TESTING FOR GENETIC DISORDERS

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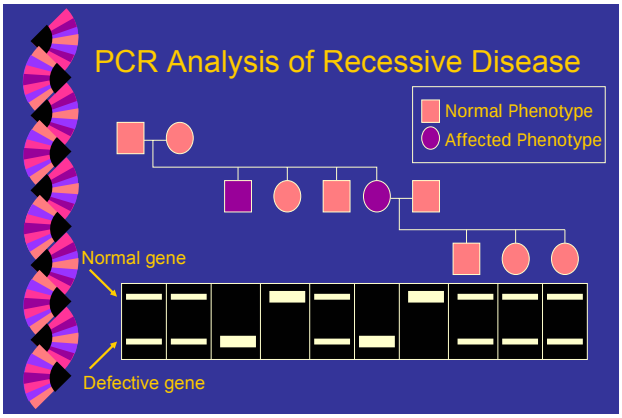
The hallmark of genetic disease is the ability to predict disease before its onset. This allows the possibility of medical or surgical intervention in order to prevent later suffering. Knowledge of breed-related genetic disease and the tests that are available permit early diagnosis and treatment.

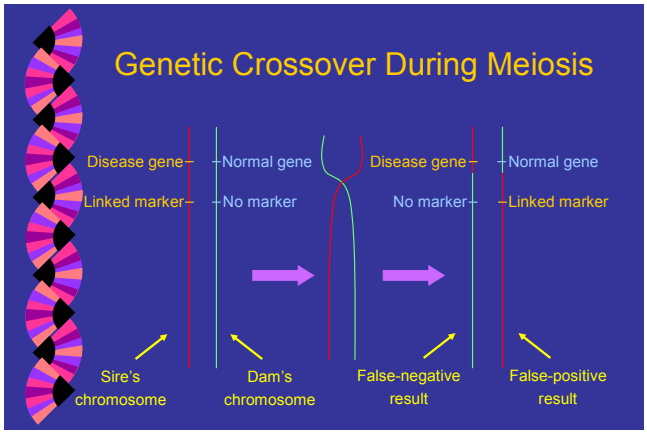
Breeders and veterinarians have been utilizing genetic tests since the beginning of domestic animal breeding. Most genetic tests measure the phenotype of an animal, or what you can see. These include radiographs, blood values, eye examinations, skin biopsies, urinalysis for crystals or metabolites, observations on structure or behavior, and ausculting for heart murmurs. Most tests of the phenotype only identify affected individuals, and not carriers. These may or may not directly relate to the genotype, or the genes regulating the defect.

Types of Genetic Tests

Direct gene tests utilizing PCR or polymerase chain reaction are a direct measurement of the genotype. (See figure; squares are males and circles are females.) These tests have to be developed specifically for each breed (or group of related breeds that share an ancestral mutation). They can be run at any age, regardless of the age of onset of the disorder. With tests for the genotype, breeders can identify affected, carrier, and genetically normal animals, and place them in appropriate pet or breeding homes.



Linkage-based DNA tests can be developed even if the defective gene causing a disorder has not been identified. Genome research has identified thousands of genetic markers, or "marker-DNA" that are spread across the chromosomes of the species. A linked-marker is a piece of DNA that lies close to the defective gene on a chromosome. If a crossover between the marker and the gene occurs during the production of egg or sperm in meiosis, the marker will no longer be linked to the defective gene. False positive and false negative results will occur (see figure). Depending on the relative distance between the marker and the defective gene on the chromosome, researchers can predict the frequency of false results for a linkage-based test; for example: 1 in 100. Due to this phenomenon, linkage test results must be compared with results from other family members to determine whether they correlate with the known genotype of relatives.



If an individual's linkage-based test for the defective gene is producing false-positive or false-negative results, all of its descendents that inherit this portion of the chromosome will also have false test results. This has been documented with families of Bedlington Terriers tested for the autosomal recessive copper toxicosis gene.

It is obvious that direct gene tests are better than linkage-based tests. However, a test with 90% or 95% confidence is better than no test at all. As genomic research progresses, researchers can identify the defective genes responsible for disorders, and can develop direct gene tests to replace linkage-based tests. The defective gene for copper toxicosis in the Bedlington Terrier has now been identified, and a direct gene test is now possible.

As the majority of genetic disorders are recessive or have a major recessive component, the identification of carriers is important for effective management. As additional DNA tests are developed for disorders, the role of genetic counseling becomes more important. Without these tests, the number of individuals that can be identified as carriers is low, even though many may be suspect due to having affected relatives. Breeds have closed gene pools; in other words, the diversity of genes in a given breed is fixed. The number of individuals removed from consideration for breeding based on concerns regarding a specific genetic disease is usually low. While this has slowed the management of genetic disease, it has also prevented genetic drift and diversity problems for pure breeds.

Once a genetic test is developed, it allows breeders to positively determine if an individual is a carrier of a defective gene. The typical response of a breeder on finding that their animal is a carrier is to remove it from a breeding program. If a majority of breeders do this, it puts the breed's gene pool through a genetic bottleneck that can significantly limit the diversity of the breed.

History has shown that breeders can be successful in reducing breed-wide genetic disease through testing and making informed breeding choices. However, there are also examples of breeds that have actually experienced more problems as a result of unwarranted culling and restriction of their gene pools. These problems include; 1) reducing the incidence of one disease and increasing the incidence of another by repeated use of males known to be clear of the gene that causes the first condition, and 2) creating bottlenecks and diminishing diversity by eliminating all carriers of a gene from the breeding pool, instead of breeding and replacing them. These occur due to the focus on the presence or absence of a single gene and not the quality of the whole animal.

The goal of genetic testing is to allow the superior genes of a breeding individual to be propagated, even if the animal is a carrier. One defective gene that can be identified through a genetic test, out of tens of thousands of genes is not a reason to stop breeding. If an owner would breed an individual if it tested normal for a genetic disease, then a carrier result should not change that decision.

Owners of carrier animals who are of breeding quality in other health, temperament, performance and conformation aspects should be bred to normal testing mates. This prevents the production of affected offspring. The breeder should be counseled to test the offspring prior to

placement; to determine whether a pet or breeding home is appropriate. The goal is to replace the carrier parent with a quality, normal testing offspring that carries on the lineage of the breeding program.

If the only quality offspring is also a carrier, then breeders can use that offspring to replace the original carrier. The breeder has improved the quality of the breeding stock, even though the defective gene remains in the next generation. The health of the breed does depend on diminishing the carrier frequency and not increasing it. Breeders should therefore limit the number of carrier-testing offspring placed in breeding homes. It is important to carry on lines. A test that should be used to help maintain breed diversity should not result in limiting it.

By breeding and not selecting against carriers, breeders are selecting for a carrier frequency of fifty-percent; higher than most breed averages. Each breeder must assess the frequency of the defective gene in their own breeding stock and determine their own rate of progress. As each breeder reduces the number of carrier breeding stock, the frequency of the defective gene for the breed will decrease.

Researchers are tempted to use the Hardy-Weinberg law to calculate a carrier frequency in the population based on the observed frequency of affected individuals. It must be understood that the law is only valid if based on a population in Hardy-Weinberg equilibrium. This requires no selection, and all members of each generation to have an equal chance at reproduction, in equal frequencies. These parameters do not exist in domestic animal breeding scenarios. While it is recognized that the frequency of carriers of recessive defective genes will far exceed that of affected individuals, there is no mathematical relationship between the two in domestic animal breeding. This has been shown in analyzing the results of generations of genetic testing for breed specific disorders. Valid estimates of the frequency of carriers in the population can be determined through population-wide genetic testing or estimated through pedigree analysis.

We know that most individuals carry some unfavorable recessive genes. The more genetic tests that are developed, the greater chance there is of identifying an undesirable gene. However, an animal is not a single gene, an eye, a hip, or a heart. Each individual carries tens of thousands of genes, and each is a part of the breed's gene pool.

No breeder wants to produce animals with genetic disorders. As most disorders do not have genetic tests at the present time, the knowledge of the affected or normal status of close relatives is necessary for informed breeding decisions. As veterinarians, we should counsel breeders to be open about both positive and negative test results in their breeding stock, and support open genetic health registries.

Our Role With Genetic Registries

There are several genetic registries that have been established to assist dog breeders with genetic disease control. The development of genetic registries for cats, on the other hand has been slow. The Canine Eye Registry Foundation or CERF (http://www.vmdb.org/cerf.html) is a closed database showing only normal eye examination results by ACVO boarded veterinarians. The Orthopedic Foundation for Animals (OFA: www.offa.org) has semi-open registries for hip dysplasia, elbow dysplasia, autoimmune thyroiditis, congenital cardiac disease, and patellar

luxation. As veterinarians, we should counsel our clients to check off the boxes for open disclosure of test results on the OFA submission forms, for listing on the OFA website. If you do not have the new OFA forms with the check-off boxes, they can be downloaded from the OFA website.

The Canine Health Information Center or CHIC (www.caninehealthinfo.org) is an open health database that has been established by the AKC Canine Health Foundation and the Orthopedic Foundation for Animals. National parent clubs decide to participate in the CHIC program, and determine the testable genetic disorders for their breed. (For example, hip evaluation, CERF examination, and thyroid testing.) Owners and breeders can search online for dogs in the CHIC database, and view their test results. If a dog completes the recommended testing panel, it receives a CHIC number regardless of whether it passes all of the tests. CHIC is about health consciousness, not health perfection. As more testable disorders are identified, few dogs will be normal for all tests.

The usefulness of registries to control genetic disorders improves with open registries, so that breeders can utilize the information on breeding animals and their close relatives to make informed breeding decisions. This requires an atmosphere of cooperation and understanding between breeders, for the benefit of the breed. Breeding practices do not cause defective genes. No one wants to produce affected animals, or propagate a genetic disorder. If breeders are reluctant to identify affected individuals, then the usefulness of open registries will be limited.

There is a tendency for breeders to breed to the top-winning male. This can also occur with a popular dog that has OFA excellent hip conformation, or has produced no epileptic offspring in matings to epileptic dams. Regardless of the popularity of the breed, if a large portion are breeding to a single stud, (the popular-sire syndrome), the gene pool will drift in that individual's direction and there will be a loss of genetic diversity. Too much breeding to one individual will give the gene pool an extraordinary dose of his genes, and this will include whatever detrimental recessives he may carry, to be uncovered in later generations. Such genes have become widespread even in populous breeds due this process, called the founder's effect.

The loss of genes from a breed's gene pool occurs through selection: the use and non-use of offspring. If a popular sire is used extensively, gene frequencies, and the gene pool can shift towards his genes, limiting the breed's genetic diversity. On the other hand, dogs that are poor examples of a breed should not be used simply to maintain diversity. Related dogs with desirable qualities will maintain diversity and improve the breed.

Purebred dog breeds have closed studbooks. No new genes are available to the breed, except from infrequent mutations that are usually not desirable. Considering a breed as a whole, genes cannot be gained through selective breeding; they can only be lost. This has lead breeders to question whether a pure breed can go though hundreds of years of selective breeding and still maintain its health and viability.

Breeders underestimate the amount of diversity that can be present in a breed; even one with a limited group of founders. A molecular genetic study of the Chinook dog breed, which was

reduced to four dogs in the 1970s, showed that there was significant gene diversity and heterozygosity in the breed.

The studbook for the Thoroughbred horse has been closed for more than 300 years. However, researchers have found that on average 63 percent of the variable gene pairs are heterozygous and that 4.7 genes are potentially available to each pair. This diversity is present in spite of the fact that 95 percent of the breed traces back to a single founder male.

The Doberman Pincher breed has a problem with von Willebrand's disease; an autosomal recessive bleeding disorder. Genetic testing has found that the defective gene is present in 77 percent of Dobermans. Doberman breeders can test and identify carrier and affected dogs. They can decrease the defective gene's frequency by breeding carriers to normal-testing dogs and selecting quality, normal-testing offspring for breeding. By not just eliminating carriers, but replacing them with their normal-testing offspring, genetic diversity will be preserved.

Breeders are the custodians of their breed's past and future. "Above all, do no harm" is a primary oath of all medical professionals. Genetic tests are powerful tools, and their use can cause significant positive or negative changes. Breeders should be counseled on how to utilize test results for the best interests of the breed.

The following table lists the genetic tests that are presently available for dog and cat owners and breeders. Tests are identified as phenotypic (P), linkage-based (L) or direct (D) tests of the genotype. Information on the procedures and costs of the tests can be found on the testing facility websites listed below the table.

Disorder	**Breeds**	**Type**	**Test Facility**
Canine Leukocyte Adhesion Deficiency (CLAD)	Irish Red & White Setter Irish Setter	D	Optigen
Coat Color Gene Variations	American Cocker Spaniel Curly Coated Retriever Dalmatian Doberman Pincher English Springer Spaniel Flat Coated Retriever Gordon Setter Labrador Retriever Newfoundland Poodle Schipperke Scottish Terrier	D	VetGen
Cone (Retinal) Degeneration	German Shorthaired Pointer	D	Optigen
Congenital Hypothyroidism with Goiter (CHG)	Toy Fox Terrier	D	Michigan State University – Fyfe Lab.

Disorder	Breeds	Type	Test Facility
Congenital Stationary Night Blindness (RPE65-CSNB)	Briard	D	Optigen
Copper Toxicosis	Bedlington Terrier	L	VetGen
Cystinuria	Newfoundland Labrador Retriever	D	Optigen (Newf only) PennGen VetGen (Newf only)
Fanconi Syndrome	Basenji Norwegian Elkhound	P	PennGen
Fucosidosis	English Springer Spaniel	D	PennGen
Glanzmann's thrombasthenia	Great Pyrenees Otterhound	D	Auburn - Boudreau Lab
Globoid cell leukodystrophy	Cairn terrier West Highland White Terrier	D	Jefferson Medical Coll.
Glycogenosis (GSD) Type IV	Norwegian Forest Cat	D	PennGen
GM1-Gangliosidosis	Portuguese Water Dog	D	New York University Neurogenetics Lab
GM1 or GM2 Gangliosidosis	Korat	D	Auburn - Baker Lab
Ivermectin Sensitivity (MDR1)	Collie Other breeds	D	Washington State University
Mannosidosis	DSH Persian	D	PennGen
Methylmalonic Aciduria - Cobolamin Malabsorbtion	Beagle Border Collie Giant Schnauzer Shar Pei	P	PennGen
Mucopolysaccharidosis (MPS)	DSH Miniature Pinscher Schipperke Siamese	D	PennGen
Myotonia Congenita	Miniature Schnauzer	D	Optigen PennGen
Narcolepsy	Dachshund Doberman Pinscher Labrador Retriever	D	Optigen
Phosphofructokinase Deficiency (PFK)	American Cocker Spaniel English Springer Spaniel	D	Optigen PennGen VetGen

Disorder	Breeds	Type	Test Facility
Progressive Retinal Atrophy - Dominant	Bullmastiff (English) Mastiff	D	Optigen
Progressive Retinal Atrophy – Type A	Miniature Schnauzer	D	Optigen
Progressive Retinal Atrophy – X-Linked	Samoyed Siberian Husky	D	Optigen
Progressive Retinal Atrophy (prcd)	Australian Cattle Dog Chesapeake Bay Retriever English Cocker Spaniel Labrador Retriever Nova Scotia Duck Trolling Retrievers Poodle; Miniature & Toy Portuguese Water Dog	L	Optigen
Progressive Retinal Atrophy (rcd1)	Irish Red & White Setter Irish Setter Sloughi	D	Optigen VetGen (Irish Setter)
Progressive Retinal Atrophy (rcd3)	Cardigan Welsh Corgi	D	Michigan State Univ. Optigen VetGen
Pyruvate Kinase Deficiency (PK)	Abyssinian American Eskimo Dog Basenji Beagle Cairn Terrier Chihuahua Dachshund DSH Somali West Highland White Terrier	D	Optigen (Basenji) PennGen (All) VetGen (Basenji)
Renal Dysplasia	Lhasa Apso Shih Tzu Soft Coated Wheaton Terrier	L	VetGen
Severe Combined Immunodeficiency (SCID)	Bassett Hound Cardigan Welsh Corgi Pembroke Welsh Corgi	D	PennGen

Disorder	Breeds	Type	Test Facility
Von Willibrand's Disease	Bernese Mountain Dog Doberman Pincher Kerry Blue Terrier Manchester Terrier Papillion Pembroke Welsh Corgi Poodle Scottish Terrier Shetland Sheepdog	D	VetGen

Optigen: www.optigen.com (607-257-0301)
PennGen: www.vet.upenn.edu/penngen (215-898-8894)
VetGen: www.vetgen.com (800-483-8436)
Auburn - Baker Lab: bakerhj@vetmed.auburn.edu (334-844-5951)
Auburn - Boudreau Lab: boudrmk@vetmed.auburn.edu (334-844-2692)
Jefferson Medical College: David.wenger@mail.tju.edu (215-955-1666)
Michigan State University – Peterson-Jones:
http://www.cardigancorgis.com/about1PRAarticle.htm (517-353-3278)
Michigan State University – Fyfe Lab.: http://www.msu.edu/unit/mic/facpages/fyfeform.htm
(517-355-6463x1559)
New York University Neurogenetics Laboratory: (212-263-7637)
Washington State University:
http://www.vetmed.wsu.edu/announcements/ivermectin/ownerInfo.html (509-335-2988)