detect mutations in groups of genes that, in combination, are known to cause disease.

Benefits to Medicine, Individual Dogs

Once we can test thousands of genes at a time in one individual, we will have lots of data on each animal to use for medical purposes and for counseling breeders in their breeding selections. The owner could keep a DNA profile of their dog on a locked disc and present it to the veterinarian at the time of examination. The veterinarian could then obtain the genetic component of case history by screening the patient's DNA profile. For example, one could insert the DNA profile disc into a computer and compare it to the canine genetic database on the internet. Alternatively, perhaps the DNA profile of each purebred dog will eventually be obtainable from a secure internet database for each breed, and then accessed by the patient's veterinarian. Thus the patient's profile could be screened for all known genetic disease to date, or for genes that are associated with adverse drug reactions. This information will be useful in constructing differential diagnoses and in choosing therapeutic agents for individual patients. The individual DNA profile could also be used to counsel breeders in their breeding selections. As part of the prebreeding examination, profiles of prospective parents could be compared to the canine genetic database for undesirable mutations, and the probability of offspring being affected for known genetic diseases could be calculated by computer software. Additionally, the probability of known desirable traits in the offspring could be obtained.

Benefits to Medicine, Populations of Dogs

Once we can examine thousands of genes at a time in an individual, compare them to those of other dogs, and begin using this information to plan matings of individual dogs, we have powerful tools to reduce the frequency of, or eliminate, deleterious genes from a population. When we understand polygenic inheritance, we can potentially eliminate whole groups of deleterious genes from populations. The effect of such selection on a widespread basis within a breed could rapidly improve health within a few generations. However, until we have enough information on gene interaction, we will not know whether some of these genes have other functions that we wish to retain. And, other population effects should not be ignored. At least initially it may be best to use this new genetic information to avoid mating combinations that we know will produce affected animals, rather than to eliminate whole groups of genes from a population. This is particularly important for breeds with small gene pools, where it is difficult to maintain genetic diversity.

Finally, we will eventually have enough information about canine gene function to select for specific genes encoding desirable traits and increase their frequencies in a population. This is similar to breeding practices that have been applied to animals for hundreds of years. The difference is that we will have a large pool of objective data that we can use rapidly on many individuals at a time. This has great potential to improve the health of the dog population as a whole. However, if we or our breeder clients make an error, we can inadvertently cause harm through massive, rapid selection. Therefore, we should probably not be advising clients on polygenic traits or recommend large scale changes in gene frequencies in populations until much more knowledge of gene interaction is obtained. By then it is likely that computer modeling will be available to predict the effect of changing one or several gene frequencies in a dog population over time. And since new mutations are likely to arise in the future, these tools will be needed indefinitely to detect, treat, and eliminate genetic disorders from dog populations.

Closing

Information available from genomics will only be useful in improving canine health if veterinarians have the knowledge and skills to use it ethically and responsibly. There is a great potential to improve overall canine health through genetic selection, but also the potential to do harm if we fail to maintain genetic diversity. Our profession must be in a position to correctly advise clients on the application of this information to individual dogs as well as to populations of dogs, and particularly purebred dogs. Continuing education programs highlighting genetic testing, counseling, and applied population genetics will be useful in preparing us for this important role.

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