How to Recognize and Screen for Hereditary Diseases

Urs Giger, DACVIM, ECVIM, ECVCP; Charlotte Newton Sheppard, Professor of Medicine
Section of Medical Genetics, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Because of the increased awareness of breeders, pet owners, and veterinarians of genetic defects and the improved diagnostic abilities in clinical practice, the number of reported hereditary diseases in small animals is rapidly growing. While in humans around five thousand disorders have been described as having a genetic basis (Online Mendelian inheritance in Man [OMIM] http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM&cmd=search&term=omim, the second highest number of reported naturally occurring hereditary disorders is seen in the domestic dog ("Inherited Diseases in Dogs" [IDID], http://www.vet.cam.ac.uk/idid). Currently the literature describes about one tenth of the number seen in humans, but the disease number in dogs is rapidly rising. For several common breeds greater than 40 inherited diseases have been reported, although most defects are probably rare in any one breed (http://www.vet.cam.ac.uk/idid). Similarly about 180 disorders have been adequately documented in cats, and every year new defects are being reported. For the small animal practitioner, it can be a daunting, nearly impossible task to remember all these disorders. The recent advances to recognize and screen for hereditary diseases in companion animals will be covered and illustrated with clinical case examples.

Genetic diseases are caused by chromosomal alterations or gene mutations. Disease-causing mutations are heritable changes in the sequence of genomic DNA that alter the expression, structure, and function of the coded protein. The genotype refers to the animal's genetic makeup, reflected by its DNA sequence, whereas the phenotype relates to the clinical manifestation of specific gene(s) and environment, or both. The molecular genetic defect is now known for > 50 hereditary disorders in small animals which are listed on our web site http://www.vet.upenn.edu/pennqen. These molecular genetic changes include point mutations, deletions, and insertions in the DNA sequence that result in a missense or nonsense sequence with an altered codon sequence.

The pattern of inheritance depends mainly on two factors: 1) whether the mutation is located on an autosome (autosomal) or on the X-chromosome (X-linked), and 2) whether the phenotype, the observable expression of a genotype as a disease trait, is dominant, i.e., expressed when only one chromosome of a pair carries the mutation, or recessive, i.e., expressed when both chromosomes of a pair carry the mutation. Thus, it is the phenotype rather than the mutant gene or protein that is dominant or recessive. Whereas in humans most diseases are dominantly inherited, recessive traits are favored by the common inbreeding practices in small animals. For approximately half of the disorders suspected to be of a genetic nature the mode of inheritance remains however unknown. While the recognition and control of single gene disorder is relatively simple, identification and eradication of the more recently recognized disorders with a complex trait of inheritance are much more difficult to handle. Complex traits refer to involvement of more then one gene and also various environmental factors that can affect the development and the severity of the disease process. There are a rising number of examples of complex traits including congenital heart developmental anomalies, increased susceptibility to inflammatory, immune-mediated, and degenerative diseases, predisposition to drug reactions (pharmacogenetics) and cancer.

Clinical Signs

Gene defects can involve any gene or organ; therefore, the clinical signs of hereditary diseases are extremely variable and may mimic other acquired disorders. Some typical features, however, may raise our suspicion of a genetic disorder. In contrast to infectious diseases, intoxications, and nutritional imbalances that generally affect an entire litter, hereditary diseases often involve only a few in a litter. Furthermore, the age of onset of clinical signs for a particular gene defect is rather specific and independent of environmental factors unless it is a complex trait.

Most genetic defects cause clinical signs early in life. In fact, fetal resorptions, late abortions, and stillborns may also be caused by genetic traits but are rarely determined. Most *puppy and kitten mortality* occurs during the first week of life, shortly after the maternal homeostatic system can no longer compensate for an endogenous defect. Some neonatal kitten losses have recently been attributed to blood type incompatibility: Type A and AB kittens born to type B queens develop life-threatening neonatal isoerythrolysis when nursing and absorbing anti-A containing colostrum during the first day of life. Certain congenital malformations also may not be compatible with life, such as severe cleft palates and hernias. The term *congenital* only implies that the disease is present at birth, however, and does not necessarily mean it is hereditary.

A common presentation is *failure-to-thrive*. These animals lag behind their healthy littermates in their development; they do not gain weight at a normal rate and are generally lethargic. They are poor doers, often fade (hence the term *fading puppy or kitten syndrome)*, and finally die. Failure-to-thrive should not be confused with *growth retardation*, which refers to a proportionally stunted growth that may or may not be associated with other clinical signs. In addition to these relatively unspecific clinical signs, some defects may cause specific clinical manifestations. Easy to recognize are *developmental malformations* that involve any part of the skeleton and lead to disproportionate dwarfism, gait abnormalities, and/or facial dysmorphia. A large number of *hereditary eye diseases* have been described in dogs, some of which are not recognized until adulthood. *Neuromuscular signs* may vary from exercise intolerance to ataxia and seizures. Defects of many other internal organs are associated with unspecific clinical signs. Many disorders cause an isolated typical sign, whereas others produce a characteristic overall pattern of anomalies known as *syndromes*.

Clinical manifestations of hereditary diseases are extremely variable ranging from benign to debilitating and lethal. They are usually chronic and progressive, i.e., once an animal shows signs it probably will not recover, and often cause death at an early age. A few hereditary defects, however, result in intermittent or recurrent problems, such as hereditary bleeding disorders and primary immunodeficiencies.

Diagnostic Tests

Diagnostic tests generally are required to further support a genetic disorder in a diseased animal. Radiology and other imaging techniques may reveal skeletal malformations or cardiac anomalies, and ophthalmologic examination may further define an inherited eye disease, although some are not recognized before several years of age. Routine tests such as complete blood cell count, chemistry screen, and urinalysis may suggest some specific hematologic or metabolic disorders or rule out many acquired disorders. Furthermore, clinical function studies may more clearly define a gastrointestinal, liver, kidney, or endocrine problem. Histopathology and/or electron microscopy of a tissue biopsy from an affected animal or from the necropsy of a littermate or relative may give the first clue as to a genetic defect.

However, for many hereditary diseases specific laboratory tests are required to reach a definitive diagnosis. There are two different ways to screen animals for hereditary disorders. One could wait until an animal is diseased and a genetic cause is suspected before administering the laboratory tests that will identify the disorder. The true meaning of screening, however, is

to test all animals for genetic diseases by performing tests before clinical signs are recognized. As mentioned above this generally requires more than clinical examinations. A variety of laboratory tests, such as hematological, metabolic, and DNA tests have been developed which are not only able to identify affected/diseased animals but also asymptomatic carriers of recessively inherited disorders. The Section of Medical Genetics at the School of Veterinary Medicine of the University of Pennsylvania is one of few places that performs such tests to diagnose known as well as to discover novel hereditary disorders. (www.vet.upenn.edu/penngen)

The molecular defect has been identified for >50 hereditary diseases in companion animals, and thus DNA screening tests have been developed and are being offered by various molecular genetic laboratories beside our laboratory at Penn. These tests are mutation specific and can therefore only be used in animals suspected to have the exact same gene defect. Small animals within the same or a closely related breed will likely have the same disease-causing mutation for a particular disease, e.g., phosphofructokinase deficiency in English springer and American Cocker spaniels, but also mixed breed dogs (mother-son or father-daughter matings). However, dogs and cats as well as unrelated breeds of a species with the same disorder will likely have different mutations, as shown with X-linked muscular dystrophy and erythrocyte pyruvate kinase deficiency in various dog breeds and cats.

DNA tests have several advantages over other biochemical tests. The test results are independent of the age of the animals, thus, the tests can be performed at birth or at least long before an animal is placed in a new home as well as before clinical signs become apparent. DNA is very stable and only the smallest quantities are needed; hence, there are no special shipping requirements as long as one follows the specific instructions for biological products. DNA can be extracted from any nucleated cell, e.g., blood, buccal mucosa (cheek brushes), hair follicle, semen, and even formalinized tissue. For instance, blood can be sent in an EDTA tube or a drop of blood can be applied to a special filter paper. Buccal swabs can be obtained with a special cytobrushes (10 rotations against cheek), although this method should not be used in nursing animals, or if absolutely necessary, only after flushing the oral cavity. The DNA segment of interest is amplified with appropriate primers and polymerase chain reaction (PCR). The mutant and/or normal allele are identified by DNA sequencing and by DNA size differences directly on a gel in case of deletions or insertions or after restriction enzyme digestion for point mutations. These tests are generally simple, robust, and accurate as long as appropriate techniques and controls are used. Furthermore, they can be used not only for the detection of affected animals but also for carriers and thus are extremely valuable to select breeding animals that will not cause disease or further spread the disease-causing allele. For instance, phosphofructokinase deficiency was recognized to cause intermittent anemia and myopathy in English Springer spaniels and a DNA based test has become available in the early 1990s, there were still 4% and 1% carriers in the field trial and conformation lines, respectively, in the first randomized survey performed in 1998. If an animal with all the desirable qualities is found to be a carrier, it could be bred to a clear animal (homozygous normal), as this would not result in any affects and as long as all offspring would be tested and only clear animals were going to be used in the next generation.

For many inherited disorders, the defective gene remains unknown; however, for a few a polymorphic DNA marker that is linked to the mutant allele has been discovered. Such linkage tests were first developed for copper toxicosis in Bedlington terriers and are now available for some forms of retinopathy and renal carcinoma and nodular dermatitis in German Shepherds, and are accurate for a particular patient as long as there is a known affected animal in its family (informative family). At present, mutation-specific and linkage tests are available only for single gene defects in small animals; however, complex genetic traits may also soon be approached by these methods as they are for humans. The recent unraveling of the canine and feline genome sequences will hasten the progress in identifying disease-causing mutations for single and particularly complex disease processes.

PROGNOSIS AND THERAPY

Because the clinical consequences of the many hereditary disorders vary greatly, it is not surprising that the prognosis for survival and quality of life ranges from excellent to grave. The clinical course and outcome for a particular genetic defect is rather similar among affected animals. Some defects are recognized as a breed characteristic, such as fold ear in Scottish folds and tailless Manx (both dominant traits), or an incidental finding, e.g., microcytosis in Akitas, whereas others are progressive and lead to severe organ dysfunction and death, e.g., many lysosomal storage diseases.

At present, the therapeutic options in the treatment of hereditary diseases are limited and ethical principles need to be carefully considered. Although several structural malformations can be surgically corrected, such as cryptorchism, hernias, hepatic shunts, and a patent ductus arteriosus, these animals should not be shown or bred. In a few cases a deficient protein, cofactor, substrate, or metabolite can be supplemented to correct the defect. For instance, vitamin B12 deficiency in cachectic and lethargic Giant Schnauzers, Beagles, and Border collies with an ileal receptor defect can be helped by monthly cobalamin injections. Pancreatic enzyme supplementation and daily insulin injections are used to manage animals with exocrine or endocrine pancreatic insufficiency, respectively. Fresh frozen plasma is administered in the treatment of hereditary coagulopathies and von Willebrand disease whenever animals excessively bleed. Other enzyme and protein replacements are also experimentally attempted. On occasions a therapeutic trail may lead to a diagnosis.

Although kidney transplants have been established in clinical practice for chronic renal failure in cats, they have not been applied clinically in animals with hereditary (juvenile) renal disorders. Several hereditary disorders of hematopoietic cells have been experimentally corrected by bone marrow transplantation, e.g., pyruvate and phosphofructokinase deficiency, cyclic hematopoiesis, and interleukin-2 (IL-2) receptor defects. Furthermore, bone marrow transplantation is being attempted to deliver functional cells or active proteins to other tissues including liver, bone, and brain, e.g., in lysosomal storage disease. Finally, gene therapy, the integration of a functional gene into the patient's own defective cells, will likely be clinically feasible in the twenty-first century.

CONTROL

Much more important than the treatment of hereditary disorders is the control of these traits in breeding programs. Thus, in order to reduce the frequency or eliminate altogether a genetic defect, the further spread of the mutant gene has to be prevented in a family or entire breed. Hence prior to breeding animals should be screened for know hereditary diseases. It is obvious that affected animals of any genetic disease should not be used for breeding. This approach is simple and effectively eliminates disorders with a dominant trait. For recessively inherited disorders, however, the elimination of affected animals is not sufficient to markedly reduce the prevalence of a defect within a breed or kennel/cattery. Although it may be safest not to breed any related animals of affected animals, as requested by some kennel clubs, this practice may, because of inbreeding and narrow gene pools in some breeds, eliminate all breeders in an entire kennel or cattery, and may severely reduce the genetic diversity (gene pool) of a breed. Thus, it will be pivotal to detect carriers (heterozygotes) and truly "clear" animals (homozygous normal). Obligate carriers can be readily identified for autosomal (both parents of affected) and X-chromosomal recessive (mother of affected) disorders. As mentioned above, for some diseases, reliable carrier detection tests are available

and many breeders know about them and inform the veterinarian. For instance, carriers have half-normal (~50%) enzyme activity by functional assays, or have a normal *and* mutant DNA sequence for the diseased gene on a DNA test. Breeders should, therefore, be encouraged to screen their animals before breeding for know genetic diseases whenever carrier tests are available. Their availability is also listed on several web sites for veterinarians and breeders including www.vet.upenn.edu/penngen. Unfortunately, many breeders still mistrust these newer tests; either they were disappointed by the inaccuracy of early tests, such as the radiographic examination for hip dysplasia, or they fear that the results may become public and could hurt their business. Thus, breeders need to be educated by well-informed veterinarians. If a carrier needs to be used because of a narrow gene pool and many other desirable traits, it should be bred with a homozygously normal (clear) animal; all its offspring need to be tested, and only clear animals should be used in future breedings.

In conclusion, it is most exciting to learn about many recent advances for many hereditary disorders and genetic predispositions in small animal practice, be it for the diagnostic approach to a hereditary disease, the understanding of its pathophysiology, or its control. In addition to the clinician's responsibility to suspect a genetic disease and to appropriately diagnose it with modern specific techniques, clinicians must become involved in the control of these disorders in the breeders' kennels or catteries. Practitioners thus can make an important contribution toward controlling the further spread of mutant genes and reducing future suffering of animals.

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Urs Giger, DACVIM, ECVIM, ECVCP
Charlotte Newton Sheppard, Professor of Medicine
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School of Veterinary Medicine, University of Pennsylvania
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