THE IMPORTANCE OF CLINICAL GENETICS (Part II)

The Importance of Clinical Genetics

Editor's Note: Dr. Giger was the speaker at this year's Montgomery County weekend seminar. This edition of the News features the second half of his article, "The Importance of Clinical Genetics." The first half was printed in the Fall edition.

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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.

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 losses occur 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 malformations that involve any part of the skeleton and lead to disproportionate dwarfism, gait abnormalities, and/or facial deformities. 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.

Examples of Hereditary Diseases in West Highland White and Cairn Terriers

- Craniomandibular osteopathy
- Legg-Calve Perthes disease
- Hip dysplasia
- Myotonia congenita
- Multisystemic neuronal degeneration
- Globoid cell leukodystrophy
- Erythrocyte pyruvate kinase (PK) deficiency
- Von Willebrand disease type I
- Hemophilia B
- Cataract
- Atopy
- Idiopathic pulmonary fibrosis
- Hepatic portosystemic shunt
- Familial chronic active hepatitis
- Polycystic kidney and liver disease (AR PKD)
- Cystinuria
- Ectopic ureters

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.

A few laboratories provide special diagnostic tests that allow a specific diagnosis of an inborn error of metabolism. Inborn errors of metabolism include all biochemical disorders due to a genetically determined, specific defect in the structure and/or function of a protein molecule. Aside from the classical enzyme deficiencies, genetic defects in structural protein receptors, plasma and membrane transport proteins, and other proteins covered by this definition will result in biochemical disturbances. The laboratories' approach is to detect the failing system or to determine the specific protein or gene defect. The most useful specimen to detect biochemical disturbances is urine because abnormal metabolites in the blood will be filtered through the glomeruli (the kidney's filtering system), but fail to be reabsorbed, as no specific renal transport system exist for most abnormal metabolites.

Once the failing system has been identified, the defect can be determined at the protein level. These protein assays include the classic enzyme function tests as well as immunologic assays. Because most enzymes are present in abundant amounts, no major functional abnormalities are generally observed unless the enzyme activity is severely reduced, usually to less than 20 percent of normal value. Thus, homozygously affected animals (animals with 2 defective alleles) have very low protein activity and/or quantities, often in the range of 0 to 5 percent. These tests may also be used to detect carriers (heterozygotes), who typically have intermediate quantities at the protein level (30-70 percent), but no clinical signs. Unfortunately, protein assays require submission of appropriate tissue or fluid under special conditions to specialized laboratories along with a control sample, and are labor intensive. The Section of Medical Genetics at the School of Veterinary Medicine of the University of Pennsylvania is one of the 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 over 3 dozen hereditary diseases in companion animals, and thus DNA screening tests have been developed. 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 swabs), 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, 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 size difference 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 affecteds and 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 no 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. Examples of hereditary disorders are shown in the table on page 6.

Table 1

EXAMPLES OF HEREDITARY DISORDERS CHARACTERIZED AT THE MOLECULAR GENETIC LEVEL IN DOGS AND CATS (as of 2004)

DISORDER	**BREED**
Hematologic disorders	
Elliptocytosis (band 4.1)	Mixed breed
Pyruvate kinase (PK) deficiency	Basenji, West Highland White Terrier, Dachshund, Beagles, Eskimo, Chihuahua, Abyssinian, Somali, DSH cat
Phosphofructokinase (PFK) deficiency	English Springer and Cocker Spaniel, Mixed breed dog
Hemophilia A (Factor VIII)	Mixed breed
Hemophilia B (Factor IX)	Cairn Terrier, Labrador Retriever, Mixed breed
von Willebrand disease vWD type 1	Doberman, Manchester and Cairn Terrier, Pembroke Welsh Corgi
vWD type 2	German Shorthair & Wirehaired Pointer
vWD type 3	Dutch Kooiker, Scottish terrier
Autosomal recessive severe combined immunodeficiency	Jack Russel Terrier
X-linked severe combined immunodeficiency (SCID)	Basset, Cardigan Welsh Corgi
Leukocyte adhesion deficiency (LAD)	Irish Setter, Red & White Setter
Complement component 3 deficiency1	Brittany Spaniel
Hereditary eye diseases	
Progressive retinal atrophy	Irish setter (B-chain phosphodiesterase)
Rod cone dysplasia	Cardigan Welsh Corgi, Chesapeake Bay & Labrador Retriever, English Cocker Spaniel, Portuguese Waterdog

Stationary night blindness	Briard
prcd-PRA	Australian Cattle Dog, Chesapeake Bay Retriever, English Cocker Spaniel, Labrador Retriever, Miniature and Toy Poodle, Nova Scotia Duck Tolling Retriever, Portuguese Water Dog
Cone degeneration	German Shorthaired Pointers
Type A PRA	Miniature Schnauzers
X-linked PRA	Siberian Husky and the Samoyed
Neuromuscular diseases	
Shaking puppy syndrome	English Springer Spaniel
Dystrophin muscular dystrophy	Golden Retriever, Rottweiler, DSH cat
Mucopolysaccharidosis type I	Plott Hound
type IIIA	Wirehaired Dachshund, New Zealand Huntaway dog
type IIIB	Schipperke
type VI	Siamese cat (two mutations), Miniature pinscher
type VII	German shepherd, mixed breed, Miniature Schnauzer, DSH cat
Alpha mannosidosis	Persian, DSH cat
Gangliosidosis GM1	Siamese, Korat cat
GM2	Korat cat
Globoid cell leukodystrophy (Krabbe)	West Highland white and Cairn terrier
Glycogenosis type IV	Norwegian Forest cat
Alpha fucosidosis	English springer spaniel
Neronal ceroid lipofuscinosis	English setter
Myotonia congenita	Miniature schnauzer
Narcolepsy	Doberman, Labrador retriever
Ivermectin toxicity (MDR-1 gene)	Collie, Sheltie, Australian kettle dog
Hepatic diseases	
Hyperchylomicronemia	DSH cat
Glycogenosis type Ia	Maltese
Copper toxicosis	Bedlington terrier
Renal diseases	
Cystinuria type I	Newfoundland, Labrador retriever
Renal adenocarcinoma and	German Shepherd

nodular dermatitis	
x-linked nephropathy	Samoyed

Giger Urs (2000), "Clinical Genetics," in *Textbook of Veterinary Internal Medicine*, S.J. Ettinger and E.C. Feldman, ed. Philadelphia, PA, Saunders.

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