Veterinary Molecular Genetics

By Wendy Shepard Chisholm, V.M.D.

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With trialing on the East Coast, she encountered many questions from her "Labrador" friends as well.

"It's time to ring in the century of biotechnology…Now we're just a few years away from one of the most important breakthroughs of all time: deciphering the human genome, the 100,000 genes encoded by 3 billion chemical pairs in our DNA."10 (Time Magazine "The Future of Medicine: How genetic engineering will change us in the next century." Jan. 11, 1999). What follows is a technical discussion of genetic information important to all breeders. Most of this material I was privileged to hear at the North American Veterinary Conference, January, 1999, in Orlando, Florida. I have tried wherever possible to include explanations and definitions of medical terms, so all readers can follow the discussion.
With the recent availability of molecular markers for the canine genome, and the ongoing work to develop a comprehensive canine genetic map, we are at the beginnings of a revolution in canine health care. 1 With the control of most infectious diseases, nutritional problems, and intoxications, hereditary problems have emerged as a major problem in small animal medicine. More than 500 hereditary disorders have been recognized in dogs and cats, and represent a large, heterogeneous, and growing group.8 In the dog alone, thre are 65 genetic orthopedic diseases. With the common practice of breeding closely related animals, there is a lack of selection against genetic disorders. Many breeds of dogs are characterized by genetic diseases, many of which are inherited as autosomal recessive or apparently complex traits (autosomes are all chromosomes, excluding the X and Y sex chromosomes).
In the coming months, it is expected that diagnostic tests will be developed for many common canine diseases, as they are currently available for progressive retinal atrophy, progressive rod cone degeneration, copper toxicosis, Von Willebrands disease and more. Most recently, over a dozen hereditary disorders have been characterized with the development of the simple and accurate DNA-based polymerase chain reaction technique (PCR). Breeders will have the option and responsibility, to test key dogs in their breeding programs to determine carrier status for a variety of inherited disorders. A dog will be able to be clearly identified as being carrier or "clean" of a particular genetic disorder.

The Canine Genome and Map

For most canine diseases, the underlying genetic cause is yet to be determined. Historically, the canine genome has been difficult to study. The dog has 38 pairs of chromosomes. Standards for chromosome identification by G-banding have been established for only the largest 22 canine autosomes by the committee for the Standardized Karyotype of the Dog.1, 3 The remainder of the chromosomes are expected to be identified in the very near future. With genetic mapping of disease genes, an effort is made to find polymorphic markers which are linked to disease loci. Markers with the best predictive value are those which are located very close to the disease gene in question.

Genetic Tests

Genetic tests are not all sophisticated polymerase chain reaction (PCR) based assays.7 While some genetic tests identify the animals genotype (affected, carrier, or normal), others indicate the phenotype of the disorder. Note: (The genotype is the genetic constitution of the dog. It is unique to each dog. A comparison of genotype can determine if a dog is the offspring of a sire or dam tested, thus verifying parentage. The phenotype is the clinical appearance of the dog.)
Many traits may be complex in origin, with several genes predicated to contribute to the final phenotype. These traits often appear in the population with significant variability in the phenotype. Screening for cataracts, ausculting for heart murmurs, hip and elbow radiographs, and observation on behavioral traits are all tests of the phenotype.7
PCR based tests are true tests of the genotype. The test is specific for a defective gene, and can differentiate between genetically normal, carrier, and affected individuals. These can be performed at any age, regardless of the onset of the disorder. Offspring can be tested before placement into breeding versus non-breeding situations. Molecular screening tests, utilizing the polymerase chain reaction techniques (PCR tests), are now available to simply and accurately diagnose affected and carrier animals. Examples of PCR based tests are phosphofructokinase deficiency in Spaniels, pyruvate kinase deficiency in Basenjis, several storage diseases (fucosidosis, mucopolysaccharidoses, glycogenesis, gangliosidoses), cystinuria, and type 1 and 3 Von Willebrands disease, as well as several x-linked inherited disorders (hempohilia B, severe combined immunodeficiency).8 These tests are specific for a particular mutation and are therefore usually breed specific.
One recent successful genome screen was the mapping of the progressive rod-cone degeneration (PRA) locus to canine chromosome 9. PRA (progressive retinal atrophy) is the most widespread hereditary retinal disease leading to blindness in dogs. Phenotypically it is the canine counterpart of retinitis pigmentosa (RP) in humans. 1, 4 With the rapid progress in the field of canine genetics, the identification of genes underlying many of the inherited traits, makes the dog a unique asset for the study of mammalian genetics in general.5 The majority of the problematic genetic disorders in domestic animals have a recessive component, with in-apparent carriers being used for breeding. Most dominant genetic disorders are easier to control, as the defective traits are apprarent.
PCR based tests can also be used for linkage studies to polymorphic genetic markers if a defective gene has not been identified. If the genotype is linked to a genetic marker, the defective gene is located close to the marker on the same chromosome. While not an exact test of the genotype, marker-based tests can successfully be used to identify genotypes. Examples of marker-based tests are copper toxicosis in Bedlington Terriers, and progressive retinal atrophy (PRA), in many breeds of dogs.
Other phenotypic tests, such as enzyme storage diseases and blood factor assays, can identify heterozygous carriers of defective genes. A problem with these tests is that sometimes carriers and normal individuals cannot be separated.7 Other phenotypic tests, such as electroretinogram for PRA, or pelvic radiographs for canine hip dysplasia, only identify the affected phenotype and not carriers. Phenotypic tests may also have certain age requirements for their validity.

Polygenic Disorders

Polygenic disorders, such as epilepsy, hip dysplasia, elbow dysplasia, osteochondrois, and congenital heart defects historically have been difficult for breeders to control. For example, in canine hip dysplasia, there is no one “normal hip” gene. A number of genes must combine to produce an affected, dysplastic individual. If. Phenotypically normal parents produce affected offspring, both should be considered to carry a genetic load for the disorder. In polygenic disorders, the phenotype of the individual does not provide all of the necessary information for genetic control. Many polygenic disorders have a major recessive or dominate “trigger gene” that must be present to produce an affected individual. The trigger gene in one breed or family may be different than the gene in another. The identification of these genes will provide better control in the future.7

DNA Fingerprinting

With the application of molecular genetics to veterinary medicine, and the availability of DNA certification programs to preserve the integrity of breed registries, the ability now exists to reliably identify individuals and to deduce their relatedness to others (pedigree) by DNA analysis.9

DNA fingerprinting is being used widely to identify individuals, breeds, and strains, as well as to determine the parentage of not only domestic and wild animals, but microorganisms, insects and plants. “All forms of DNA fingerprinting are based upon detection of a specific segment of the DNA (alleles) or the relative location of repeated nucleotide sequences which are scattered randomly throughout the genome of animals.”9 The DNA alleles are inherited, approximately 50% from each parent. They provide a reliable means of identifying individuals as well as determining the pedigree of individuals, even in highly inbred populations.

With the advances in mapping the canine genome, we will have the ability to identify the genes responsible for over 350 identified, inherited diseases in dogs, as well as the genes that affect infectious diseases, cancer and reproduction.

MHC Complex

The major histocompatibility complex (MHC) is a multi-allelic group of genes present in all animals.9 It is a polymorphic system, with thousands of potential allelic combinations. The genes of the MHC are involved in controlling disease resistance, immune function, and reproduction. MHC haplotypes are associated with a number of significant diseases (arthritis, thyroid diseases, autoimmune disease, ocular disease, intestinal diseases, mastitis, some forms of cancer, and infertility). It is thought that similar disease associations can be made with specific canine MHC haplotype. The development of canine MHC genetic markers will soon allow these assocations to be identified, and thus controlled or eliminated by selective breeding.

The long term viability of any population of animals (breed or species) depends on maintaining a high degree of genetic diversity (polymorphism) in the MHC. There are powerful selective pressures to keep the MHC as diverse as possible. The MHC is responsible for “hybrid vigor”, disease resistance, as well as performance traits and heritable defects.9 As polymorphism decreases (usually related to inbreeding), the survivability of the individual, and thte long term health of the population is incrementally reduced. The loss of MHC genetic diversity is responsible for a portion of the reduced “hybrid vigor” in inbred or highly selected animals, including some dog breeds.

One of the major reasons breed registries and methods to reliably document pedigree were developed, was recognition of the detrimental effect of inbreeding on disease resistance and reproduction. However, such systems do not prevent the breeding of individuals with similar MHC haplotypes. This is a problem when the genetic basis of the breed is narrow (due to inbreeding and linebreeding). With the advances in genetic research, information will soon be available to breeders, so they can make informed decisions on breeding.

One of the most powerful applications of DNA fingerprinting is the identification of individuals and tracing their pedigree through several generations. Breeders, recognizing the importance of pedigree in selecting breeding animals, will have a reliable means to trace pedigree, to measure and record performance criteria, as well as to work to improve the overall health and maintain the “hybrid vigor” of the breed.

Open versus Closed Registries

An open genetic disease registy is a data book of genetic history for any breed and for specific genetic diseases. In an open registry, owners, breeders, veterinarians and scientists can trace the genetic history of any particular dog, once that dog and close relatives have been registered. In order to control genetic diseases, we must know how prevalent the diseases are within the breed and in any particular bloodline.6

Since June of 1990, 3 genetic registries are available to dog breeders. The Institute of Genetic Disease Control in Animals (GDC), in Davis, California, is an open registry. Here, information about each dog is automatically linked by a computer, with other relatives in the registry. This information is available to people so they can choose which bloodlines indicate a reduced risk of producing genetic disease. This information is available because the owner has signed a release so their dog may be placed in the open registry. This type of information is not available in a closed or confidential registry. Only when conscientious breeders submit all the information to the registry, on both normal and abnormal individuals, is this information available.

The GDC open registry is similar to breed registries in Europe, such as Sweden. With polygenic traits such as hip dysplasia, elbow dysplasia, epilepsy, and congenital heart defects, an excellent phenotype does not guarantee excellent genotypes or progeny. Breeding for an excellent "genotype" can only be determined after a review of as many relatives as possible. The GDC gives a report, after enough individuals in a line of dogs have been reported. For a fee of $10.00, one can obtain a report for prospective males to select for breeding. This type of information is not available in a closed or confidential registry. (Since 1990, the GDC has maintained a registry for orthopedic diseases, in 1992: soft tissue diseases were added, 1993: CMO, Perthes, and medial patella luxation were added, 1994: eyes and tumors, 1995: globoid dystrophy, 1997: tricuspid valve dysplasia, and deafness were added. These are done at the requests of breed clubs, with the GDC working with the veterinarians, dog owners and breed clubs).

Both the Orthopedic Foundation for Animals (OFA) and the Canine Eye Registration Foundation (CERF) are closed or confidential registries. They provide only phenotypic information. Information is provided if the individual is free of signs of the disease, but the status of the parents, siblings, half-siblings or progeny is unknown. And it is well-known that the mating of phenotypic, unaffected dogs, may result in offspring that are affected, unaffected, or a combination of both.

Positive Indentification of Dogs for Registration

The AKC, OFA, and GDC all recognize the importance of positively identifying an individual dog for registration. Current means for identification are tattooing, micro-chipping, and DNA fingerprinting. Retrospective pedigree evaluation of some genetic registries (e.g. dairy cattle) have shown that in many cases the reported pedigree is incorrect.9 DNA certification ensures the integrity of a registy in a way never before possible.

DNA "fingerprinting" has been required by the Australian and New Zealand Greyhound Association for registration since 1994 for all sires, and 1996 for all dams. Over 2000 greyhounds have been "fingerprinted". The system has effectively resolved disputes related to identification and pedigree. The Irish Coursing Club also has a DNA "fingerprinting" program for greyhound registrations.9 Both systems assure the validity of the sampling by having a veterinarian submit the samples. Having a licensed professional collect the sample, from a legal perspective, addresses the "chain of custody" issue when submitting the sample

The AKC and the United Kingdom Kennel Club also officially accept the use of DNA "fingerprinting" in the resolution of identity and pedigree disputes. These organizations do not require DNA analysis as a condition for registration. The AKC has a voluntary DNA certification program. There are two inherent problems with the AKC program:

1. The AKC does not address the legal problem of "chain of custody" of the sample. There is poor sample security, with anyone able to submit a sample of saliva by the cheek-swab method.
2. The second problem is the possibility of cross-contamination. With the AKC "cheek-swab" method, if a dog licks another dog, the sample may be cross-contaminated with DNA from the saliva of the second dog.

Research scientists currently working on genetic markers for inherited eye diseases, only accept blood samples submitted by a veterinarian, to avoid the possibility of either of these problems (chain of custody and cross-contamination) from occurring.

Recommendation to Breeders and Parent Breed Clubs

Because attempts to control inherited diseases have largely failed, in part due to inaccurate reporting of a pedigree to genetic registries, breed registration organizations are beginning to adopt the use of DNA "fingerprinting" to protect pedigree integrity. "DNA fingerprinting provides the best method to measure relative genetic relatednes."9 It allows the breeder to compare genetic composition of breeding animals, allowing them to maintain as much hybrid vigor as possible by avoiding inbreeding. DNA fingerprinting provides the ability to map specific performance traits and genetic diseases to the responsible genes. Being able to identify carriers of specific genetic diseases, these systems have the ability to influence breeding programs in order to select for disease resistance.

The most important factor in the control of genetic disease is to know the status of the entire litter from which the problems came rather than the status of the individual parents. Genetic counselors advise owners to breed from phenotypically normal individuals where the majority of full-siblings are also phenotypically normal. While some genetic tests (PCR) accurately identify an animal's genotype (affected, carier), others indicate the phenotype of the disorder.

On the other hand, a "relative risk" pedigree analysis identifies the minimum age of the mutated defective gene in the population, providing a closest common ancestor analysis. The minimum age of the defective gene in the population helps to identify the genetic spread of the defective gene in the gene pool. The closest common ancestor analysis in pedigrees does not identify carriers of a defective gene, and its use for this purpose (witch-hunting or finger pointing) is counterproductive. This point can not be over emphasized. However, the closest common ancestor analysis can be sued in a positive manner with genetic counseling. For example, carriers of a genetic trait (as determined by a blood test), used in breeding, should be accompanied by the recommendations to replace carrier breeding stock with normal testing offspring. This selects against the

defective gene, but allows a breeding program to progress without limiting genetic diversity. Recommendations to eliminate all carriers and affected individuals from breeding can significantly limit genetic diversity.

Genetic diversity concerns are also compounded with the widespread use of frozen and fresh shipped semen, where individual males can have a profound input on a breed's gene pool. This has become especially evident with the "favorite sire syndrome", with detrimental recessive genes becoming widespread due to prolific breeding of popular sires in many breeds. Any major shift in breeding choices to a limited number of males will restrict genetic diversity and increase the possibility of fixing undetected, defective, recessive genes in the population. Breed wide genetic disease control programs should monitor the frequency of the defective genes in the population, and work to diminish them without affecting the overall genetic diversity of the gene pool.

High frequency defective genes require breed-wide counseling, so that selective pressure does not significantly shift the gene pool. Rare defective genes, regardless of their genetic spread, should be closely controlled. The test and slaughter system should no longer be used. We cannot afford to eliminate every affected dog, and carrier dog from breeding, but instead have to learn to live with genetics.

In some cases there may be a truly extraordinary dog, who exemplifies the breed standard, and is found to be a carrier of a highly undesirable trait. Owners and breeders will have to make the difficult decision how to modify their breeding program, and what sort of risks they are willing to take. From a veterinarian's perspective, clients will expect accurate risk assessment for their dogs, and will want guidelines on how best to proceed with their breeding programs.

Some breed clubs have their own genetic registries. OFA, CERF and GDC are examples of multi-breed registries. The Canine Genetics Laboratory, Baker Institute, Cornell University developed and established the DNA tests for hereditary eye disorders. The Josephine Deubler Genetic Disease Testing Laboratory, at the University of Pennsylvania, offers biochemical, hematologic and molecular genetic tests for many hereditary disorders of companion animals. One example of successful control of a genetic problems by a breed club is the control of PRA in Irish Setters by a blood test. While PRA was once a major problem in the breed, the availability of the blood test has reduced the carrier rate to 7%.

Conclusion

Genetic disease control must be balanced with the need to breed individuals whose form, structure and function, and performance, improves the breed. Breeders should select for healthier breeding stock, while slowly working aways from genetic defects. And the most successful endeavors to map disease genes will be those which are based on high quality diagnostic data from veterinarians. "Having absolutely accurate information about dogs in a family affected, and how the disease state is expressed, will be the key to unraveling the genetics of any canine disease trait."1

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