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Conference Director:

Dr. Jerold S. Bell

Director of the Clinical Veterinary Genetics course at
Cummings School of Veterinary Medicine at Tufts University.

How to Recognize and Screen for Hereditary Diseases

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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/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, http://www.angis.org.au/databases/BRIX/omia. 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/penngen. 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 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 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 hematopoetic 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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Congenital Defects of Kittens

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Introduction

Congenital defects are abnormalities of structure, function or metabolism that are present at birth. A defect may cause physical impairment or it may cause the death of the kitten, before or after birth. Congenital defects in stillborn kittens often go unrecognized because few stillborns are submitted for complete necropsy. Many congenital defects are cosmetic or minor, while others may cause serious impairment of health. Not all congenital defects are heritable.

Congenital defects may be:

- Obvious at birth, e.g. cleft palate
- Found only with diagnostic testing or at necropsy, e.g. diaphragmatic hernia
- Subtle abnormalities found only with sophisticated testing, e.g. lysosomal storage diseases

Prevalence of Congenital Defects

It has been suggested that cats have fewer congenital defects than many other domestic animal species, such as the dog, cow or horse. Approximately 3-5% of all human infants are born with a congenital defect and many childhood deaths in North America (30% or more) are due to congenital malformations. Congenital defects are also a significant contributor to neonatal mortality in the cat.

There are a few studies of congenital defects in pedigreed cats in the literature. *Cave et al* noted that congenital disease was more common in pedigreed kittens than in the non-pedigreed kittens in their necropsy study of 274 kittens aged up to 16 weeks. But the difference was not statistically significant and no individual breed of cat was significantly predisposed to congenital diseases in their data.

In a survey of 3,468 pedigreed kittens by *Scott et al (1978)*, 6.8% had malformations. Individual breeds ranged from no defects reported to 17% (Colorpoint Shorthair) and 19% (Manx) of kittens affected. *Scott et al (1979)* reported 4.2% of Burmese kittens from one cattery had congenital defects, as well as 12.7% of Persian kittens from four catteries. The types of abnormalities reported included heart defects, open fontanelles, gastroschisis, eye and eyelid defects and gastrointestinal tract defects.

The most recent survey of congenital defects in pedigreed cats is found in *Sparkes et al*. In that analysis of 14 breeds in the UK, 14.9% of the litters included one or more kittens with congenital defects, ranging from 6% of the Devon Rex litters to 31% of the Tonkinese litters.

Some statistics have been published for congenital defects in non-pedigreed cats in research colonies. *Young* noted 2.8% of 633 kittens had congenital defects in a study of a specific pathogen free (SPF) colony. These congenital defects included intestinal and urinary tract abnormalities, chest and hind leg deformities, umbilical hernia and other defects. *Lawler and Monti* found 10.7% of 477 kittens in a minimal disease colony had congenital defects. The most common defect was cleft palate and several kittens were noted to have multiple defects. *Addie et al* reported that less than 1% of 280 kittens from an SPF colony had congenital defects.

Fetal Development

Feline fetal development can be divided into three stages:

- Pre-implantation (days 0-12)
- Embryogenesis (days 12-24)
- Fetal growth (day 24 to term)

The "critical period" is the stage during which each developing organ or structure is most sensitive to disruption. For most organs and structures, the critical period occurs during embryogenesis, in the third and fourth weeks of gestation. At the end of embryogenesis, the fetus is about 1/2 inch long. Developmental errors that occur during the first two weeks of gestation are usually lethal. It is also important to note that a defect in the development of one organ system or structure can result in the abnormal development of other organs or structures.

A teratogen is anything that disrupts normal fetal development, e.g. a drug or chemical. The timing of exposure of the fetus and the dose are important factors in determining outcome. Embryos are susceptible to teratogens, but this susceptibility tends to decrease as the critical developmental period for each organ system passes. This makes the fetus increasingly resistant to the effects of teratogens with age, with the exception of structures that differentiate late in gestation, such as the cerebellum, palate and urogenital system.

Causes of Congenital Defects

Congenital defects may be heritable, and the inheritance pattern or gene(s) responsible may or may not be known. A few congenital defects are due to chromosomal abnormalities, such as pseudohermaphroditism.

Many congenital defects are not heritable, but caused by other factors, such as:

1. Infections *in utero*
    - Usually viral, e.g. panleukopenia virus as a cause of cerebellar hypoplasia
2. Drugs
    - e.g. griseofulvin as a cause of cleft palate
    - Little is known about the effects of most drugs during pregnancy in the cat
    - It is best to avoid drug therapy in pregnant queens unless benefits outweigh potential risks
3. Chemicals, environmental toxins
4. Hyperthermia
    - e.g. fever, high ambient temperatures, resting on heating pads, radiators or hot air vents, etc.
5. Poor intrauterine environment
    - Inadequate development of the placenta
    - Cystic endometrial hyperplasia/pyometra complex
6. Nutritional factors
    - e.g. taurine deficiency as a cause of musculoskeletal defects

In some cases, defects may be caused by interplay of both environmental and genetic factors.

What Breeders Can Do

When a kitten with a congenital defect is born, breeders naturally want to know what caused the defect and whether the defect is heritable. Investigation of congenital defects requires asking and answering important questions:

- Is there a breed or familial predisposition (suggesting a hereditary cause)?
- Are there multiple defects in one kitten or multiple defects in the litter (suggesting a non-hereditary cause)?
- What were the results of any previous matings of the parents with each other?
- What were the results of any previous matings of the parents with other partners?
- Are there any potential contributing factors in the management or diet of the queen?
- Was the queen ill during pregnancy?
- Was the queen given any drugs or vaccinations during pregnancy?

It is important for breeders to monitor the health of their breed by:

- Collecting health data, including kitten morbidity/mortality data, on a regular basis
- Keeping excellent cattery records, including information on every litter and every kitten
- Communicating openly with each other and working together in breed clubs/groups
- Acting co-operatively to work on new defects as they emerge to:
    - Determine the prevalence of a new defect
    - Investigate the clinical aspects of the defect
    - Collate pedigrees
    - Work with clinicians, specialists, and geneticists to characterize the defect and its possible inheritance and develop a screening test

An important part of investigating congenital defects is performing necropsies on affected cats or kittens that die or are euthanized. Too often, many affected kittens have died and were not submitted for necropsy by the time a problem becomes apparent. For informative necropsies, the entire body should be submitted to a qualified pathologist within 24 to 48 hours. The body should be refrigerated and not frozen if possible. If there is a long delay expected before the body can be examined, the veterinarian can take samples of all major organs (or collect entire organs) for fixation in formalin and freeze the rest of the body. It is also important to supply the pathologist with the complete medical history of any affected kittens.

Molecular genetics is the marriage of classic genetics and molecular biology techniques. New laboratory techniques allow researchers to search for markers for genetic traits and diseases, and even to identify defective genes themselves. While relatively few specific genes responsible for diseases and defects in cats have been identified to date, feline geneticists have made rapid progress in recent years. For example, genes responsible for polycystic kidney disease, hypertrophic cardiomyopathy, and spinal muscular atrophy have been identified, allowing for the development of commercially available genetic tests.

Breeders and veterinarians can assist in the search for genes causing congenital defects by identifying individuals with abnormalities and having the foresight to bank samples that can be analyzed later on. Early in the course of an investigation, hundreds or thousands of tests must be run, and large DNA samples are necessary. Examples of samples that can readily be used as abundant sources of DNA include frozen reproductive organs from spay and neuter surgeries and frozen whole blood samples (but not serum or plasma). Formalin-fixed tissues are very poor sources of DNA. Buccal (cheek) swabs are also good DNA sources, but provide smaller amounts of DNA and so are best used once a genetic test is available.

Kitten Health Projects

The Internet provides an opportunity to collect data from a large group of breeders anywhere in the world in a simple and straightforward manner using web-based submission forms. Several breed-specific, prospective studies are currently underway or in data analysis. It is very important to gather enough data to know what is normal within individual breeds so that breeders and veterinarians alike can recognize what is abnormal. Such studies also allow the scientific collection of information important to breeders, such as common congenital defects or diseases within each breed, in a confidential manner. Breeders have shown a great willingness to collect data and use it to improve breeds and breeding practices. Such great stores of information and knowledge as breeders possess should not be overlooked in our efforts to better the health of cats.

A summary of data collected on congenital defects in several breeds is presented in Table 1. Some congenital defects are found in many breeds, such as thoracic wall defects (flat chest, pectus excavatum), gastroschisis, umbilical hernia and cleft palate. Other defects seem to be associated with certain breeds, such as craniofacial defect (Burmese), ocular dermoids (Birmans), and eyelid coloboma (Ragdolls). Most of these defects do not have a known inheritance pattern, and no screening tests are available to detect carriers should they be due to recessive genes. Most of the defects have not yet received attention from researchers or geneticists.

For more information on these breed-specific health studies, see:
http://catvet.homestead.com/BreedProjects.html

Table 1: Congenital Defects in Some Pedigreed Breeds

Breed	# Litters	% Litters with at least one congenital defect	Examples of congenital defects
Bengal	181	18	FCK, PE, CP, UH, SYN
Birman	217	12	DER, UH, SYN, CP, GAS
Burmese - Traditional	94	15	FCK
Burmese - Contemporary	54	80	HD, DER, FCK
European Burmese	66	15	FCK
Devon Rex	204	13	FCK, CP, UH
Egyptian Mau	52	23	UH
Havana Brown	27	11	UH, FCK
Manx	31	13	GAS
Munchkin	54	11	CP, UH, GAS
Norwegian Forest Cat	124	5	FCK, CP
Ocicat	128	25	FCK, PE, XIPH
Ragdoll	199	13	CP, COL
Sphynx	104	9	CP, PE, UH

COL = eyelid coloboma
CP = cleft palate
DER = dermoids (nasal and ocular)
FCK = flat chest defect
GAS = gastroschisis
HD = craniofacial (head) defect
PE = pectus excavatum
SYN = syndactyly
UH = umbilical hernia
XIPH = everted xiphoid process

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Molecular Nutritional Approach to Managing Osteoarthritis

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Overview:

Osteoarthritis (OA), or degenerative joint disease, is the most prevalent joint disorder in dogs, affecting up to 20% of the adult dog population.[1] Mild OA may result in subtle gait changes or intermittent lameness. As the disease progresses, the dog may become less active, show visible lameness, have difficulty rising or laying down, express pain, or have difficulty posturing to urinate or defecate.

OA is associated with inflammation and increased degradation or loss of proteoglycans from the extracellular matrix, resulting in a morphologic breakdown in articular cartilage.[2]

There is no known cure for OA, so treatment is focused on controlling pain, improving joint function and slowing the degenerative process within the joint.[3] Standard medical care usually involves weight management, controlled exercise, and anti-inflammatory and analgesic medications. In addition to medical therapy, dietary management can play an important role in the clinical management of dogs with OA.

Molecular view of OA:

Selection and implementation of appropriate therapies for a patient with OA are dependent not only on an understanding of the clinical and gross pathologic changes associated with OA, but also an understanding of the cellular and metabolic pathways involved. In recent years, advances in canine genetics and genomics have lead to powerful tools by which nutrition researchers can examine cellular response to OA. Using these tools, a comprehensive view of the cells response to OA can be determined. Several key experiments now reveal a better understanding of the biology of OA, and help in understanding the best nutritional management of the arthritic dog.

While osteoarthritis (OA) is perceived as a structural disease, the underlying pathology and chronic changes occur at a cellular and molecular level. The imbalance between anabolic and catabolic factors leading to degradation of articular cartilage in OA involves many factors at the molecular level. Gene expression analysis reveals changes in the expression of various structural proteins of the extracellular matrix, inflammatory cytokines, catabolic and anabolic enzymes and cell signaling molecules.[4]

Coupled with numerous OA gene-expression studies, it is clear that inflammatory pathways play a critical role in the chondrocytes response to injury and subsequent progression toward repair or toward arthritis. When compared to normal cartilage, OA-affected cartilage behaves somewhat like an activated macrophage, with up-regulation in expression of interleukin (IL)-1, IL-6 and IL-8 genes. Further examination of either mRNA or specific protein levels (via ELISA) demonstrated arthritis-associated elevations in prostaglandin (PG)E2, tumor necrosis factor (TNF)-α, nitric oxide, and matrix metalloproteinase (MMP)-1, -2, -3, -9, -10 and -13.[5-7] These data suggest a direct connection between the elevation of these inflammatory markers and the structural changes seen in the arthritic joints.

A primary target of medical treatment in OA is inhibition of the cyclooxygenase (COX) enzymes, especially the COX-2 enzyme, via use of non-steroidal anti-inflammatory drugs (NSAIDs).[8-10] The use of COX-2 selective inhibitors can decrease prostaglandin E_2 (PGE2) concentrations and block inflammatory pathways involved in OA, as well as reduce pain and lameness.[8, 9, 11-14]

Nutritional opportunities in OA:

Another means of reducing PGE2 and production of inflammatory mediators is through the use of dietary long chain omega-3 (n-3) polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA; 20:5n-3). The primary omega-6 fatty acid in cell membranes is arachidonic acid (AA; 20:4n-6), which serves as the precursor for the production of PGE2, thromboxane A2 and LTB4, potent inflammatory mediators in OA. If the diet is enriched with long chain n-3 PUFA, specifically EPA and docosahexaenoic acid (DHA;22:6n-3), part of the AA in cell membranes will be replaced by these n-3 fatty acids.[15-17] EPA then may be used instead of AA for the production of eicosanoids, resulting in a different and less inflammatory set of compounds (e. g. PGE_3, TXA_3 and LTB_5 instead of PGE_2, TXA_2 and LTB_4).[15, 16]

Dietary n-3 PUFA also suppress the pro-inflammatory mediators IL-1, IL-2 and TNF in cartilage tissue.[18, 19] Thus, the substitution of omega-3 for part of the omega-6 fatty acids should result in a reduction in inflammation that would be beneficial in inflammatory conditions, including OA.

A review of studies in arthritic humans indicated that most showed positive results from long-chain n-3 PUFA supplementation.[20] Recent research in dogs supports many of these prior studies in

humans confirming clinical benefits of dietary n-3 fatty acids in OA. Twenty-two dogs with OA of the hip were given a fatty acid supplement marketed for dogs with inflammatory skin conditions (DVM Derm Caps, DVM Pharmaceuticals, Miami, FL).[21] When dosed according to the manufacturer's recommended dosage, 13 of 22 dogs had noticeable improvement in their arthritic symptoms within two weeks.[21] Another, uncontrolled study evaluated dogs with naturally occurring OA of the elbow, using force-plate analysis, before and after being fed a diet enriched with n-3 PUFA. Improvements in vertical peak force were observed within 7 to 10 days on the diet. (Budsberg SC 2004, unpublished)

In yet another study, dogs fed a diet enriched with n-3 PUFA following corrective surgery for ruptured cruciate ligaments showed a significant decrease in synovial fluid PGE2.[22] Synovial fluid MMP-2 and MMP–9, enzymes which degrade structural proteins in cartilage, also were decreased in these dogs compared to dogs fed the control diet.

Summary:

Osteoarthritis is a disease characterized by an imbalance in catabolic and anabolic factors affecting the degradation and synthesis of the extracellular matrix. New techniques are allowing researchers to characterize this disease and evaluate potential therapeutic and nutritional agents at the cellular and molecular level. Proinflammatory mediators and inflammatory cytokines play a central role in the gene-expression changes, and resulting biochemical changes seen in the arthritic articular chondrocyte. Oral administration of EPA has been shown effective in the nutritional management of osteoarthritis in several species.

Additional Detail

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Sequencing the Dog & Cat Genome and its Implications

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Two main objectives exist for sequencing the genomes of dogs and cats:

1. Even through the human genome has been sequenced, we still have a hard time identifying all the functional elements, such as genes and regulatory elements (sequences turning on and off genes) in the human genome. Fortunately, genes and regulatory elements are highly similar between mammals, whereas non-functional sequence has changed between species. Thus, by sequencing and comparing the genomes of many mammals we can find the functional elements. An initiative is now ongoing to sequence as many as 20 mammals in the next year or two.

2. Identification of disease mutations becomes much less laborious if one already has access to the genome sequence and knowledge of the genes and other functional elements. Since disease gene mapping has been ongoing for many years in both dogs and cats, this resource will greatly facilitate canine and feline genetics. Particularly the dog has a breed structure that suggests that identifying genes for common diseases such as cancer and diabetes might be much easier than in humans. The genome sequence is accompanied by a map of the variation (markers) within and between breeds, which can be used to identify disease genes. Understanding of the patterns of variation in breeds will facilitate effective use of these markers and will lead to disease gene identification important for both canine and human health.

The dog genome:

- The genome sequence of a female boxer has been generated. Each position in the genome was sampled ~7.5 times, which means that the genome is relatively complete (~99%).
- The genome has been compared to other mammalian genomes such as human, mouse and rat.
- The genes have been identified and the majority of these have a corresponding gene in other mammalian genomes. Thus, a disease gene identified in one species can be studied also in other mammals, where it is likely to cause similar disease.
- A number of possible regulatory elements have been identified by comparison of the mammals. Mutations in these elements are more likely to cause disease than those found elsewhere in the genome.
- A SNP map of several million single base variants (markers) has been generated. Small amounts of sequence from 10 breeds, 4 wolves and one coyote was compared to the boxer sequence to generate this map.
- The structure of variation within and across breeds suggests that mapping disease genes in breeds that have a high incidence of a particular disease will be feasible.
- The Broad Institute is currently collecting samples from dogs affected with osteosarcoma, hemangiosarcoma, mealanoma, mammary carcinoma, lymphoma and mast cell tumors at this point. We also need older unaffected dogs to use as controls (see www.broad.mit.edu/mammals/dog/ for more details). Now that the genomic tools are in place sample collection is the key step towards identifying canine disease genes.

The cat genome:

- The genome sequence of a female cat will be ready in early fall of 2005. For this project each position has been sampled only ~2 times. Thus we expect the sequence to cover only ~80% of the cat genome.
- The genome will be compared to the other mammals to identify genes and other functional elements.
- A small effort to study the variation within the cat population is being discussed.
- The sequence will permit easier identification of disease genes.

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Strategies to Control Canine Hip Dysplasia: The Need for Veterinarian and Breeder Alliance

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ABSTRACT:

Genetic control of CHD requires: a) a knowledge of the principles of quantitative genetics, b) an accurate screening method keyed to a phenotype with optimal heritability, c) an organized screening program based on a proven screening phenotype, d) a centralized database containing essential phenotypic and pedigree information, and e) trust and cooperation between breeders and the veterinarians who perform the screening procedure.

THE RELATIONSHIP BETWEEN PHENOTYPE AND GENOTYPE:

Controlling diseases of complex inheritance (aka, polygenic diseases) like CHD requires a concerted and coordinated effort on the part of breeders and veterinarians. As importantly, the integrity of the test used to screen for CHD is central to reducing the frequency of hip disease (figure 1).

The principal objective of selective breeding is to maximize the pairing of good genes by breeding dogs not overtly affected with (and preferably, not susceptible to) CHD. The purpose of the screening test is to evaluate hip phenotype (that which you can see or measure) as an estimate of the genotype (that which you can't 'yet' see or measure). The relationship between phenotype and genotype is embodied in the concept of heritability represented by the symbol, h^2. Heritability denotes the reliability of the phenotype in predicting the genotype. A high heritability, say approaching 1, means that the phenotype closely reflects the genotype. Or put in other words, all the variation in the phenotype is explained by the genes. Environmental factors, such as diet or exercise, have no influence on the phenotype. In contrast, a heritability of 0 means that a disease is not influenced whatsoever by genes and accordingly variation in expression is purely environmental.

Heritability is mathematically defined as the ratio of additive genetic variation to the total phenotypic variation of a given trait ($h^2=V_G/V_P$). And the total phenotypic variation, V_P, in turn is defined as the sum of the genetic variation, V_G, plus the variation owing to environment factors, V_E, (sometimes termed nongenetic factors). Then,

$$h^2=V_G/ (V_G + V_E)$$

One can see from this simple relationship that when environmental factors do not influence the trait of interest (i.e., V_E is small) then, h^2 approaches 1 and the trait or disease of interest can be considered purely genetic. Similarly, as environmental factors cause increasingly more variation in the phenotype, h^2 approaches 0.

Examples of environmental factors that contribute to variation in the denominator of the heritability relationship above include diet, exercise, gender, age, and diagnostic error. These factors increase the total phenotypic variance in the denominator of this relationship, V_P, and therefore they have the effect of lowering estimates of heritability, the significance of which will be emphasized later. So, polygenic traits are influenced by both environmental and genetic effects. For example, a dog's weight is partly influenced by environmental factors in terms of how much it is fed and how much exercise it gets. However, it is known that body weight can also be influenced by genetic factors, i.e., obese parents tend to have obese offspring.

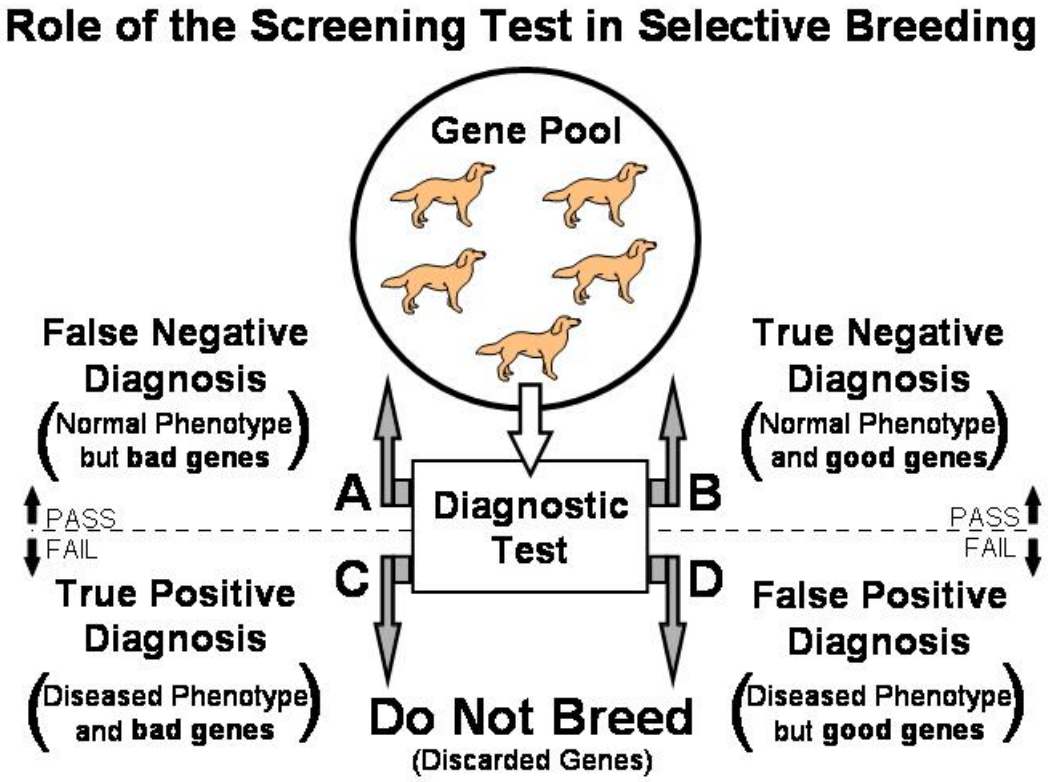

FIGURE 1: The Role of a Screening Test in Improving the Gene Pool. The objective of any screening test for a genetic disease is to lower the frequency of 'bad genes' in the gene pool. This entails using 'what can be seen', as a result of the diagnostic test (the phenotype) to estimate 'what can not be seen' (the genes). Dogs are permitted to enter the gene pool based on normal results of the test (arrow A or B). A perfect test (arrows B and C only) would be capable of accurately separating 'good genes' from 'bad genes' on the basis of the phenotype alone (i.e., the test result), thereby quickly and effectively ridding the gene pool of bad genes (arrow B → good genes enter the gene pool: arrow C → bad genes are not returned). Unfortunately, no screening test is 100% accurate. For example, a test result may wrongly exclude from breeding a dog that tests positive for a diseased phenotype even though it harbors good genes (arrow D). This would be an unfortunate missed opportunity, as some good genes would not re-enter the gene pool. However, this mistake would not appreciably harm the gene pool. Of much greater potential damage to the gene pool is a test result that indicates a dog has a normal phenotype (negative) but which, in fact, harbors many bad genes (arrow A). Such a mistake would recycle bad genes through the gene pool, resulting in a steady-state level of disease in the offspring derived from that gene pool, despite the best efforts at selection (e.g., breeding excellent to excellent). The frequency of disease coming from the gene pool will depend on the sensitivity of the test to detect bad genes. This sensitivity is directly related to the **heritability** of the phenotype used for screening, therefore the higher the heritability, the better the test, and the more rapid the genetic change.

IMPORTANCE OF HERITABILITY:

Worldwide, the predominant mode of choosing breeding stock is to make selections based on the individual animal's hip phenotype, so-called mass selection. It must be stressed, however, that this is not the most effective method to select breeding candidates. More rapid genetic change can be accomplished if the hip phenotypes of relatives are incorporated into the selection decisions. By incorporating data from relatives, one can calculate so-called 'breeding values' for each individual dog. Although this method facilitates more accurate selection decisions, it is not widely employed because of the need for extensive record keeping coordinated with the availability of accurate pedigree information. The breeder should recognize, however, that there are better tools to help in making selection decisions than just resorting to the individual dog's phenotype, eg, hip score.

The common practice of selecting breeders by using only the individual animal's phenotype makes knowledge of the magnitude of heritability of utmost importance. Why is heritability so important in this regard? Because, for a quantitative trait, the rate of expected genetic change in the next generation, (□G), from mating a dog and a bitch is equal to the product of the heritability, (h^2), times the selection pressure that is applied (Relationship 1 below). Selection pressure is defined as the deviation of the parental mean, eg. hip laxity, from the population mean. [1]

□G = h^2 x ($Avg_{Parents}$ - $Avg_{Pop.}$) **(1)**

Where:

□G = the expected change in average litter phenotype after one generation

h^2 = heritability of phenotype, eg. DI or subjective hip score

$Avg_{Parents}$ = average hip phenotype of the parents

$Avg_{Pop.}$ = average hip phenotype of the population from which parents were derived.

Therefore, the higher the heritability of a specific trait and the greater the selection pressure applied, the more rapid the expected genetic change per generation of breeding. Estimates of heritability above 0.4 make feasible selection based on the individual phenotype. These concepts applied to PennHIP data are illustrated in the actual mating of 2 tight-hipped German Shepherd dogs (Figure 2). In this example, extreme selection pressure has been applied because the sire and dam are drawn from the tightest 5th percentile of the breed. One can see that the mean hip laxity of the litter derived from these 2 parents is 0.27. From formula 1, it is possible to calculate the 'realized heritability' of any metric, for example, DI**.** The GSD population average DI is 0.39 and the parental average DI is 0.2, therefore the selection pressure applied was 0.19 DI units. Again, the average DI for the 9 puppies was 0.27. Therefore the realized heritability from this single mating can be found by rearranging terms in Relationship 1:

h^2 = □G/($Avg_{Parents}$ - Avg_{Pop})

Plugging in data from the mating in Figure 2

h^2 = (0.39-0.27)/(0.39-0.20) = 0.63

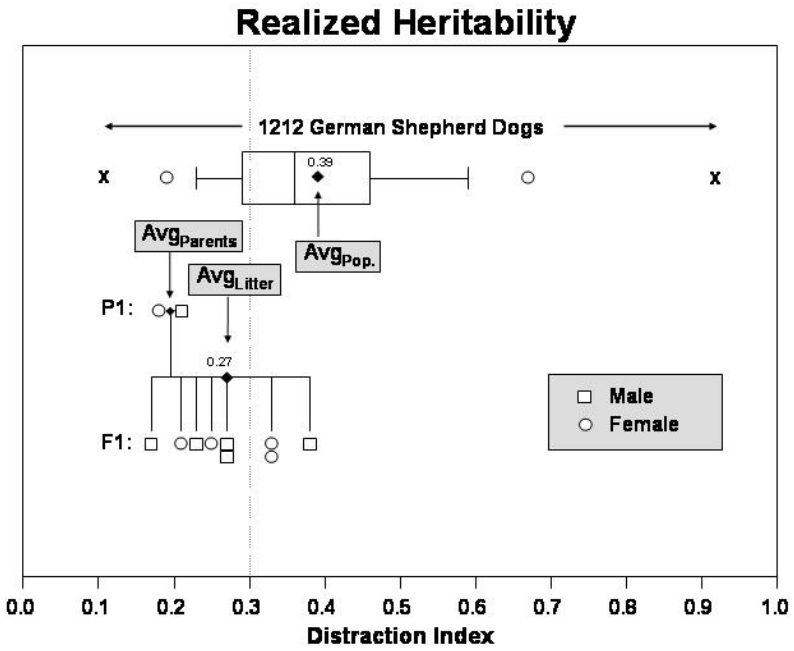

FIGURE 2: Calculation of Realized Heritability from a Single Mating. See text for full description. This illustration shows the relative relationships of passive hip laxity of, 1) the German Shepherd dog breed at large, 2) the dog and bitch, P1, and, 3) the litter, F1. Note that the mean litter DI moved approximately 60% of the distance from the mean of the GSD population toward the mean of the parents. Plugging these averages in hip laxity into Relationship 1 yields a realized heritability of approximately 0.6. It is notable that all 9 puppies showed hip laxity below the average for the breed and that hip laxity in 6 of the 9 puppies fell below a DI of 0.3, indicating little to no susceptibility to DJD.

Currently there are no published estimates of heritability for subjective (OFA) hip scores for the most popular breeds of dogs. A retrospective analysis from the OFA showed heritability in 4 less common dog breeds to average 0.26.[4] Phenotypes with heritability of this magnitude would be considered to be lowly heritable[1] meaning that genetic change will be slow (only 25% of of the applied selection pressure will be passed on in each generation of breeding- see Relationship 1). These figures are corroborated by 2 well-executed studies of subjective hip score (OFA-type scoring), which yielded similar estimates of heritability of 0.22 [2] and 0.43 [3] for German Shepherd Dogs.

The magnitude of selection pressure applied is the other important factor in relationship 1 above. With successful application of selection pressure, offspring within generation will begin to look similar, eg, more dogs will be normal, meaning that the phenotypic and genotypic variance will get smaller. However, with decreasing variation in the hip phenotype (for example, in subjective score), there may come a point, a steady state, at which little additional incremental selection pressure can be applied by using the subjective score as a selection criterion. That is, if the application of maximum selection pressure (e.g., breeding 'excellent' to 'excellent', see arrow A in Figure 1) still produces affected progeny, no more genetic progress can be expected (short of incorporating estimated breeding values in making selection decisions, mentioned above). Such has been the experience of The Seeing Eye, Inc. after 17 years of selection against hip dysplasia using a subjective scoring scheme similar to, but more strict than, that of the OFA.[5]

Heritability of a given phenotypic trait is a property of the population under study. Therefore heritability of each trait or diagnostic phenotype must be calculated for each breed and each population of dogs. An example of a calculation of realized heritability was illustrated in Figure 2, however, estimates of heritability can also be calculated by other methods. For example, the upper limit for heritability of DI can be estimated as the intraclass correlation coefficient for longitudinal repeatability of hip score, e.g., DI measurements, over time.[1] In one study of German Shepherd Dogs, the intraclass correlation coefficient of repeatability of DI was between 0.67 and 0.74, indicating a high upper limit of heritability for DI and in line with the realized heritability calculated above.[6] In contrast, in the same study the longitudinal repeatability of subjective score over the same interval from 4 months of age to 24 months of age was 0.08 and not statistically significant.

Heritability can also be estimated by analyzing resemblance between parents and offspring in terms of hip laxity (DI). To accomplish this, a regression analysis of litter mean DI phenotype, can be plotted against parent mean DI, to yield a line whose slope is an estimate of heritability. This method was employed in the publication of heritability estimates from the OFA, mentioned above.[4] Using a similar method, estimates of heritability of DI (unpublished) for a group of German Shepherd Dogs was between 0.42 and 0.65, and the upper limit for heritability of DI among a group of Labrador Retrievers was 0.92. For Golden Retrievers the estimate for heritability of hip laxity from an analysis of 265 dogs comprising 47 litters was 0.64 .[7] For comparison, the estimate of heritability for subjective hip score (slope of regression line) in the study of Golden Retrievers was 0.22 and not statistically significant.

The most valid estimates of heritability of DI or subjective hip score are derived by incorporating knowledge of relevant phenotypes in the context of the full pedigree. The Seeing Eye, Inc. has maintained a closed colony of dogs intended for use as dog guides for the blind. Leighton, et al invoked rigorous mathematical methods that incorporated the full pedigree structure, and found the heritability of DI to be 0.46 for German Shepherd Dogs and 0.46 for Labrador Retrievers.[8] The corresponding heritability estimates for subjective hip score (determined by a board-certified veterinary radiologist) were lower at 0.34 for German Shepherd Dogs and 0.34 for Labrador Retrievers. This low heritability of subjective hip score in German Shepherd dogs is supported by a recent study from Finland by Leppanen et al.[9] Applying BLUP (best linear unbiased prediction) procedures to analyze 10,335 GSDs from 1985 to 1997 these investigators found that using subjective hip score as a selection criterion over this 12-year time interval failed to produce genetic improvement.

Heritability analyses using these newer, more sophisticated statistical methods are needed for all hip screening methods applied to all breeds of dogs. Results thus far are promising that the heritability of DI will be considerably higher than the heritability of subjective hip scoring. These findings have great clinical significance owing to the abundant evidence linking hip laxity as measured by DI with osteoarthritis of the hip. (Figure 3).[10] Currently there are no published estimates of the heritability of other diagnostic hip phenotypes including the DLS score[11], the DAR score[12], and the scores of Fluckiger[13], Barlow test, Bardens test, or Ortolani test. Such studies are necessary to determine the relative merit of these diagnostic tests as candidate hip screening methods for selecting breeding stock.

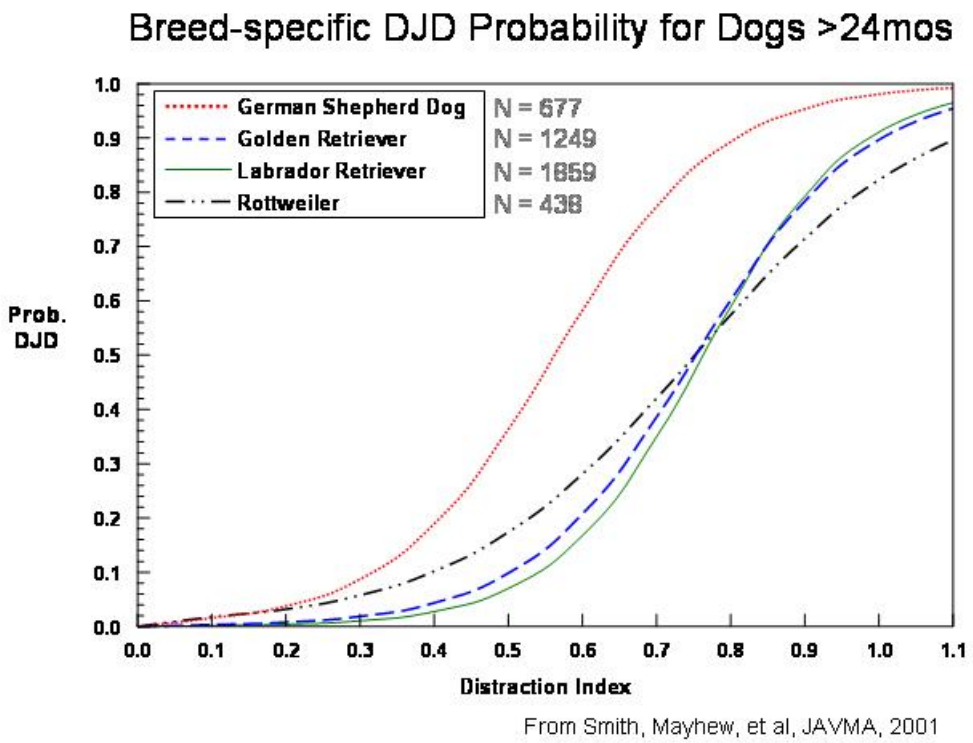

FIGURE 3: Breed Specific DJD Probability based on DI for Dogs ≥ 24 Months of Age. Probability of radiographic evidence of degenerative joint disease (DJD) as a function of distraction index (DI) for dogs ≥ 24 months old of 4 common breeds. Note the spatial shift to the left for the German Shepherd Dog breed indicating an increased probability of DJD for any given DI compared to the three other breeds.

SELECTION PRESSURE TO PRODUCE RAPID GENETIC CHANGE IN CHD:

Breeders cannot influence the magnitude of the heritability of the phenotype, but they can control the magnitude of applied selection pressure (i.e., the difference between the mean of the parents and the mean of the population at large, see Relationship1). Therefore, to the extent that breeders select breeding candidates, they can control the rate of improvement in hip phenotype in each generation. For the most rapid genetic change, the breeder can decide to mate only the tightest-hipped dogs within the breed (those with the lowest DI) and then continue to inbreed for tight hips. This approach would maximize the difference between the parent average and population average (i.e., the selection pressure, the second term on the right side of Relationship 1, would be large). There would therefore be a greater expected change in each generation assuming constant heritability. This approach, however, creates concern that founding a breeding program on only a few dogs, and inbreeding on these dogs, would reduce the overall genetic diversity in the gene pool and could contribute to the loss of some desirable traits or the expression of some undesirable traits. This reality affects some breeds more than others. For example less than 5% of golden retrievers have hip laxity in the 'tight-hipped' range below a DI of 0.3. If one were to require that breeding candidates conform to this standard and must come from this small pool of dogs, the result would be a serious reduction in genetic diversity, not to mention that the strategy would neither be practical nor unacceptable to breeders.

To avoid these potential problems accompanying this 'extreme' selection, a 'moderate' approach has been suggested to go hand in hand with PennHIP testing, particularly in breeds with few or no

members having tight (DJD-unsusceptible) hips. In such breeds it is recommended that breeders choose breeding stock from the tightest half of the breed, thereby maintaining an acceptable level of genetic diversity while still

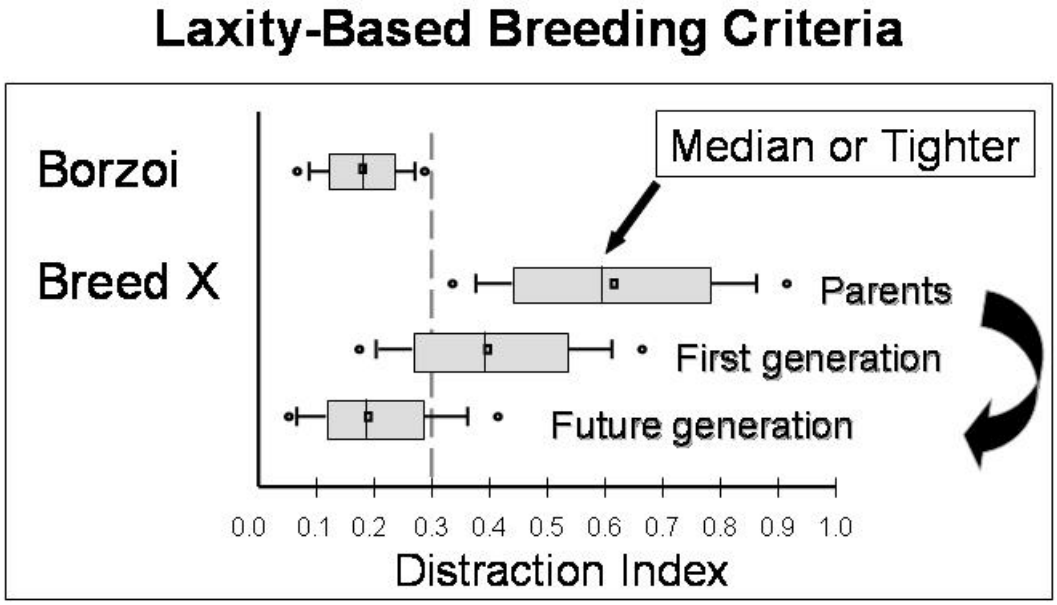

FIGURE 4: Proposed Minimum Laxity-based Breeding Criteria. By using the generational median (or mean) as the minimal criterion for breeding, one can expect genetic change to occur. Breed X displays a range and distribution of hip laxity not unlike the golden retriever breed, for example. Genetic change toward tighter hips can be expected in each subsequent generation by breeding dogs in the tighter half of the distribution (and preferably much tighter). The goal of this strategy is to tighten the hips of Breed X until matching the range and distribution of hip laxity of the Borzoi. Obviously, based on Relationship 1, the tighter the parents, the greater the selection pressure, and the more rapid the expected genetic change toward hip improvement. This logic follows well-established principles of quantitative genetics.

applying meaningful selection pressure (Figure 4). Clearly the more selection pressure applied, the more rapid the genetic change. The PennHIP database ranks each dog relative to other members of the breed making it possible for the breeder to identify dogs whose DI will apply meaningful selection pressure (Figure 5). By applying at least moderate selection pressure, eventually the average of the population will shift with each generation toward tighter hips, increasingly tightening the minimum standard for breeding. By following these time-tested principles, eventually, fewer dogs will be at risk for developing DJD. Understandably, more rapid genetic change could be achieved by imposing greater selection pressure or by using estimates of breeding value from incorporation of the pedigree. These strategies are recommended for the aggressive breeder wishing to achieve the most rapid hip improvement. Even absent these measures, however, the principle of mass selection if linked to a highly heritable phentoype, such as the PennHIP DI, holds great promise for reducing the frequency and severity of DJD in future generations of dogs.

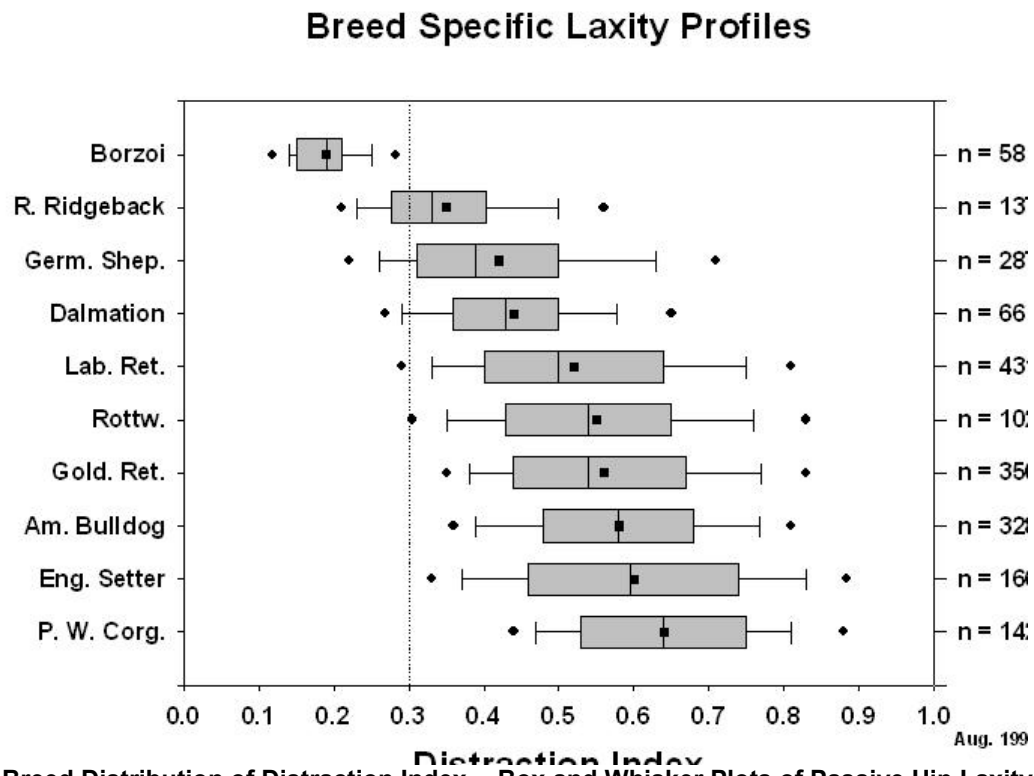

FIGURE 5: Breed Distribution of Distraction Index -- Box and Whisker Plots of Passive Hip Laxity by Breed. Data is drawn from the PennHIP database (Aug. 1998) and shows breed-specific passive hip laxity. Note that the Borzoi breed has no members with hip laxity greater than a DI of 0.3. Note also that the golden retriever breed has few if any members with hip laxity less than a DI of 0.3. The obvious objective of selective breeding is to move the laxity profiles of the looser CHD-prone breeds, like golden retrievers, into the hip-laxity range approximating that of the Borzoi, a breed of dog that has an extremely low incidence of CHD.

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FIGURE LEGENDS:

FIGURE 1: The Role of a Diagnostic Test in Improving the Gene Pool. The objective of any diagnostic test for a genetic disease is to lower the frequency of 'bad genes' in the gene pool. This entails using 'what can be seen', as a result of the diagnostic test (the phenotype) to estimate 'what can not be seen' (the genes). Dogs are permitted to enter the gene pool based on normal results of the test (arrow A or B). A perfect test (arrows m B and C only) would be capable of accurately separating 'good genes' from 'bad genes' on the basis of the phenotype alone (i.e., the test result), thereby quickly and effectively ridding the gene pool of bad genes (arrow B → good genes enter the gene pool: arrow C → bad genes are not returned). Unfortunately, no diagnostic test is 100% accurate. For example, a test result may wrongly exclude from breeding a dog that tests positive for a diseased phenotype even though it harbors good genes (arrow D). This would be an unfortunate missed opportunity, as some good genes would not re-enter the gene pool, however, this mistake would not appreciably harm the gene pool. Of greatest potential damage to the gene pool is a test result that indicates a dog has a normal phenotype (negative) but which, in fact, harbors many bad genes (arrow A). Such a mistake would recycle bad genes through the gene pool, resulting in a steady-state level of disease in the offspring derived from that gene pool, despite the best efforts at selection (e.g., breeding excellent to excellent). The frequency of disease coming from the gene pool will depend on the sensitivity of the test to detect bad genes. This sensitivity is directly related to the heritability of the phenotype used for screening, therefore the higher the heritability, the better the test, and the more rapid the genetic change.

FIGURE 2: Calculation of Realized Heritability from a Single Mating. See text for full description. This illustration shows the relative relationships of passive hip laxity of, 1) the German Shepherd dog breed at large, 2) the dog and bitch, P1, and, 3) the litter, F1. Note that the mean litter DI moved approximately 60% of the distance from the mean of the GSD population toward the mean of the parents. Plugging these averages in hip laxity into Relationship 1 yields a realized heritability of approximately 0.6. It is notable that all 9 puppies showed hip laxity below the average for the breed and that hip laxity in 6 of the 9 puppies fell below a DI of 0.3, indicating little to no susceptibility to DJD.

FIGURE 3: Breed Specific DJD Probability based on DI for Dogs ≥ 24 Months of Age. Probability of radiographic evidence of degenerative joint disease (DJD) as a function of distraction index (DI) for dogs ≥ 24 months old of 4 common breeds. Note the spatial shift to the left for the German Shepherd Dog breed indicating an increased probability of DJD for any given DI compared to the three other breeds.

FIGURE 4: Proposed Minimum Laxity-based Breeding Criteria. By using the generational median (or mean) as the minimal criterion for breeding, one can expect genetic change to occur. Breed X displays a range and distribution of hip laxity not unlike the golden retriever breed, for example. Genetic change toward tighter hips can be expected in each subsequent generation by breeding dogs in the tighter half of the distribution (and preferably much tighter). The goal of this strategy is to tighten the hips of Breed X until matching the range and distribution of hip laxity of the Borzoi. Obviously, based on Relationship 1, the tighter the parents, the greater the selection pressure, and the more rapid the expected genetic change toward hip improvement. This logic follows well-established principles of quantitative genetics.[1]

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Canine Health Information Center: Practical Applications for Breeders

Eddie Dziuk
Orthopedic Foundation for Animals
Columbia, MO 65201

CHIC—The Canine Health Information Center

The Canine Health Information Center, also known as CHIC, is a centralized canine health database jointly sponsored by the AKC/Canine Health Foundation (AKC/CHF) and the Orthopedic Foundation for Animals (OFA). The program was originally conceptualized by the AKC Delegate Parent Club and Canine Health Committees. The AKC CHF and OFA took on the project, and implemented it in the Fall of 2001 with eight pilot breeds participating. Today, nearly one third of the AKC Parent Clubs have joined CHIC, and nearly 20,000 dogs have met the individual breed health testing requirements and been assigned CHIC numbers.

The CHIC Mission Statement is quite simply "To provide a source of health information for owners, breeders, and scientists, that will assist in breeding healthy dogs." The specific program goals are:

- To work with parent clubs in the identification of health issues for which a central information system should be established.
- To establish and maintain a central health information system in a manner that will support research into canine disease and provide health information to owners and breeders.
- To establish scientifically valid diagnostic criteria for the acceptance of information into the database.
- To base the availability of information on individually identified dogs at the consent of the owner.

The CHIC database is a tool that collects health information on individual animals from multiple sources. As more phenotypic and genetic screening tests become available and breeders make greater use of these tests, it is important that a database exists to capture this data. CHIC satisfies this need by functioning as a centralized pool of data. CHIC is about encouraging testing and health awareness and recording the results. It is not necessarily about 'normalcy' nor should it be misconstrued as an award program for normal dogs. Dogs with abnormal results are eligible for CHIC numbers as long as their results are in the public domain so that the breeders can benefit from the information in making more informed breeding decisions.

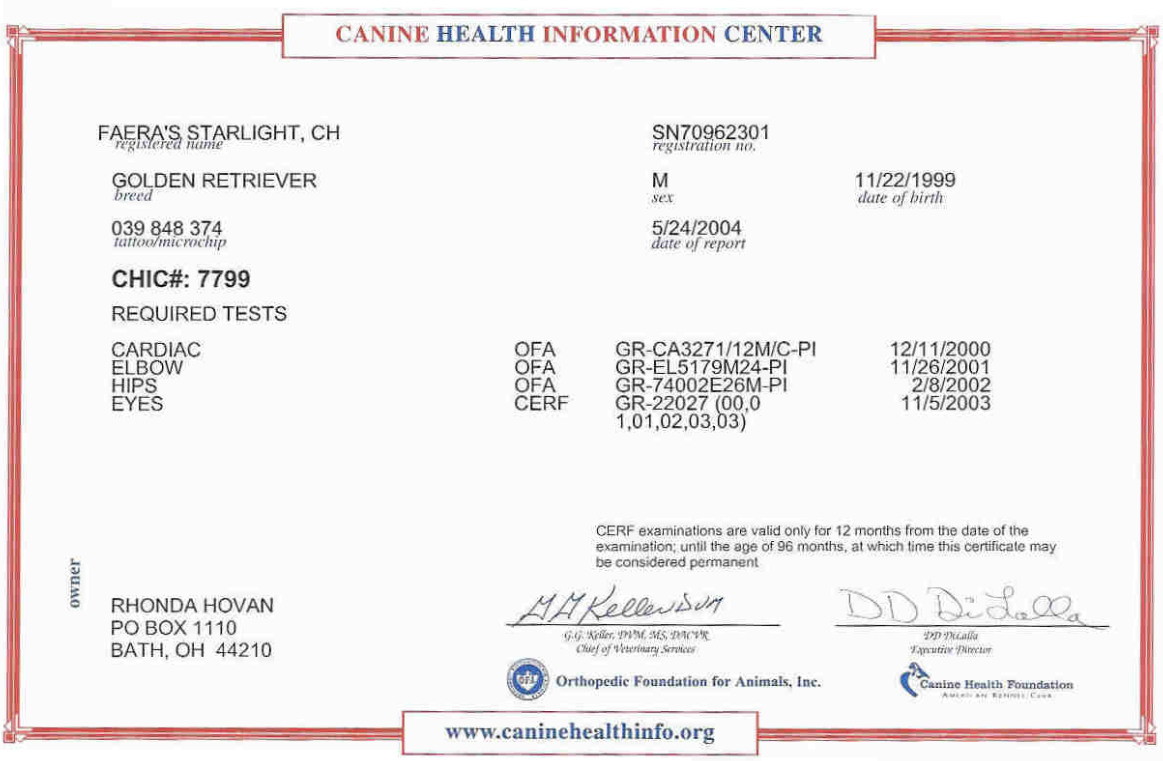
Core to the CHIC philosophy is the realization that each breed has different health concerns. Not all diseases have known modes of inheritance, nor do all diseases have screening tests. Some screening tests are based on phenotypic evaluation, others on genetic testing. With all these variables, a key element of CHIC is to customize or tailor the CHIC requirements to the needs of each breed. These unique requirements are established through input from the parent club prior to the breed's entry into the CHIC program. Breed specific requirements typically consist of the inherited diseases that are of the greatest concern and for which some screening test is available. Each parent club also drives specific screening protocols. As an example, one parent club may allow cardiac exams to be performed by a general practitioner. Another parent club may require the exam to be performed by a board certified cardiologist. A club may also use the CHIC program to maintain information on other health issues for anecdotal purposes. Later, as screening tests become available, the disease may be added to the breed specific requirements.

Regardless of breed, each dog must be permanently identified in order to have test results included in CHIC. Permanent identification may be in the form of microchip, or tattoo.

CHIC operates an informed consent database. All information regarding test results remains confidential unless the owner specifically authorizes release of the information into the public domain. Owners are encouraged to release all test results realizing it is in the ultimate health interests of the breed and the information greatly increases the depth and breadth of any resulting pedigree analysis. For those not quite ready to accept open sharing of information, there is still value in submitting their results. All test information entered into the database is available in aggregate for research and statistical reporting purposes, but does not disclose identification of individual dogs. This results in improved information on the prevalence of the disease, as well as information regarding progress in reducing the incidence of the disease.

A CHIC number is issued when test results are entered into the database satisfying each breed specific requirement, and when the owner of the dog has opted to release the results into the public domain. The CHIC number by itself does not imply normal test results, nor should it be interpreted as a 'stamp of approval" for breeding. The CHIC number only indicates that all the required breed specific tests were performed and the results made publicly available.

When a CHIC number is issued, a CHIC report is generated. The CHIC report is a consolidated listing of the tests performed, the age of the dog when the tests were performed, and the corresponding test results. As new results are recorded, updated CHIC reports reflecting the additional information are generated. For example, if a breed requires annual CERF examinations, an updated CHIC report will be generated every time updated CERF results are entered.



CANINE HEALTH INFORMATION CENTER

FAERA'S STARLIGHT, CH
registered name

SN70962301
registration no.

GOLDEN RETRIEVER
breed

M
sex

11/22/1999
date of birth

039 848 374
tattoo/microchip

5/24/2004
date of report

CHIC#: 7799

REQUIRED TESTS

CARDIAC	OFA	GR-CA3271/12M/C-PI	12/11/2000
ELBOW	OFA	GR-EL5179M24-PI	11/26/2001
HIPS	OFA	GR-74002E26M-PI	2/8/2002
EYES	CERF	GR-22027 (00,0 1,01,02,03,03)	11/5/2003

CERF examinations are valid only for 12 months from the date of the examination; until the age of 96 months, at which time this certificate may be considered permanent

owner

RHONDA HOVAN
PO BOX 1110
BATH, OH 44210

G.G. Keller, DVM, MS, DACVR
Chief of Veterinary Services

DD DiLella
Executive Director

Orthopedic Foundation for Animals, Inc.

Canine Health Foundation

www.caninehealthinfo.org

Figure 1, a sample CHIC report.

Once included in the CHIC program, the breed specific requirements are dynamic. As health priorities within a breed change, or as new screening tests become available, the breed specific requirements can be modified to reflect the current environment

Health testing by itself is only the first step in attempting to reduce the incidence of genetic disease in our companion animals. It is important to take the next step and record the results in genetic health registries so that the data is preserved and others may benefit from it. Finally, the website brings things full circle by making the information easily accessible to the public via the internet. The CHIC website is located at www.caninehealthinfo.org. The website contains basic information on CHIC such as its mission and goals, and maintains a listing of the participating breeds and approved breed specific test protocols. The CHIC website also provides a search engine to locate dogs that have been issued CHIC numbers, their test dates, and the results of their tests. The website has been designed to seamlessly integrate with the existing OFA website.

The OFA and CHIC search engines allow queries to be very broad or very specific. Search criteria include registration numbers, registered names (including full name, first part of name, any part of name), breed, sex, birthdate (or range), specific disease registry, specific diagnostic rating, and report date (or range). Any combination of these search criteria can be specified resulting in a variety of potential matches.

Figure 2, Screen Shot from the OFA website displaying the search criteria screen. In this example, the kennel name "Faera" was entered as the search criteria.

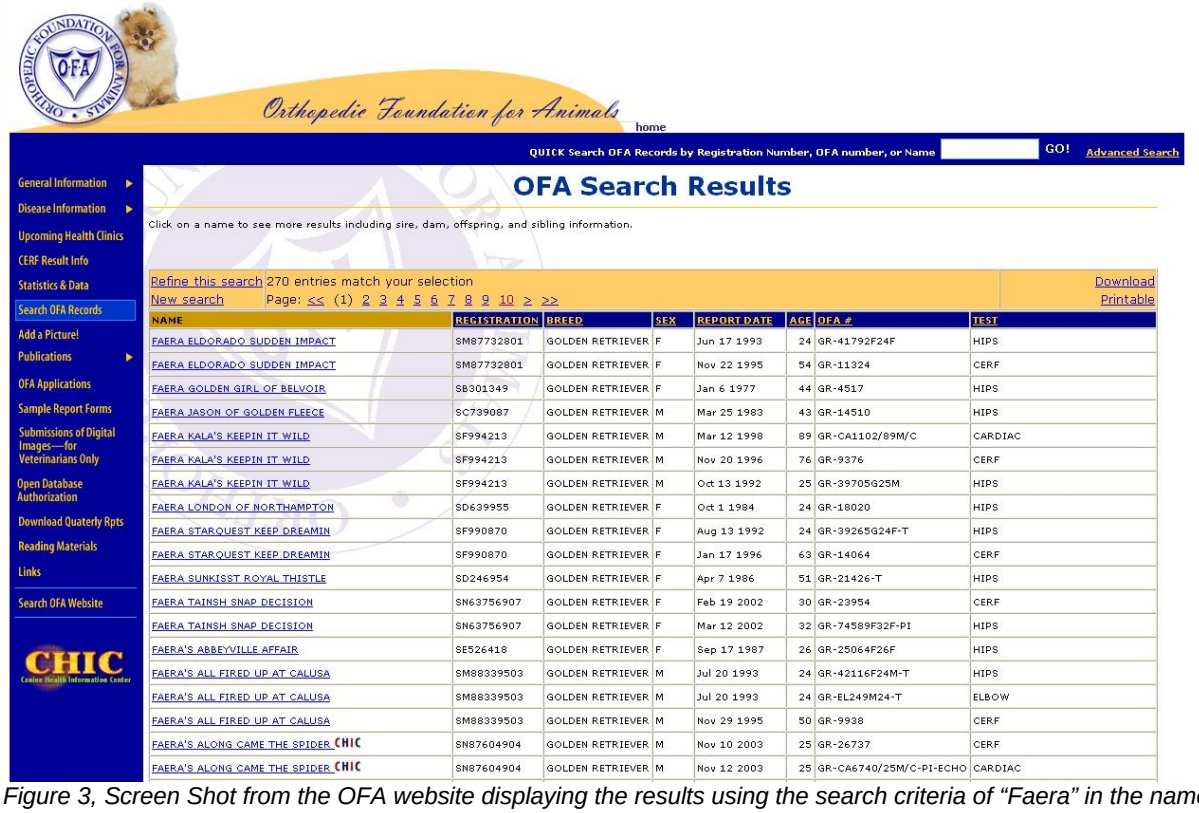


Figure 3, Screen Shot from the OFA website displaying the results using the search criteria of "Faera" in the name field.

Figure 4, Screen Shot from the CHIC website displaying similar search results using the criteria of "Faera" in the name field.

Once an individual dog is selected through the search options, detailed information is displayed, including health screening done, age at the time of testing, and test results. In addition, wherever possible, the database does a pedigree query, and displays the dog's sire and dam, siblings (both full and half), offspring, as well as any of *their* recorded health test results. All displayed names are hotlinked, so the user can easily browse from dog to dog.

FAERA'S ALONG CAME THE SPIDER CHIC

Registration: SN87604904 **Sire:** SN70962301
Breed: GOLDEN RETRIEVER **Dam:** SN24738202
Sex: M ***Titles:**
Color: GOLDEN **CHIC #:** 15706
Birthdate: Sep 15 2001 **Addtl. Reg. #**

No photo on file

OFA Number	Registry	Report Date	Age	Final Conclusion
GR-80825G25M-PI	HIPS	Oct 29 2003	25	GOOD
GR-EL8281M25-PI	ELBOW	Oct 29 2003	25	NORMAL
GR-26737	*CERF*	*Nov 10 2003 * 25*		*NORMAL. TESTED: 03*
GR-CA6740/25M/C-PI-ECHO	CARDIAC	Nov 12 2003	25	NORMAL - CARDIOLOGIST, ECHO

** CERF Certification is valid for one year from the date of the exam.*

Sire/Dam	Registration	Birthdate	Sex	Relation	CARDIAC	ELBOW	CERF	HIPS
FAERA'S I AM...I SAID CHIC	SN24738202	May 9 1995	F	Dam	GR-CA1129/33F/C-T	GR-EL1402F24-T	GR-16309	GR-56773G24F-T
FAERA'S STARLIGHT CHIC	SN70962301	Nov 22 1999	M	Sire	GR-CA3271/12M/C-PI	GR-EL5179M24-PI	GR-22027	GR-74002E26M-PI

Half Siblings(Sire)	Registration	Birthdate	Sex	Relation	CARDIAC	ELBOW	CERF	GDC ELBOWS	GDC HIPS	HIPS
CHARISMA'S RED WHITE N BOO	SN82449405	Mar 26 2001	F	Half(Sire)		GR-EL7327F24-PI				GR-78927F24F-PI
CHARISMA'S HOLLYROCK BOOLVARD CHIC	SN82449402	Mar 26 2001	M	Half(Sire)	GR-CA5367/19M/C-PI	GR-EL9702M37-PI	GR-25119			GR-79144F24M-PI
CHARISMA'S CARABOO COFFEE CHIC	SN82449401	Mar 26 2001	F	Half(Sire)	GR-CA5368/19F/C-PI	GR-EL9703F37-PI	GR-25121			GR-79145F24F-PI
CHARISMA BOOTLOOSE N FANCY FREE	SN82449407	Mar 26 2001	F	Half(Sire)		GR-EL7331F24-PI				GR-78933F24F-PI
CHARISMA BOOBERRIES 'N CREAM	SN82449404	Mar 26 2001	F	Half(Sire)		GR-EL7508F24-NOPI				GR-79252G24F-NOPI
HARBORVIEW HOLD THE LINE CHIC	SN82931601	Apr 25 2001	M	Half(Sire)	GR-CA8259/14M/C-PI	GR-EL7859M26-PI	GR-28429			GR-80000G26M-PI
HARBORVIEW U BETTER HOLD ON CHIC	SN82931602	Apr 25 2001	F	Half(Sire)	GR-CA8260/14F/C-PI	GR-EL7860F26-PI	GR-28430			GR-80001G26F-PI
LAURELL'S FIRE N ICE CHIC	SN83400907	May 4 2001	M	Half(Sire)	GR-CA5102/15M/C-PI	DEGENERATIVE JOINT DISEASE I UNILATERAL LEFT	GR-25698			MILD UNILATERAL LEFT
LAURELL'S STAR FIRE DIAMOND	SN83400905	May 4 2001	F	Half(Sire)	GR-CA5266/16F/C-PI	GR-EL7647F24-PI	GR-24989			
LAURELL'S FIRE OPEL	SN83400902	May 4 2001	F	Half(Sire)		GR-EL7581F24-PI				GR-79395G24F-PI
LAURELLS ANNIEGETYOURGUNFIRE	SN83400903	May 4 2001	F	Half(Sire)		GR-EL8356F26-PI				MODERATE
SUNBEAMS CATCH A TIGER BY THE TAIL CHIC	SN88712501	Jun 17 2001	M	Half(Sire)	GR-CA6641/25M/C-PI	GR-EL7855M24-PI	GR-27791			GR-79988G24M-PI
WOODWALK RUNS LIKE A DEER CHIC	SN84679801	Jun 28 2001	F	Half(Sire)	GR-CA6847/26F/C-PI	GR-EL8064F25-PI	GR-24311N			GR-80395E25F-PI
STARDUCKS HOLD THE PICKLES	SN86532301	Jun 29 2001	F	Half(Sire)		GR-EL7882F24-NOPI				
SUNKYST JUST BY CHANCE	SN85371101	Jun 30 2001	M	Half(Sire)	GR-CA5514/17M/C-NOPI	GR-EL8625M30-PI				
CONFETTI ONCE IN A BLUE MOON CHIC	SN84831706	Jul 4 2001	F	Half(Sire)	GR-CA8555/41F/C-PI	GR-EL8027F25-PI	GR-28699			GR-80314G25F-PI

Figure 5, Screen Shot from the OFA website displaying individual dog record. Whether searching from the OFA site or the CHIC site, the individual dog records such as the one above are seamlessly accessed from either.

Another unique feature is the vertical pedigree analysis which encourages breeders to truly analyze the depth and breadth of a pedigree in a vertical fashion rather than the simple more traditional horizontal method.

Hip Status Vertical Pedigree

Printable Hips Elbows Cardiac Thyroid

FAERA'S ALONG CAME THE SPIDER SN87604904

Return to info display

FAERA'S ALONG CAME THE SPIDER
subject **"GOOD"**

Sibs(0)

Offspring(0)

FAERA'S STARLIGHT
sire ***"EXCELLENT"***

Sibs(2)
GOOD(2)

FAERA'S I AM...I SAID
dam **"GOOD"**

Sibs(13)
GOOD(8)
FAIR(5)

TWIN-BEAU-D'S PETERBUILT
paternal grandsire "FAIR"

Sibs(3)
GOOD(1)
FAIR(2)

FAERA'S SWEET CAROLINE
paternal granddam **"GOOD"**

Sibs(13)
GOOD(8)
FAIR(5)

FAERA'S FUTURE CLASSIC
maternal grandsire **"GOOD"**

Sibs(6)
GOOD(2)
FAIR(4)

FAERA'S SHILO LUEREE FIRE
maternal granddam "FAIR"

Sibs(17)
GOOD(12)
FAIR(5)

The OFA database is not directly linked to the AKC or any other registry. Parent, Offspring, and Sibling information is limited to dogs contained in the OFA database, and where the sire/dam information has been filled out on the application so that subsequent relationships can be determined. Titles are included as a courtesy and are limited to those provided on the dog's application.

Figure 6, Screen Shot from the OFA website displaying vertical pedigree analysis information. Where available the pedigree shows not only the animals in the direct pedigree line, but summarizes information on sibling data.

The CHIC program offers a variety of benefits to breeders, buyers, parent clubs, and researchers. For breeders, CHIC provides a reliable source of information regarding dogs they may use in their breeding programs. In the future, breeders can begin to analyze the pedigrees of a proposed breeding for health strengths and weaknesses as well the traditional analysis of conformation, type, and performance strengths and weaknesses.

For buyers, the CHIC program provides accurate information about the results of a breeder's health testing. For diseases that are limited to phenotypic evaluations, there are no guarantees. However, the probability that an animal will develop an inherited disease is reduced when its ancestry has been tested normal. Further, as more DNA tests become available and the results are entered into CHIC, the CHIC database will help breeders predict whether progeny will be clear, carriers, or affected.

For parent clubs considering establishment of health databases on their own, CHIC provides the answer with no upfront investment required by the club. The CHIC infrastructure is supplied and maintained by the OFA. The data is maintained in a secure environment by trained staff. The services are not subject to the time, technology, and resource constraints that parent clubs might face on their own. This frees parent clubs to focus on the tasks of identifying health concerns, educating their membership, raising funds for research, and encouraging participation in the CHIC program.

For researchers, CHIC provides confidential and accurate aggregate information on multiple generations of dogs. CHIC information will also be useful for epidemiological studies enhancing our knowledge of health issues affecting all breeds of dogs.

For everyone interested in canine health issues, CHIC is a tool to monitor disease and measure progress.

The Orthopedic Foundation for Animals is a nonprofit 501(c)(3) foundation formed in 1966 with the following objectives:

1. *To collate and disseminate information concerning orthopedic and genetic diseases of animals.*
2. *To advise, encourage and establish control programs to lower the incidence of orthopedic and genetic diseases.*
3. *To encourage and finance research in orthopedic and genetic disease in animals.*
4. *To receive funds and make grants to carry out these objectives.*

The AKC/Canine Health Foundation is a 501(c)(3) nonprofit organization formed in 1995 with the following mission: To develop significant resources for basic and applied health programs with emphasis on canine genetics to improve the quality of life for dogs and their owners. The AKC/Canine Health Foundation is the largest funder of exclusively canine health research in the world.

Strategies for Identifying and Managing Complex Genetic Disorders

A.M. Oberbauer, Ph.D.
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Davis, California, USA

The basic objective of all breeders is to improve on a breed, thus the axiom of "breed the best to the best." The last part of the adage, "and hope for the best" oft quoted by thoroughbred breeding legend C.V. Whitney, sums up the historical breeding perspective. Until genetic tests exist allowing breeders to identify, and then select for, particular traits, physical traits and disorders must be dealt with from a probability/likelihood viewpoint rather than a breeding certainty. While Mendelian inherited traits are more easily tracked, and possibly dealt with from a breeder's point of view, disorders that are genetically complex in their regulation often are of most concern.

Selective breeding either for or against a trait or disorder requires that the trait/disorder be under genetic control. Generally the first evidence that a trait has a genetic component is empirical: a disorder having greater prevalence among related individuals leads breeders to consider that the disorder is familial and controlled in part by genetic contributions. With some disorders, affected dogs appear in to be segregating through the generations. Alternatively, the disorder may appear sporadically in the pedigree. Familial data can be analyzed statistically to estimate the proportion of genetic vs. environmental contribution to the phenotypic expression of a trait. This heritability value provides an estimate of the extent of genetic control over a trait. Information from many different families representing multiple geographical locations yields the most accurate heritability estimate because a range of environmental influences are then evaluated. Further, the magnitude of the heritability estimate can be predictive of a breeder's ability to effectively select against the disorder.

Although a high heritability estimate has been associated with Mendelian inheritance, as more genetic studies are completed that association has become less robust; that is especially true if environmental factors are shared amongst dogs affected with a particular disorder. To assess mode of inheritance an additional statistical analysis must be done. Complex segregation analysis models whether a trait is best described as governed by a single gene inherited in a Mendelian fashion or if the disorder is more polygenic with multiple genes contributing to its expression. If it is the latter, the disorder is more difficult to eradicate due to the seemingly sporadic nature of the disorder's expression. Thus, knowledge of the mode of inheritance is critical to the success of selective breeding away from a particular genetic disorder.

In complex segregation analysis, the phenotypic data representing expression of the disorder are fit to proposed models of inheritance (single gene, few genes, many genes, many genes with a single major gene, etc.) and then the how well the data "fit" is compared among the models. That is, if a particular mode of inheritance is specified, will that model generate the observed frequency of the disorder that is seen in the actual data? The different models are then statistically compared in a maximum likelihood analysis which permits the investigator to determine what mode of inheritance is most consistent with the actual recorded data. These analyses also account for environmental factors that may influence the expression of the disorder.

Genes regulating disorders inherited in a classical Mendelian fashion can be identified with linkage analysis as discussed in other presentations at this conference. While the identification and characterization of Mendelian genetic disorders are by no means simple, similar characterizations for complex disorders present a more complicated challenge to identify the underlying genes. That is due in part, as noted above, to the lack of a clear pattern to the transmission of the disorder through the generations and to the interaction of the genes with particular environmental exposures. This ambiguity in the transmission of the disorder poses problems for predicting the risk of an individual to either contract or pass on the disorder.

Complex disorders regulated by a few genes are sometimes referred to as "oligogenic" and those regulated by a large number of genes interacting with environmental influences are referred to as "polygenic" or "multifactorial". Complex disorders are also often referred to using Mendelian terminology with additional terms such as "autosomal recessive with incomplete penetrance" or "autosomal dominant

with modifiers." The variability in terminology merely underscores the multifaceted interaction of the genetic and environmental contribution to the expression of a complex disorder. In all cases, regardless of terminology, more than one gene regulates the expression of the disorder and to best select against a particular disorder requires greater knowledge than for simple disorders.

Human complex genetic disorders recently have received a great deal of attention, partly due to the relative straightforward approach necessary to identify the gene underlying a Mendelian disease. In fact, a recent review states "Nearly every Mendelian genetic disorder has now been mapped to a specific gene or set of genes" (Mayeaux, 2005). With the conquest of simple disorders, the challenge of complex disorders coupled with the availability of new genetic tools has appealed to scientists. Further, most disorders appear to be complexly inherited. This has led to more comprehensive statistical approaches and improved technology being recruited to detect the chromosomal regions behind complex disorders.

Information derived from the complex segregation analysis can guide the characterization of the genetic regulation. For example, as mentioned, one potential mode of inheritance model that can be tested is many genes with a single major gene. Further model modifications can include whether that single major gene is inherited in a Mendelian fashion. A major gene is defined as one whose contribution has a significantly large influence on the expression of a trait. (Of note, other definitions more narrowly define major gene as one that is necessary and sufficient to result in expression of the disorder).

The presence of a major gene can facilitate the investigation and localization of the genetic components behind the disorder. The technical approach for a disorder in which a major gene exerts a large effect on expression is similar to that for a simple Mendelian disorder. Complications to the detection of the chromosomal region behind a disorder arise in the *accuracy* of the diagnosis of the disorder in individuals and, as plagues all genetic studies, if the disorder is late onset, the appropriate censoring of the data (e.g., is the five year old unaffected or merely "preaffected" and not yet expressing the disorder?). Disorders regulated by a major gene offer a great probability of success in actually identifying the single causal gene the significantly influences the expression of the disorder. The mutation within that gene can then be used to develop diagnostic breeding tools to aid in selection of superior broodstock.

In contrast, disorders that are polygenic with all involved genes contributing somewhat equivalently to the expression of the disorder have been, historically, extremely problematic in terms of identifying and developing DNA based selection tools for those genes. One reason for this is that naturally occurring polymorphisms in a gene may be indistinguishable from mutations. When many different genes are known to contribute to the expression, but the identity of the genes is unknown, then attempting to correlate DNA changes with the expression of the disorder is particularly difficult. In an attempt to circumvent this hurdle, scientists have created inbred lines of animals (e.g., cattle) to minimize the inherent genetic polymorphism and accentuate the genetic change that results in particular traits of interest. These studies are often referred to as quantitative trait loci (QTL) studies due to the generalized quantitative nature of complex traits.

One application of studying hereditary health disorders in dogs is in advancing our understanding of human conditions. But in most cases, advances to dog and cat genetic studies rely upon findings in mouse, livestock, or human studies. For instance, current emphasis is being placed on identifying the genes regulating human diabetes, obesity, and autism (Mayeux, 2005; Veenstra-VanderWeele et al., 2004). Thus, we can look to success in these species as indicators of successful characterization and genetic marker development for complex traits. For example, in livestock, identifying the DNA regulating marketable traits is desirable. Six separate chromosomes have been identified as regulating the thickness of fat on market cattle (Li et al., 2004). However, it is important to note that few QTL study results have been successfully implemented into mammalian breeding schemes. There remain, however, many lessons to be learned from current, and past, studies of complex traits in these species.

Although current approaches focus on identification of the underlying DNA with the objective of developing DNA based tests, the development of DNA based tests for fully polygenic complex disorders will take a great deal of time and resources. Until such genetic tests become available, breeders need to work to minimize the frequency of complex disorders in their breeds. Once a reliable estimate of

heritability and then mode of inheritance is established, breeders need to act upon that information prior to the establishment of DNA tests and marker assisted selection.

The Orthopedic Foundation of America (OFA) and Canine Eye Registry Foundation (CERF) are both voluntary registries to assist breeders in selecting breeding stock that are free of phenotypic dysplasia or eye abnormalities. These data, while useful, are limited in that a quantitative value of risk associated with using a particular dog in a breeding program is not delineated. Further, the owners must assent to publishing information from their dogs and not all of an owner's dogs are assessed thereby limiting the utility of the available data.

Yet minimizing the expression of typical complex disorders, such as deafness, hip dysplasia, behavioral abnormalities, epilepsy, and some forms of cancer, is an important goal that needs to be addressed sooner than genetic testing can be made available. Methods to cope with breeding away from complex disorders can be addressed by looking at past studies of quantitative trait improvement breeding schemes in other species. Perhaps the best documented breeding approach to improve quantitative traits (i.e., complex traits) is the National Dairy Herd Improvement Association (DHIA) whose goal, when the association was founded over 85 years ago, was to provide a voluntary record keeping system for dairy producers. As an offshoot of that, volumes of data were chronicled representing a sire's genetic contributions to milk production and reproduction parameters, all of which are complex traits. These data are analyzed and used to derive **breeding values** for any given sire which allows for expansive improvement in complex traits: a 12-fold increase in milk production when producers participate and utilize the DHIA program in their breeding programs (National Milk Producers Federation. 1993).

Assigning genetic merit to individual animals requires a comprehensive database for as many animals and traits as possible yielding the "so-called" depth and breadth of pedigree information that is considered invaluable to informed breeding decisions. Details on many complex traits could be compiled and then animals assigned a breeding value for each trait. The precise raw data would not be revealed but the composite breeding values would be available. Note, because dogs and cats are not bred for any **single** trait, an animal with a low or unfavorable breeding value for one trait/disorder may be exceptional for a different trait. Therefore, breeders could weigh the pros and cons of doing a particular breeding due to the differential weight breeders apply to various attributes. That is, particular sires complement particular dams potentially expanding the number of sires used which may avoid the "popular sire syndrome".

Applying breeding values to assign merit for different traits is being applied to the breeding of service dogs. This has been ongoing for a sufficient number of years that this breeding approach can be evaluated subjectively: a greater number of dogs are being placed with people with disabilities than previously indicating the success of the breeding program. In addition, the use of breeding values was modeled to predict the successful reduction of epilepsy incidence in the Belgian Tervuren (Famula & Oberbauer, 1998).

The future holds great promise in the availability of genetic selection tools based upon genetic mutations even for complex disorders. However, it is not prudent for breeders to wait upon the development of such genetic tests if a disorder can be minimized by informed phenotypic selection. Further, some complex disorders are regulated by so many individual genes that complete characterization of all regulating mutations is unlikely. If a disorder is known to be genetically regulated, even complex disorders can be reduced in the population by generating and using breeding merit scores. Though that requires cooperation among breeders, the benefits for a breed are immense.

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Recent Progress on the Genetics of Canine Epilepsy and Addison's Disease

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Our laboratory has been studying the genetics behind epilepsy and Addison's disease in several dog breeds for a number of years. This report will summarize some of the current findings regarding these two genetic disorders beginning with epilepsy.

DeLahunta (1977) characterizes a seizure as "a transitory disturbance of brain function" representing enhanced and synchronous activity of neurons (March,. 1998). In many instances, the seizure reflects an isolated incident and never recurs. *Recurrent* seizure activity is what defines epilepsy. Epilepsy is then categorized based upon the cause of the seizures. Seizures can be sequelae to other physiological conditions such as infections, cancer, or hypoglycemia. Seizures can also be the consequence of exposure to environmental toxins or the result of trauma. When a particular initiator for the seizures can be identified, then the epilepsy is referred to as **secondary** or **acquired** epilepsy. When no underlying cause can be detected, the epilepsy is considered **primary** or **idiopathic** epilepsy. In both categories of epilepsy, the actual neurological disturbance is similar, but the initiator differs.

Idiopathic epilepsy is an extremely common neurological disorder in dogs and cats. The Canine Epilepsy Research Project, a consortium of researchers from the Universities of Missouri and Minnesota, reports having DNA submissions of epileptic dogs from 85 different dog breeds of genetically diverse backgrounds. Epilepsy in cats, while much less prevalent, is still a serious condition (Kline, 1998), although the preponderance of feline seizures represent acquired or secondary epilepsy (Parent and Quesnel, 1996).

Seizures can be mild with symptoms of generalized confusion and "gazing" or seizures can be severe (grand mal) with loss of consciousness, spastic muscle movements, bowel and urinary incontinence, and involuntary salivation. Seizure frequency can vary from several times per day to more intermittent episodes occurring perhaps once every few years (Thomas, 2000). Although the duration of a seizure is generally short, the after effects may linger for hours to days following the episode itself. Further, the occurrence of one seizure may potentiate future seizures (March, 1998). Regardless of precisely how the seizures are manifested, the effects of idiopathic epilepsy are distressing for the dog and the owner, and possibly even hazardous in the larger breeds.

Many diverse dog breeds experience seizures and while the majority of breeds are considered to have a genetic component to the epilepsy, the details of inheritance in most breeds are not well described. For any sound inheritance study, the first step to characterizing the genetic contribution is to obtain sufficient phenotypic and pedigree data from related dogs. One significant complication to the study of epilepsy is the ambiguity of diagnosis. Idiopathic epilepsy diagnosis is one of exclusion in that no underlying cause for the seizures can be identified. Therefore, it is vital that the families studied transmit very definable, recognizable seizure activity through the generations. Further, any study needs to account that age of seizure onset, while typically between 2-4 years of age, can vary so designating a dog as "unaffected" needs to be done judiciously.

In our studies we have restricted our analyses to dogs that exhibit grand mal seizuring on the premise that owners can readily identify that seizure form. We are presently studying epilepsy in the Belgian Tervuren, Belgian Sheepdogs, Poodles, English Mastiffs, and Giant Schnauzers. By far we have made the most progress on the Belgian breeds as we have been studying those for the greatest length of time. However, the approach our laboratory has taken with the Belgians is the same as for the other breeds. We have collected phenotypic (health status) and pedigree data, along with DNA samples, on more than 1850 Belgians (with an average of 12.75% classified as epileptic). These samples complement an earlier "phenotype-only" study of ~ 1000 Belgians. From these data we have determined, statistically, that the heritability for seizures in the Belgians is on the order of 0.77 to 0.83 (Famula et al., 1997; Famula and

Oberbauer, 1998) indicating a large genetic component to the expression of seizures. As an aside, the heritability estimate for epilepsy in the English Mastiff is likewise, extremely high. The data for the Belgians were then subjected to complex segregation analyses to characterize the mode of inheritance which suggested, polygenic with a single major gene of large effect inherited as an autosomal recessive influencing the expression of the disorder.

These findings formed the rationale in undertaking a full scale genome scan of the Belgian DNA to identify a genetic region linked to the seizure phenotype. We initially scanned a small cohort of related dogs with microsatellite markers offering reasonable genome coverage (Oberbauer et al., 2003). We have since expanded the number of dogs evaluated as well as the extent of genomic coverage. Five genomic regions have been identified as potentially linked to the expression with one region exhibiting fairly robust LOD scores. LOD scores are defined as the log (base 10) of a likelihood ratio between two conditional probabilities, one being that linkage exists and the other being that the marker and phenotype are unlinked. In human studies, LOD scores in excess of 3.0 are considered "significant" for linkage (Shete and Amos, 2002). One genomic region has consistently yielded LOD scores hovering around 3.0. We are currently expanding the number of dogs analyzed and the number of genetic markers with the intent to enhance the LOD scores and improve the confidence of linkage. With linkage established, the DNA will be sequenced and candidate genes within that region will be investigated for causal mutations. The ultimate objective is to generate a genetic marker test to allow breeders to identify dogs that carry a mutation permissive for the expression of epilepsy.

Similar heritability and complex segregation studies have been ongoing in other breeds (e.g., Patterson et al., 2005). In January of this year, a research team in Canada uncovered the causal mutation for a very specific form of epilepsy (progressive myoclonic epilepsy inherited as an autosomal recessive) that plagues miniature wirehaired dachshunds (Lohi et al., 2005). The genomic scanning approach resulted in the development of a genetic test that is now available for breeders of the miniature wirehaired dachshund.

The current research on the genetics of Addison's disease, or hypoadrenocorticism as it is more accurately termed, also shows a very strong genetic contribution to the expression of the disorder. In Addison's disease, the adrenal cortex fails to synthesize and release adequate quantities of two classes of steroid hormones. Mineralocorticoids that regulate electrolyte balance and corticosteroids that regulate many aspects of metabolism and the stress response are greatly reduced in dogs with Addison's.

Similar to epilepsy, Addison's disease can be characterized as being either primary or secondary. As the name implies, primary reflects an insufficiency due to a defect or atrophy in the adrenal gland itself. Secondary Addison's disease reflects the condition where the impaired adrenal cortex function is the consequence of some other identifiable cause; for example, a deficiency in adrenocorticotropin hormone (ACTH), the hormone that stimulates the adrenal gland to function.

As noted above, with primary Addison's, the adrenal cortex gradually deteriorates and becomes incapable of hormonal production (Kaufman, 1984); this deterioration is speculated to be a consequence of the immune system failing to distinguish that the adrenal cortex is a self tissue (Smallwood and Barsanti, 1995; Greco and Harpold, 1994; Weller et al., 1996). Thus, Addison's disease is often a late onset disorder with diffuse symptoms that include generalized fatigue, inappetence, gastrointestinal upset, and weight loss. Primary Addison's seems to be the most prevalent form and is diagnosed by providing exogenous ACTH and evaluating the adrenal cortex's ability to secrete glucocorticoids. If ACTH fails to induce glucocorticoid production and release, the adrenal cortex is considered defective and the animal is determined to be a primary Addisonian.

The existence of Addisonian dogs repeatedly appearing in pedigrees of certain dogs led breeders to speculate that Addison's disease is inherited. Although Addison's disease occurs in the dog population as a whole (Little et al., 1989), within certain breeds there has been a higher than expected incidence noted. We have determined the heritability and mode of inheritance if feasible in the Standard Poodle, Great Dane, West Highland White Terrier, Bearded Collie, Portuguese Water Dog, and Leonberger. Using an approach identical to that described for the epilepsy work, we have estimated heritability for

those breeds with sufficient data submission. In all breeds except the Great Dane (which currently lacks the necessary numbers of dogs enrolled in the study) the heritability for Addison's disease is greater than 0.7 indicating a very large degree of genetic regulation.

Complex segregation analyses confirm the genetic component and suggest that the best fit mode of inheritance is autosomal recessive with modifying genes. In other words, Addison's appears to be polygenic but with a major controlling gene. The lesser genes likely regulate the age of onset and the progression of the disorder. Of note, is that there is no sex affect in any of the breeds reflecting an equal number of males and females diagnosed with Addison's disease.

Based upon these findings, we have approached the genetic linkage study in three separate ways. 1) Using multigenerational families of Poodles and Portuguese Water Dogs, we are scanning for linkage between the disorder phenotype and genetic markers linked to candidate loci. The candidate genes were chosen for their involvement in normal immune function and cell recognition. At this point in time, no linkage among the genes tested was discerned. 2) For Poodles, Bearded Collies, and Portuguese Water Dogs, we are using eight highly unrelated dogs of each breed in a homozygosity screen using 327 microsatellite markers that offer 9 MB genome coverage. The theory is that since the major gene is autosomal recessive, the unrelated dogs should show homozygosity in the chromosomal region that regulates the expression but not in other chromosomal regions. This is because, on the whole, the dogs chosen are not closely related and therefore should share little DNA similarity other than in that one region (Lohi et al., 2005). Several chromosomal regions indicate further investigation is warranted and we will target those regions with additional markers. 3) Using multigenerational families of the above breeds we will use the 327 markers in a full genome scan. We believe this approach will yield chromosomal regions significantly linked to the Addisonian phenotype. The linkage, followed by gene identification and genetic sequencing to identify the precise mutation will enable the development of a diagnostic DNA based test for breeders to integrate into their breeding program. We are hopeful that the diversity of breeds under study will result in a test that is of utility to all breeds.

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Genetics of Canine Cancer

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Overview of the Issue

Cancer is a disease of genes. Although the vast majority of cancers occur sporadically, risk is variably influenced by heritable factors. Dogs and people share a common environment and are susceptible to a similar range of cancers, although there are some significant differences between both species. This lecture will review the genetic basis and pathology for cancer in general, and will discuss advances in the search for cancer susceptibility genes in dogs.

Introduction

Cancer is the leading cause of death in humans under the age of 85, as well as the leading cause of disease-related death in dogs. As such, it has gained exceptional importance in our society. Both genetic and environmental factors have major effects on the temporal occurrence of cancer, and there is thus a new emphasis to learn more about how these factors influence cellular and molecular changes in cancer. Dogs and people are susceptible to many of the same types of cancer and the natural history (incidence, age of onset, location, progression, outcome) of many cancer types is similar in both species. Our pet dogs share our environment closely, allowing us to examine not only the heritable risk factors, but also those associated with the environment. Moreover, when compared to humans, dogs have shorter generational life spans (as many as five or more related generations frequently co-exist), extended pedigrees with detailed family histories, and more homogeneous genetic backgrounds, which provide unique opportunities to address questions about the origin and behavior of cancer. The answers we obtain studying cancers of dogs will contribute to our ultimate goals to design strategies for prevention and treatment of cancer in both dogs and people.

Cancer is a "genetic" disease

To understand the implications of cancer, one must first realize that cancer is not a simple disease. Rather, the term cancer describes a large number of diseases whose only common feature is uncontrolled cell growth and proliferation. A very important concept that is now universally accepted is that "*cancer is a genetic disease, although it is not always heritable*." Tumors arise from cells that accumulate mutations which eliminate normal constraints of proliferation and genetic integrity. These mutations provide cells a selective growth advantage within their environment. This is essentially the same evolutionary phenomenon that we call "natural selection", albeit on a microscopic scale. Various theories have been proposed to explain the genetic basis of cancer. One explanation invokes stochastic (random) events – the inherent error rate of enzymes that control DNA replication during each division introduces about 1 in 1,000,000 to 1 in 10,000,000 mutations for each base that is replicated during each round of replication. The genome consists of many millions of base pairs, so each daughter cell is likely to carry at least a few mutations in its DNA. In other words, *the single most important risk factor for cancer is life*. Yet, most of these mutations are silent; that is, they do not present any problems to the cell's ability to function, but others can disable tumor suppressor genes or activate proto-oncogenes that respectively inhibit or promote cell division and survival. An alternative hypothesis is that mutations are not stochastic, but rather "directed" due to the presence of a "mutator phenotype," where the factors that control DNA replication and repair are inherently prone to more errors than would be expected by simple stochastic events in particular individuals. This leads to different cancer predispositions, which would be higher than the mean in such individuals, and might explain why not all people (or dogs) exposed to similar environmental carcinogens develop the same forms cancer at the same rate. There is evidence to support both mechanisms (stochastic and directed) in people and animals.

In both cases, loss of function of tumor suppressor genes and gain of function of oncogenes appear to contribute disproportionately to the origin of tumors. Tumor suppressor genes encode proteins that constrain cell division, promote cell death, or are essential to maintain the integrity of DNA. These genes can even help eliminate renegade cells that have initiated the path to cancer; thus, mutations that disable tumor suppressor genes contribute to the development and progression of tumors. In a broadly oversimplified approach, tumor suppressor genes can be grouped in three categories. One that includes *p53* and *ATM*, among others, is responsible for controlling DNA repair. Cells can undergo spontaneous

mutations, and these tumor suppressor genes must ensure that the mutant cells do not divide until the errors in their DNA sequence are repaired. Another that includes various cyclin-dependent kinase inhibitors such as *INK4* and even some proto-oncogenes such as *Ras* (see below) controls cellular aging. Each cell in the body has the potential for a finite number of divisions, and these genes prevent further replication when that number has been reached. A third serves to counteract the function of growth-promoting genes and survival genes. Among these are *RB* and *PTEN*. Inactivation of tumor suppressor genes such as those listed above increases the risk of cellular transformation that can result in various types of cancer. Moreover, cancers that arise due to other mutations but that retain the function of these tumor suppressor genes may respond more favorably to therapy, making these promising targets for genetic therapy of cancer. Proto-oncogenes are the polar opposites of tumor suppressor genes. They encode proteins that promote cell growth and survival. In most cases, these genes are "turned on" and "turned off" as needed to maintain an adequate balance of cell division. However, when these genes are targets of mutation, they may gain independent function that cannot be "turned off", leading to the development of cancer. It is very important to note that, even though there are numerous prototypical tumor suppressor genes and oncogenes (or cellular proto-oncogenes), many different genes can function in one or the other category (and sometimes both), depending on the context in which they are expressed!

Genetics of Canine Cancer

A Heritable Cancer Syndrome of Dogs

We seek to define genetic lesions that underlie the pathogenesis of cancer in dogs. Mutations of specific genes that increase the probability, or risk, that an individual (actually a cell) will develop a tumor. In some cases, mutations occur in reproductive cells and are passed on in the germ line. Identification of such mutations should help us predict relative cancer risk in individuals (or the likelihood of individuals to produce progeny with elevated cancer risks), allowing us to invest in practices to modify the environment that may reduce or eliminate the risk (cancer prevention). The investigation of cancer susceptibility in families or breeds of dogs is of critical importance to dog breeders and dog owners alike. Unlike other heritable conditions, genetic susceptibility to cancer may not manifest in disease until a dog has reached middle age, and long after it has achieved breeding potential. When present, this genetic susceptibility may be due to a process called loss of heterozygosity. Individuals inherit two copies of each gene upon conception, one from the sire, and one from the dam. Each of these gene copies is called an "allele." A family or breed may have through the course of time, lost a functional allele of a "tumor suppressor gene" through mutation. The affected individuals are heterozygous (that is, they have two different alleles, and only one is functional). These individuals may not develop disease (cancer), unless the second, functional copy of the gene in question is mutated in a cell that retains the capacity to divide. Even in the best of circumstances, genetic analysis can only predict the probability or provide a relative risk, rather than a definitive assessment of whether or not the individual will in fact develop cancer. In an elegant series of research papers, Ostrander's and Lingaas's groups recently reported an example of a heritable cancer syndrome (renal cystadenoma and nodular dermatofibrosis or RCND) in German Shepherd Dogs, where the gene defect was traced to a novel tumor suppressor gene called *BHD* (folliculin) [1,2]

Cancer Syndromes with Significant Heritable Influence

There are various cancers whose prevalence in specific breeds indicates the risk is modulated in large part by inherited factors. Perhaps the best studied among these is bladder cancer in Scottish terriers. Recent work by Knapp, Glickman, and others [3,4] shows that the risk for this disease is almost 20-fold greater in Scottish terriers than the average for all dogs. However, the occurrence of the disease seems to be influenced by environmental factors such as exposure to herbicides, as well as by body composition. The genes that modulate risk in this breed remain to be determined, and are possibly linked in a complex mode of inheritance that could make their identification challenging. For this reason, mapping studies comparing risk among Scottish terriers and other susceptible breeds might be a fruitful endeavor.

Cancer Syndromes Mediated by Somatic Mutations

In most cancers fo dogs and humans, mutations occur "sporadically", that is, they alter the DNA of non-reproductive (somatic) cells. Generally, these mutations arise in susceptible individuals upon exposure to certain environmental insults; but the risk is not necessarily shared by relatives of the affected individual. Nevertheless, identifying the patterns of mutations associated with specific tumor types is likely to provide information to obtain a more accurate prognosis, and to develop more effective treatments. One example of this process has been extensively studied by London and her colleagues, who described a common

mutation of the c-Kit protein in canine mast cell tumors. Although the presence of this mutation is associated with a worse prognosis, it offers a potential target for treatments to improve the outcomes of dogs with this disease [5,6]

Cancer Syndromes Associated with Environmental Factors

Perhaps the best example of this type of cancer is mammary tumors of female dogs. More than 30 years ago, Dorn and colleagues showed a significant association of mammary tumors with intact hormonal status in female dogs. Based on their work and others, it is estimated that ovariectomy can reduce the risk of this disease by more than 200-fold, demonstrating the key role of estrogen (and possibly progesterone) in the pathogenesis of malignant mammary tumors in the dog. While in many ways this resembles mammary tumor biology of women, the story in cats is vastly different [7]. Recent work focuses on the role of other oncogenes, such as IGF-1 in these tumors [8].

Cancer Syndromes with Complex Inheritance Patterns

Another approach that has been successfully used to identify genes that contribute to cancer is the study of recurrent chromosomal abnormalities. Historically, this approach is responsible for the identification of the vast majority of tumor-associated genes (in humans). Until recently, major technical obstacles dampened the study of canine chromosomes, but many were overcome by the work of Breen and his colleagues, who developed reagents and adapted techniques to define a consensus karyotype for the dog [9-11]. Using this information, our groups have documented breed specific prevalence (and hence, presumed patterns of inheritance) for canine non-Hodgkin lymphoma (NHL) [12]. As importantly, we have shown that pathognomonic molecular abnormalities of both NHL and leukemias of dogs and humans are conserved, indicating the underlying basis for these diseases is firmly embedded in the genome and may result from peculiar aspects of mammalian evolution.

Summary

We can confidently state that the genetic basis of cancer is now beyond question. It is estimated that at least five mutational events are required for overt malignant transformation, and genomic instability seems to be necessary to establish a self-renewing population of cells (possibly cancer stem cells) whose progeny expand to cause clinical disease. Ultimately, a subpopulation endowed with metastatic properties that is drug resistant leads to death of the cancer patient. A major focus of contemporary cancer genetics is to define whether such properties are inherent to cancer stem cells or whether they arise by natural selection and clonal evolution. Current knowledge and available molecular tools allow us to predict prognosis and response to therapy in some cancers of companion animals, and we believe the availability and usefulness of such tools in clinical practice will expand rapidly. Hence, as we improve our understanding of fundamental mechanisms that account for malignant transformation and tumor progression, we will be able to design even better strategies for cancer prevention and therapy.

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Feline Genetic Disorders and Genetic Testing

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Overview of the Issue

Genetic testing is becoming more prevalent in many companion animal species and is an increasingly important diagnostic tool for veterinarians. At least seven[1-5] genetic tests for diseases and phenotypic traits have become available for the cat within the past 12 months alone. The low-resolution genetic sequencing of the cat genome should greatly facilitate the development of additional tests at an even faster pace. DNA-based tests for several inborn errors of metabolism have been available for cats for many years[6-10], however, DNA-based tests for more common diseases that afflict large breed populations, such as hypertrophic cardiomyopathy and polycystic kidney disease, have been recently announced and currently are, or may soon be, commercially available from more than one testing facility[3,11]. Genetic tests for several recessive coat color variations can be used by breeders to develop more efficient breeding programs. A DNA marker panel for cat parentage and identification has been internationally standardized and recognized, allowing the verification of pedigrees and individuals worldwide. Different laboratory techniques can be used to assay the same particular genetic mutation, thus, veterinarians and breeders need to recognize the inherent differences of these assays and the associated error rates. In addition to the important value of genetic tests to the breeder and veterinarian, these potential error rates for genetic testing will be discussed. The newly available tests for the domestic cat will be used as examples for these discussions. Breeders and veterinarians will learn the pros and cons of genetic testing and be reminded of the cooperation required between investigators, veterinarians and breeders to develop a test for the commercial market.

Introduction

The reduction of genetic variation is an inherent concern for any domesticated breed or isolated population. Loss of variation tends to promote inbreeding depression within a breed, which may be expressed as health issues and defects. Generally, all breeds experience population bottlenecks, founder effects, selection, reduced migration, inbreeding, and random genetic loss. During the development of animals that will “breed true” and produce individuals with desired characteristics and phenotypes, deleterious or undesired traits can also increase in frequency within breed populations along with the desired, breed defining traits. These accidental traits can “hitch-hike” along with good traits within a breed via random chance, by being in close physical proximity on a chromosome to a desired characteristic, or by producing a desired trait in the carrier state. Thus, although breeders may have accidentally caused the increase in frequency of deleterious traits, with the advent of DNA-testing, breeders have a highly accurate means to determine trait status, even prior to trait onset, and hence they have the responsibility and a more effective means to properly manage deleterious traits within the population.

Along with the breeder, veterinarians have the important role of supporting proper breed management decisions. Veterinarians need to determine when a genetic test is appropriate, interpret the results for each genetic test, understand the inherent limitations and errors of a test, and provide additional health information to support informed breeding decisions. Each aspect must be considered, along with others more pertinent to the breeder, for a sustainable and healthy breeding program.

Phenotypes and Phenocopies

The phenotype is the outward appearance of an individual. What an individual looks like is a combination of environment and genetically inherited affects. Both desired and undesired traits can be mimicked by a wide variety of combinations of nature and nurture, thus, the initial challenge to breeders and veterinarians is to confirm that a phenotype is due to the expected interactions of genes and the environment. A trait that is clearly inherited as a single gene recessive trait, such as brown coat color in the domestic cat, can also be mimicked by poor nutrition. This mimicry is known as a phenocopy.

Commonly, a health issue can be accidentally lumped into the same category and be misreported within breeds. Polycystic kidney disease (PKD) in cats is associated with cystic kidneys and frequently a cystic liver. The disease often causes renal failure at an early age. Breeders can make the error of considering renal failure a differential for PKD instead of confirming that the renal failure is due to severe kidney cysts.

Renal cysts alone are not a clear diagnosis for PKD, as the normal population can have minor and asymptomatic kidney or liver cysts. Thus, breeders and veterinarians need to be reminded that a proper list of differentials needs to be considered in order to differentiate between a true phenotype and a false phenocopy.

Hypertrophic cardiomyopathy (HCM) is a disease that often has false reports by breeders. Not all heart murmurs are due to HCM, however this is a common leap as HCM is reported at a high frequency in some breeds. Proper differentials are needed to decipher dilated cardiomyopathy (DCM) and other cardiac issues from HCM, and not all abnormal echocardiograms are a result of HCM. Hence a little knowledge can often lead to false conclusions in the breeding world.

Hallmarks for Genetic Diseases

DNA-tests have been developed for simple, single gene traits in the cat. Simple or single gene traits have a predictable inheritance because their mode of inheritance is clearly, dominant, recessive, or co-dominant. These traits may or may not be sex-linked. Inherited diseases can be deciphered from sporadic or idiopathic phenocopies by several hallmarks common to genetic traits. Firstly, genetic traits will tend to have a higher prevalence within a breed. Thus, particular breeds should be recognized as high risk for some diseases. Secondly, the trait will have a highly consistent presentation. The presentations may be multiple, but fairly consistent. Thirdly, if appropriate, the trait will have bilateral presentation. PKD is defined by having multiple cysts in both kidneys. Thirdly, the disease onset will be early for the type of disease. Hence, renal failure in a 4 year old Persian cat with bilateral kidney cysts is likely PKD. Lymphomas are common in cats, however, mediastinal lymphoma in the Oriental Shorthair cat breed generally afflicts individuals by 2 years of age. This early onset disease with in a breed that always presents as a mediastinal tumor is highly indicative of a heritable condition.

Disease Heterogeneity

Along with phenocopies, genetic diseases must be categorized clearly due to disease heterogeneity. In humans there are several forms of kidney disease, which have polycystic kidneys. At least three different genes can cause polycystic kidney disease and lead to renal failure. In humans, the different genes have different modes of inheritance. Approximately 85% of human PKD is caused by a dominant mutation in the gene that makes polycystin-1, *PKD1*. However, *PHKD1* causes recessive, jeuvenile onset of PKD and *PKD2* (polycystin-2) causes a milder form of the autosomal dominant disease. The clinical presentation of these three forms of PKD is so overlapping that DNA-tests are often required to decipher which form of the disease is present.

In addition to 85% of PKD being caused by the gene *PKD1* in humans, the disease is also caused by different mutations within the gene. Hence, most newly identified human families tend to have a new, sporadic mutation. Thus, genetic testing for PKD in humans is confined to within particular families or very limited populations and a variety of PKD alleles occur within the human population.

Genetic Test Development

Phenocopies and disease heterogeneity are common and recognized complications to the development of a genetic test, however, explaining these aberrations to a breeder often causes more distress as the information can be quickly misrepresented and falsely spread in the breeder community. Genetic tests are generally developed within a particular breed or population, and then slowly expanded to other breeds and populations once scientific standards have been rigorously performed.

The newly developed PKD test for cats is an excellent example for defining standards for genetic test development. The Persian breed is one of the original breeds of the cat fancy and is recognized in a wealth of colors and varieties. Being an older breed, this may predict that Persians have been bottlenecked and inbred for a longer period of time than other cat breeds, suggesting a more inbred population. However, the large amount of color variations and varieties and the large worldwide popularity of the breed suggest a large foundation stock, less genetic loss by random chance, and increased migration, as compared to other breeds. Each of these aspects of the breed all lead to increased genetic diversity. Thus, although Persians are a breed, it is difficult to predict if a disease may be due to a single mutation that has descended and spread through the population, or if Persians are more similar to a Caucasian human population, which is large and outbred, and hence they may have more than one cause of a common phenotype.

Such is the concern with a disease like PKD, where it is known to be caused by several different genes and even different alleles within the same gene for a large outbred population, like humans. Several large pedigrees of Persian cats were used to identify the mutation for feline PKD. However, in the world of genetic testing, finding the mutation is only the first step. Although the pedigrees used to find the mutation were large, they represented cats from only a few Persian lines. Hence, the next task was to confirm that Persian PKD was caused by the same mutation in all Persians. All Persians not only implies cats in the United States, but cats from throughout the world. Thus, clinical and research veterinarians and testing facilities from throughout the world had to cooperate to confirm that the Persian PKD mutation was consistent throughout the world and what is considered and mutation that is "identical by descent".

Immediately upon the announcement of the PKD test, different cat breeds "came out of the woodwork" and expressed concerns about PKD. Several breeds represent Persian varieties, such as Exotic Shorthairs and Himalayans. These breeds can be clearly included in the genetic testing. Some other breeds are predictable candidates for testing, such as breeds that have recently used Persians, like Scottish Folds and Selkirk Rexes. But, when more esoteric breeds, such as Maine Coons, British Shorthairs, and Burmese, also came forward, the investigators now had to become familiar with not only breeding practices for a given association within their home country, but nearly every breeding practice for every association for every country. Only rigorous testing standards can help decipher the breed relationships and support the transfer of a genetic test to different breeds. One breed, the British Shorthair, has been examined in detail for PKD and has helped develop the standards used by our laboratory. This study required the interaction and cooperation of breeders, researchers and veterinarians from the United States, Australia and Europe.

Genetic Testing Standards

To enable the PKD test to be used in other breeds, our laboratory has set rigorous standards that consider the possibilities of phenocopies, disease heterogeneity, sporadic mutations, "de novo" mutations and breeding practices. In order to transfer a genetic test to a different breed, the following criteria have been established and we attempt to follow these standards before releasing tests for other breeds:

1) Standard clinical signs and diagnoses must first be confirmed. In the case of PKD, proof of cystic kidneys by ultrasound has been the gold standard. For PKD, the cysts need to be multiple in one kidney or present in both kidneys. Once these diagnoses were confirmed, samples were considered as "voucher" specimens for DNA testing.
2) The DNA test had to match the clinical diagnoses.
3) Once an individual was confirmed as positive by clinical and genetic diagnoses, related individuals were collected to confirm the mode of inheritance. This step is taken to help prove if a mutation is a new, sporadic event, or an inherited mutation. New sporadic events pose less risk to the population than an inherited mutation. Proving the mode of inheritance also supports that the disease is caused by the same mutation and potentially inherited from Persians.
4) A variety of cats from different lines need to be examined to support the test for the breed. For British Shorthairs, we were cautious as to whether this mutation was due to outcrossing with Persians, or a "*de novo*" mutation within the breed. Hence, some cats could have PKD from Persian crosses, while others have a new mutation. Some breeders perform unacceptable outcrosses, thus this step also helps to determine breed risk.
5) Families from new breeds were proven by parentage testing. Known and unknown accidents happen, thus, all research pedigrees are confirmed by parentage testing.
6) Some percentage of the breed must be examined. Hence, again, is this a localized problem, or an inherent problem within the breed. Different breeding practices for different associations and countries need to be considered. Hence, PKD in British Shorthairs from Australia may not imply that all British Shorthairs in other countries are at risk if Persians have not been a common outcross. This percentage is a slippery slope and should ideally represent the effective breeding population. This aspect of the testing may be the most difficult to establish.

7) The breed should have a clearly defined outcrossing program with cats that have a high risk for the disease, such as Persians, Exotic Shorthairs and Himalayans. Parentage testing should be used to confirm this outcrossing.

Genetic Assays

A genetic test is based on a mutation that causes a trait or markers that are highly associated with a trait. Associated markers inherently have a higher error rate than mutation tests and this information should be published by the facilities offering association tests. Many laboratory techniques can be efficiently used to assay for mutations and each technique have its own potential errors. Thus, even though a mutation test should be as close to 100% accurate as possible, the errors in the type of assay will lower the accuracy.

A complete review of different laboratory methods used for genetic testing is beyond the scope of this discussion, however, a few cases can be presented. Recently, the mutation that causes the "pointing" phenotype in Siamese and other breeds of cats has been published. This mutation disrupts a restriction enzyme site and hence the restriction enzyme, *MYL4*, can be used to assay for the mutation. Restriction fragment length polymorphisms are very routine and a low cost and low technology method for genetic testing. However, a Dominant white Siberian cat was tested for "points" and clearly should have been a "pointed" cat, however, this cat had yellow eyes. The dominant white would have masked the pointing phenotype, but all "pointed" cats should have blue eyes. Sequence analysis of this cat revealed that the cat had only one allele for "points" and the other allele wildtype, however, a different nucleotide in the restriction site was altered. This DNA variation did not cause any amino acid change, hence does not cause a phenotypic affect in the cat and is just a random DNA variant. Thus, the genetic sequencing of this cat proved that the Dominant white Siberian cat only carries points, which the genetic assay suggested the cat was pointed. In this particular case, coat and eye colors helped to identify a false positive test. In the case of other traits, these other clues may not be available, hence, the veterinarian needs to understand the possible error of a genetic test.

Direct sequencing is generally the "gold standard" for confirm DNA mutations, but is a laborious and expensive endeavor, thus most genetic tests are not routinely evaluated by sequencing. But even with sequencing and any other PCR-based assay, DNA variants in the primer regions may cause "allele drop-out" and hence cause an individual to appear homozygous when they are actually heterozygous, but the second allele has not been amplified. Various higher through-put assays are now preferred over RFLP testing, but in the end, the veterinarian needs to recognize that a mutation test will still have an error rate that is more associated with the testing method and the efficiency of the laboratory.

Basically, veterinarians should always ask about error rates and never accept 100% accuracy as an answer. Breeders and veterinarians should not be bashful about asking labs to reveal their methods, provide the supportive data and reveal their efficiency. With cat DNA testing in its infancy, strong efforts should be made by researchers, veterinarians and breeders to promote accurate testing by efficient testing facilities.

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Genetic Counseling and Breeding Management of Hereditary Disorders

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Overview of the Issue

With each new generation, breeders ask, "How can I continue my line and improve it?" Aside from selecting for conformation, behavior and general health, breeders must consider how they are going to reduce the incidence of whichever genetic disorders are present in their breed. There are no answers that will fit every situation. There are, however, guidelines to preserve breeding lines and genetic diversity while reducing the risk of producing animals that carry defective genes, or are affected with genetic defects.

Historically, genetic counseling has ranged from recommendations to not repeat a mating and outbreed, to recommendations to eliminate all relatives of affected animals from the breeding pool. Neither of these two extremes serves the best long-term interest of breeds. Repeated outbreeding to attempt to dilute detrimental recessive genes is not a desirable method of control. Recessive genes cannot be diluted; they are either present or not. Outbreeding can prevent the production of affected animals, but it will propagate and further disperse the detrimental recessive genes. In most purebred animal systems, breeders are working with closed studbooks. Breeders must consider how selection affects genetic diversity in the gene pool.

Practical genetic disease control recommendations will vary based on several factors including; the size of the breeding population, widely dispersed versus recently mutated defective genes, high frequency versus low frequency defective genes, the mode of inheritance, and the availability of tests for carriers.

With widely dispersed or high frequency defective genes, it must be recognized that carriers are spread across the gene pool. Eliminating unique breeding lines because some individuals carry a single defective gene may adversely affect gene pool diversity more than a process that allows a limited number of carriers to reproduce. Conversely, with recently mutated or low frequency defective genes, it is advisable to strictly limit breeding, so as to not spread the defective gene further in the population.

There are no breeding recommendations that will fit every situation. There are, however, guidelines that can be used to preserve breeding lines and genetic diversity while reducing the risk of producing carrier or affected individuals. Protocols for genetic counseling and breeding management of genetic disorders should be based on the known mode of inheritance, and the availability of genetic tests.

Without genetic tests, breeders can still reduce the carrier risk in their matings. A quality individual that is found to be a carrier can be retired from breeding and replaced with a quality offspring. The genes of the retired individual can thus be preserved through the selected offspring, but the carrier risk can be reduced by up to half. To further limit the spread of the defective gene, the offspring should be used in only a limited number of carefully planned matings, and should also be replaced with one or two representative offspring. With this vertical mating scheme, you are maintaining the good genes of the line, reducing the carrier risk with each generation, and replacing, not adding to the overall carrier risk in the breeding population.

If gene tests are not available, the storage of frozen semen is important for quality males with high-risk pedigrees. If tests evolve that can differentiate carrier from genetically normal animals, offspring from frozen semen matings can be reintroduced into the gene pool. Both DNA (from blood or cheek swabs) and semen should be stored to utilize this method.

Breeding Practices

We should understand how matings manipulate genes within breeding stock. First comes understanding dogs and cats as species, then as genetic individuals. There is little similarity between a Chihuahua and a Saint Bernard, or between a Himalayan and a Sphynx. However, we must understand that while

established breeds are separate entities among themselves, they all are genetically the same species. While a mating within a breed may be considered outbred, it still must be viewed as part of the whole genetic picture: a mating within an isolated, closely related, interbred population. Each breed was developed by close breeding and inbreeding among a small group of founding ancestors, either through a long period of genetic selection or by intensely inbreeding a smaller number of generations. This process established the breed's characteristics and made the individuals in it breed true.

Pure-breeds have closed stud books. This means that the diversity of genes in the breed is fixed. Genes cannot be gained through breeding, only lost. In some cat breeds, cats who meet the phenotypic standard of the breed may be introduced into the gene pool. This, of course is an added source of genetic diversity for the breed.

Tens of thousands of genes interact to produce a single individual. All individuals inherit pairs of chromosomes; one from the dam, and one from the sire. On the chromosomes are genes; so all genes come in pairs. If both genes in a gene pair are the same gene (for instance, “aa” or “AA”) the gene pair is called homozygous. If the two genes in a gene pair are unlike (for instance, “Aa”) the gene pair is called heterozygous. Fortunately, the gene pairs that make a cat a cat and not a dog are always homozygous. Similarly, the gene pairs that make a certain breed always breed true are also homozygous. Therefore, a large proportion of homozygous non-variable pairs - those that give a breed its specific standard - exist within each breed. It is the variable gene pairs, like those that control color, size and angulation that produce variations within a breed.

One method to gauge the genetic diversity of a population is to measure the average inbreeding coefficient (or Wright’s coefficient) for a breed. The inbreeding coefficient is a measurement of the genetic relatedness of the sire and dam. If an ancestor appears on both the sire and dam’s side of the pedigree, it increases the inbreeding coefficient. The inbreeding coefficient gives a measurement of the total percentage of variable gene pairs that are expected to be homozygous due to inheritance from ancestors common to the sire and dam. It also gives the chance that any single gene pair can be homozygous.

The types of matings chosen for breeding animals will manipulate their genes in the offspring, affecting their expression. Linebreeding is breeding individuals more closely related (a higher inbreeding coefficient) than the average of the breed. Outbreeding involves breeding individuals less related than the average of the breed. Linebreeding tends to increase homozygosity. Outbreeding tends to increase heterozygosity. Linebreeding and inbreeding can expose deleterious recessive genes through pairing-up, while outbreeding can hide these recessives, while propagating them in the carrier state.

Most outbreeding tends to produce more variation within a litter. An exception would be if the parents are so dissimilar that they create a uniformity of heterozygosity. This is what usually occurs in a mismating between two breeds, or a hybrid, like a Cockapoo. The resultant litter tends to be uniform, but demonstrates "half-way points" between the dissimilar traits of the parents. Such litters may be phenotypically uniform, but will rarely breed true due to the mix of dissimilar genes.

One reason to outbreed would be to bring in new traits that the breeding stock does not possess. While the parents may be genetically dissimilar, a mate should be chosen that corrects the breeding animal's faults but phenotypically complements its good traits. It is not unusual to produce an excellent quality individual from an outbred litter. The abundance of genetic variability can place all the right pieces in one individual. Many top-winning show animals are outbred. Consequently, however, they may have high heterozygosity and may lack the ability to uniformly pass on their good traits to their offspring. After an outbreeding, breeders may want to breed back to individuals related to their original stock, to attempt to solidify newly acquired traits.

Linebreeding attempts to concentrate the genes of specific ancestors through their appearance multiple times in a pedigree. It is better for linebred ancestors to appear on both the sire's and the dam's sides of the pedigree. That way their genes have a better chance of pairing back up in the resultant offspring. Genes from common ancestors have a greater chance of expression when paired with each other than when paired with genes from other individuals, which may mask or alter their effects.

Linebreeding on an individual may not reproduce an outbred ancestor. If an ancestor is outbred and generally heterozygous (Aa), increasing homozygosity will produce more AA and aa. The way to reproduce an outbred ancestor is to mate two individuals that mimic the appearance and pedigree of the ancestor's parents.

To visualize some of these concepts, the pedigree of a Gordon Setter, Laurel Hill Braxfield Bilye will be used. The paternal grandsire, CH Loch Adair Foxfire, and the maternal grandam, CH Loch Adair Firefly WD, are full siblings, making this a first-cousin mating. The inbreeding coefficient for a first cousin mating is 6.25%, which is considered a mild level of inbreeding.

In Bilye's pedigree, an inbreeding coefficient based on four generations computes to 7.81%. This is not significantly different from the estimate based on the first-cousin mating alone. Inbreeding coefficients based on increasing numbers of generations are as follows: five generations, 13.34%; six generations, 18.19%; seven generations, 22.78%; eight generations, 24.01%; ten generations, 28.63%; and twelve generations, 30.81%. The inbreeding coefficient of 30.81 percent is more than what you would find in a parent-to-offspring mating (25%).

The total inbreeding coefficient is the sum of the inbreeding from the close relatives (first cousin mating), and the background inbreeding from common ancestors deep in the pedigree. Such founding ancestors established the pedigree base for the breed. **The background inbreeding has far more influence on the total inbreeding coefficient than the first-cousin mating, which only *appears* to be its strongest influence.**

Knowledge of the degree of inbreeding in a pedigree does not necessarily help you unless you know whose genes are being concentrated. The relationship coefficient, which can also be approximated by what is called the *percent blood* coefficient, represents the probable genetic likeness between the individual whose pedigree is being studied, and a particular ancestor. It is a measurement of the average percentage of genes the individual and the ancestor should have in common.

We know that a parent passes on an average of 50% of its genes, while a grandparent passes on 25%, a great-grandparent 12.5%, and so on. For every time the ancestor appears in the pedigree, its percentage of passed-on genes can be added up and its "percentage of blood" estimated. **In many breeds, an influential individual may not appear until later generations, but then will appear so many times that it necessarily contributes a large proportion of genes to the pedigree.** This can occur in breeds, due either to prolific ancestors (usually popular sires), or a small population of animals originating the breed. Based on a twenty-five generation pedigree of Bilye, there are only 852 unique ancestors who appear a total of over twenty-million times.

In Bilye's pedigree, CH Afternod Drambuie has the highest genetic contribution of all of the linebred ancestors. He appears 33 times between the sixth and eighth generations. One appearance in the sixth generation contributes 1.56% of the genes to the pedigree. His total contribution is 33.2% of Bilye's genes, second only to the parents. **Therefore, in this pedigree, the most influential ancestor doesn't even appear in a five-generation pedigree.**

Foundation dogs that formed the Gordon Setter breed also play a great role in the genetic makeup of today's dogs. Heather Grouse appears over one million times between the sixteenth and twenty-fifth generations, and almost doubles those appearances beyond the twenty-fifth generation. He contributes over ten percent of the genes to Bilye's pedigree. Any detrimental recessive genes carried by Heather Grouse or other founding dogs, would be expected to be widespread in the breed.

The average inbreeding coefficient of a breed is a measurement of the breed's genetic diversity. When computing inbreeding coefficients, you have to look at a deep pedigree to get accurate numbers. An inbreeding coefficient based on 10-generation pedigrees is standard, but requires a computerized pedigree database to compute.

The average inbreeding coefficient for a breed will be based on the age and genetic background of the breed. A mating with an inbreeding coefficient of 14 percent based on a ten generation pedigree, would be considered moderate inbreeding for a Labrador Retriever (a popular breed with a low average

inbreeding coefficient), but would be considered outbred for an Irish Water Spaniel (a rare breed with a higher average inbreeding coefficient).

Looking at the historical pedigrees of Bull Terrier breeding dogs (males and females that have five or more registered offspring), we find that for dogs born in the decade 1970-1979, the average ten generation inbreeding coefficient was 23.11% +/- 6.04%. For Bull Terriers born 1980-1989, this number is 21.54% +/- 5.69%. For 1990-1999, the average inbreeding coefficient is 19.01% +/- 6.23. It is obvious that the 10 generation inbreeding coefficient of the Bull Terrier breed is going down with each decade. This shows that the breeders are utilizing the diversity of the gene pool, and not breeding themselves into a corner with popular sires.

Of course, the actual diversity of genes and inbreeding in the breed is not going down It is just that the earlier ancestors producing background inbreeding are falling beyond the 10^{th} generation, and are no longer included in the computation. As long as the health and vitality of the breed is being maintained, and there is no epidemic of breed-related disease from detrimental recessives, this pure-bred population should be able to be maintained.

Most breeds start from a small founding population, and consequently have a high average inbreeding coefficient. If the breed is healthy and prolific, the breadth of the gene pool increases, and the average inbreeding coefficient can go down over time. Some dog breeds were established on a working phenotype, and not on appearance. These breeds usually start with low inbreeding coefficients due to the dissimilar backgrounds of the founders. As certain quality individuals are linebred on to create a uniform physical phenotype, the average inbreeding coefficient can increase.

There is no specific level or percentage of inbreeding that causes impaired health or vigor. If there is no diversity (non-variable gene pairs for a breed) but the homozygote is not detrimental, there is no effect on breed health. The characteristics that make a breed reproduce true to its standard are based on non-variable gene pairs. There are pure-bred populations where smaller litter sizes, shorter life expectancies, increased immune-mediated disease, and breed-related genetic disease are plaguing the population. In these instances, prolific ancestors have passed on detrimental recessive genes that have increased in frequency and homozygosity. With this type of documented inbreeding depression, it is possible that an outbreeding scheme could stabilize the population. However, it is also probable that the breed will not thrive without an influx of new genes; either from a distantly related (imported) population, a natural landrace population, or crossbreeding.

Diversity Issues

Fortunately, most breeds do not find themselves in the position of this amount of limited diversity and inbreeding depression. However, the perceived problem of a limited gene pool has caused some breeders to advocate outbreeding of all individuals. Studies in genetic conservation and rare breeds have shown that this practice actually contributes to the loss of genetic diversity. By uniformly crossing all “lines” in a breed, you eliminate the differences between them, and therefore the diversity between individuals. Eventually, there will not be any “unrelated line” to be found. Everyone will have a mixture of everyone else’s genes. This practice in livestock breeding has significantly reduced diversity, and caused the loss of unique rare breeds.

A fallacy of using outbreeding to maintain genetic diversity is the belief that the diversity of a breed must be maintained in every single animal. Breeders must concentrate on the specific goals of breeding (selecting for the health and quality of the breed), versus the tools used to get there (outbreeding, linebreeding, etc.) Selecting breeding stock simply to produce the lowest possible inbreeding coefficient is not a goal that will guarantee a quality animal. Animals who are poor examples of the breed should not be used simply to maintain diversity. Related individuals with desirable qualities will maintain diversity, and improve the breed.

The process of maintaining healthy “lines” or families of animals, with many breeders crossing between lines (outbreeding) and breeding back (linebreeding) as they see fit maintains diversity in the gene pool. **It is the varied opinion of breeders as to what constitutes the ideal representative of the breed, and their selection of breeding stock that maintains breed diversity.**

A basic tenet of population genetics is that gene frequencies do not change from the parental generation to the offspring. This will occur regardless of the homozygosity or heterozygosity of the parents, or whether the mating is an outbreeding, linebreeding, or inbreeding. This is the nature of genetic recombination. Selection, and not the types of matings used affect gene frequencies and breed genetic diversity.

If two parents are both heterozygous (both Aa) for a gene pair, on the average, they would produce 25% AA, 50% Aa, and 25% aa. (These are averages when many litters are combined. In reality, any variety of pairing up can occur in a single litter.) If a prolific male comes out of this litter, and he is homozygous aa, then the frequency of the "a" gene will increase in the population, and the frequency of the "A" gene will decrease. This is known as the popular sire syndrome. Of course, each individual has thousands of genes that vary in the breed, and everyone carries some deleterious recessive genes. The overuse of individual breeding animals contributes the most to decreased diversity (population bottlenecks), and the increased spread of deleterious recessive genes (the founders effect). Again, it is selection (use of this stud to the exception of others), and not the types of matings he is involved in that alters gene frequencies. Breeders should select the best individuals from all lines, so as to not create new genetic bottlenecks.

Decisions to linebreed, inbreed or outbreed should be made based on the knowledge of an individual's traits and those of its ancestors. Inbreeding will quickly identify the good and bad recessive genes the parents share, based on their expression in the offspring. However, unless there is prior knowledge of what the offspring of milder linebreedings on the common ancestors were like, the litters (and buyers), may be exposed to extraordinary risk of genetic defects. In matings, the inbreeding coefficient should only increase because of specific linebreeding (increasing the percentage of blood) to selected ancestors.

Breeders should not set too many goals in each generation, or the selective pressure for each goal will necessarily become weaker. Genetically complex or dominant traits should be addressed early in a long-range breeding plan, as they may take several generations to fix. Traits with major dominant genes become fixed more slowly, as the heterozygous (Aa) individuals in a breed will not be readily differentiated from the homozygous-dominant (AA) individuals. Desirable recessive traits can be fixed in one generation because individuals that show such characteristics are homozygous for the recessive genes. Individuals that pass on desirable traits in numerous matings and generations should be preferentially selected for breeding stock. This prepotency is due to homozygosity of dominant (AA) and recessive (aa) genes. However, these individuals should not be overused, to avoid the popular sire syndrome.

Breeders should plan their matings based on selection toward a breed standard, based on the ideal temperament, performance, and conformation, and should select against the significant breed related health issues. Using progeny and sib-based information to select for desirable traits and against detrimental traits will allow greater control.

Breeding Recommendations to Manage Genetic Disease

Based on the mode of inheritance of a disorder, and the availability of genotypic or phenotypic genetic tests, breeding management recommendations can be used to prevent or reduce the frequency of carrier or affected offspring.

Autosomal Recessive Disorders

In the case of a simple autosomal recessive disorder for which a test for carriers is available, the recommendation is to test breeding-quality stock, and breed carriers to normal-testing individuals. This prevents affected offspring from being produced. The aim is to replace the carrier breeding-animal with a normal-testing offspring that equals or exceeds it in quality. Breeders may not produce this offspring in a single mating, and may still have to breed another carrier in the next generation, as long as it is again bred to a normal testing animal. Breeders don't want to diminish breed diversity by eliminating quality animals from the gene pool because they are carriers. Additional carrier testing offspring should not be placed in breeding homes; as the goal is to reduce the frequency of the defective gene in the population. As each breeder tests and replaces carrier animals with normal-testing animals, the problem for the breed as a whole diminishes.

The problem with simple autosomal recessive disorder for which no carrier test exists is the propagation and dissemination of unapparent carriers in the gene pool. Breeders must assess whether each individual animal in their breeding program is at high risk of being a carrier. This requires knowledge of the carrier or affected status of close relatives in the pedigree. An open health registry that is supported by the parent club makes it easier for breeders to objectively assess these matters. By determining the average carrier-risk for the breeding population, breeders can select matings that have a projected risk that is lower than the breed average.

If a breeding animal is at high risk of being a carrier, the best advice is to breed to an individual that has a low risk. This will significantly diminish the likelihood that affected animals will be produced, and can reduce by up to half the risk that there will be carriers among the offspring. Using relative-risk assessment as a tool, breeders should replace higher-risk breeding animals with lower-risk offspring that are equal to or better than their parents in quality. A negative aspect of pedigree analysis is that it selects against families, regardless of an individual's normal or carrier status. On the other hand, it allows for the objective risk assessment and continuation of lines that might otherwise be abandoned due to high carrier-risk.

Breeding an individual only once and replacing it with an offspring allows breeders to improve their chances of moving away from defective genes and also limits the dissemination of defective genes. When dealing with disorders for which carriers cannot be identified, the number of offspring placed in breeding homes should be kept to a minimum.

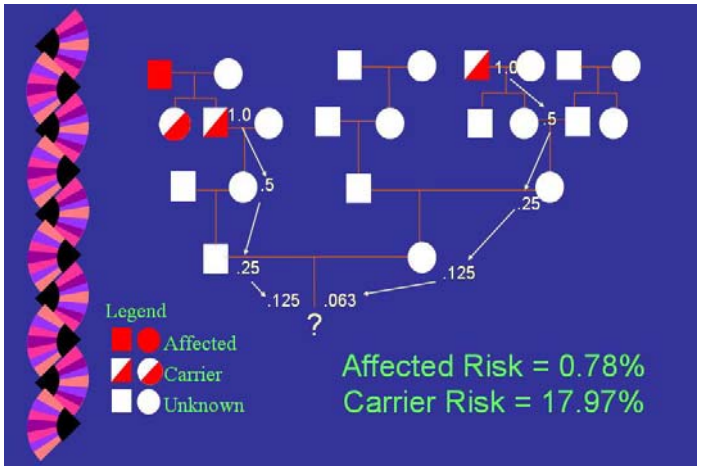
Autosomal Dominant Disorders

Autosomal dominant genetic disorders are usually easy to manage. Each affected animal has at least one affected parent, but it can be expected that half of the offspring of an affected animal will be free of the defective gene. With disorders that cause death or discomfort, the recommendation is to not breed affected animals. To produce the next generation of a line, a normal full sibling of an affected animal can be used, or the parent that is normal can be used.

A problem with some autosomal dominant disorders is incomplete penetrance. In other words, some individuals with the defective gene may not show the disorder. Roughly half their offspring, however, may be affected. If a genetic test is available, this is not a problem. Otherwise, relative-risk assessment can identify which individuals are at risk of carrying incompletely penetrant dominant genes.

Sex-Linked Disorders

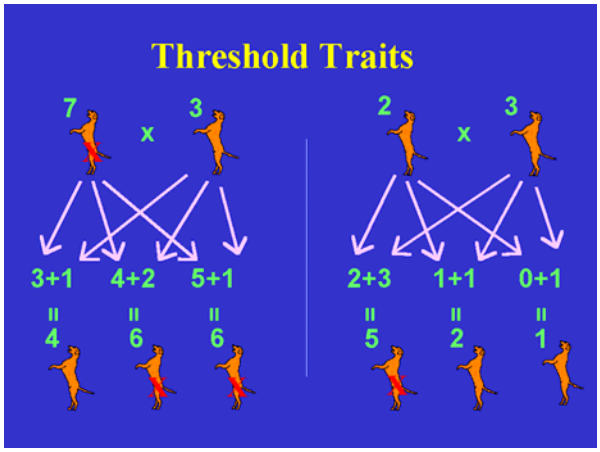
For sex-linked (also known as x-linked) recessive defective genes for which carrier tests exist, breeders should follow the same "breed and replace" recommendations as are outlined above in the discussion of autosomal recessive disorders. If there is no test, the defective gene can be traced through the pedigree. If a male is affected, he would have received the defective gene from his carrier mother. All of his daughters will be carriers, but none of his sons. By using relative-risk assessment to breed him to a female that is at low risk of being a carrier, you can prevent affected offspring, and select a quality son for replacement.



There are rare instances in which a female is affected with a sex-linked disorder. In such cases, she would have received the defective gene from both parents; specifically, an affected father and a mother who is either a carrier or is affected herself. If an affected female is bred, all the sons will be affected, and all the daughters would be carriers, so affected females clearly should not be bred. A normal male that is a littermate to an affected female, however, would be able to carry on the line without propagating the defective gene. Rare sex-linked dominant disorders are managed the same way as autosomal dominant disorders. The difference is that affected males will *always* produce all affected daughters.

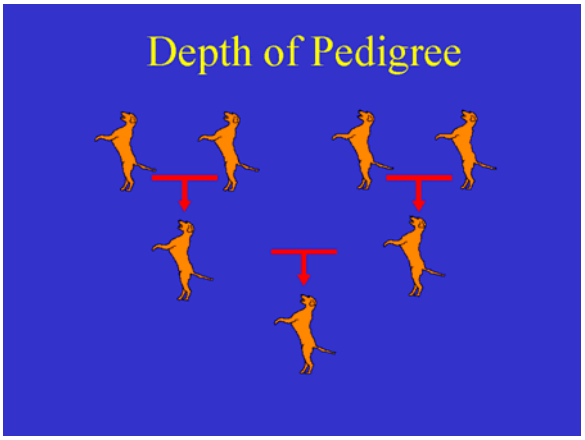
Polygenic Disorders/Complex Inheritance

To manage polygenically controlled disorders genetically, they must be considered as threshold traits. A number of genes must combine to cross a threshold producing an affected individual. As the presence of individual genes may not be all that is required to express the defect, these genes are considered liability or susceptibility genes. If phenotypically normal parents produce affected offspring, both should be considered to carry a subclinical genetic load of liability genes that combined to cause the disorder. In the theoretical mating above), consider that five additive hip dysplasia liability genes must combine to produce an affected dog.

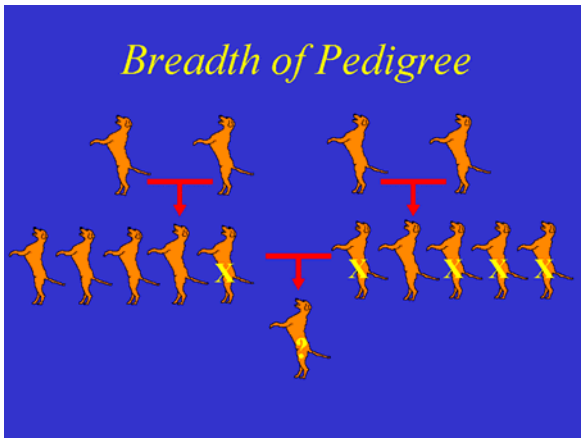


In addition to quantitative or additive genes, many polygenic disorders can have a major recessive or dominant qualitative gene that must be present to produce an affected individual. Molecular genetic research to identify these genes can allow better management of polygenic disorders such as hip dysplasia, epilepsy, and congenital heart anomalies. If one exists, the "trigger" gene in one breed or family may be different from the gene in others. Consequently, the development of a genetic test in one breed may not prove useful in all breeds.

In polygenic disorders, all individuals are not affected due to the same gene combinations. Breeders must break down affected phenotypes into traits that more directly represent the genes that control them. As environmental aspects also affect the expression of many polygenic disorders, they too need to be identified and controlled.



The primary reason for diminished progress against polygenic disorders is that breeders have been selecting for depth of pedigree; generations of phenotypically normal parents and grandparents. In polygenic disorders, the phenotype of the individual does not directly represent its genotype. The phenotype of the full brothers and sisters more directly represent the range of genes present in the breeding individual. In other words, with polygenic disorders the breadth of the pedigree (that is, consideration of all siblings of individuals in the pedigree) is as important, if not more important than the depth of the pedigree (consideration only of parent-offspring relationships). This can be evaluated through open health registries, such as CHIC (www.caninehealthinfo.org).



Phenotypically normal individuals from litters with a high incidence of disease are expected to have a higher compliment of liability genes that is closer to the disease threshold than the average for the breed. This is why it is important to screen both pet and breeding animals from litters for polygenic disorders. By counseling owners to select for breadth of phenotypically normal littermates of breeding animals, and of parents of breeding animals, all breeds should realize a decrease in polygenically controlled disorders. In addition, the offspring of breeding individuals should be monitored to see which are passing the disorder with higher frequency. By

evaluating all of these aspects of polygenic disorders, we are helping breeders produce healthier animals.

Unknown Inheritance
For disorders without a known mode of inheritance or carrier test, breeders should be counseled to use the same control methods as with polygenic disorders. Animals with a low genetic load for the disorder should be selected for breeding, through the results of examinations of first-degree relatives (littermates, parents, and offspring). If there are multiple generations of normalcy in the breadth of the pedigree, then you can have some confidence that there is less risk that liability genes are being carried. Quality individuals with higher risk should be bred to individuals with lower risk, and replaced with a quality offspring. This recommendation should be repeated in the next generation, to further lower the risk of producing affected or carrier animals.

Putting Genetic Counseling into Practice
In 2004, an article was published in the journal Preventative Veterinary Medicine (Vol. 63, pages 39-50) that discussed a genetic counseling program for Boxer breeders in the Netherlands. Objective breeding and risk values were computed for proposed matings submitted by bitch owners based on relative risk assessment of four genetic disorders: cryptorchidism, epilepsy, knee-problems (ACL, meniscus, osteo-arthritis), and cleft lip or palate.

The results showed that the combined risk values for all four disorders together had no effect on sire selection. Based on epilepsy alone, the lowest risk sire-dam combinations were selected three times as frequently than others. However, there was no significant effect on selection based on the other disorders.

When questioned about their selection choices, many breeders stated that they were familiar with the backgrounds of the dogs selected, and questioned the higher, objectively determined risk assessments. Many also questioned the genetic basis of knee problems and overestimated the environmental aspects, even though a genetic basis has been established in the Boxer breed (Am J Vet Res. Aug 2001;62(8):1198-1206). Breeders were more likely to blame the sire when high risk assessments for a certain dam-sire combination were calculated. Although most breeders argued for open communication about health problems, they were reluctant to have risk assessments published with the dog's id-number.

As evidenced from the above study, it is obvious that it will take a change in mindset of breeders to effectively deal with genetic disorders. The acceptance of objective scientific data, removing the stigma of genetic disorders, and a willingness to use open health databases will allow progress with managing genetic disease.

It is distressing to breeders when a genetic disorder is confirmed. Positive and practical genetic counseling recommendations will maintain breed lines and genetic diversity, and improve the overall health of breeds. The total elimination of defective genes will probably be impossible for most breeds. The use of these guidelines can assist breeders in making objective breeding decisions for genetic-disease management, while continuing their breeding lines. The individual breeder can use genetic tests to; 1) identify carriers, 2) work to breed away from the defective gene(s), and 3) ensure (through testing) that the defective gene(s) is not reintroduced in future matings. Each breeder will have their own rate of progress, depending on the frequency of the defective gene(s) in their own breeding animals, and which desirable individuals are carriers.

Genetic Relationships of Cat Breeds

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Overview of the Issue

Cat Diversity and Domestication

Despite the fact that nearly 70 million cats inhabit US households (U.S. Pet Ownership & Demographics Sourcebook, AVMA, 2002), little is known about the origins of the domestic cat. Equally scarce is information regarding the origins of the various cat breeds and their relationship to each other. The cat was more recently domesticated than most other companion animals and livestock species, but no molecular studies have been focused on deciphering domestic feline origins. The popularity of cats in Egyptian societies has lead to the assumption that cats were domesticated in Africa. Archeological evidence and early writings, however, place domestic cats in Asia and Eastern Europe prior to the rise of the cat in the Egyptian Empire. Recently, intact remains of a cat were identified at a Neolithic human burial site in Cyprus, which was dated at approximately 9,000 years ago, 5,000 years prior to the rise of cats in Egyptian culture [1]. Our objectives are to determine the domestication site of the cat, the relationship of wildcat populations to modern populations and breeds, and the population structure of modern cat breeds. We hypothesized that the cat was initially domesticated by civilizations in the Fertile Crescent, as a means of rodent control for crop stores and refuse sites.

Possible Progenitors: the Wildcats

The wildcat (*Felis sylvestris* Schreber, 1777) is commonly defined by four subspecies [2], the domesticated subspecies (*F. s. catus* Linnaeus, 1758), and the African *(F. s. libyca* Foster, 1780), European (*F. s. silvestris*) and Asian (*F. s. ornata* Gray, 1830) wildcats, however, many other sub-species have been noted and due to their widespread distribution, the taxonomy of wildcats is still under debate although domestication events have been proposed. The phylogeny of felids [3-5] has been investigated, however, these studies did not focus on the most recent relationships of modern domestic cats to their progenitors. More recent morphological and genetic analyses have shed some light on the relationships of small European and African wildcats, and wildcats to domesticated cats, although the major focus of these studies have been on introgression and hybridization of wildcats with domestic cats and the resulting concerns with wild population conservation.

Cats in Archeology

Skull and jaw dimensions are currently the most distinguishing features between wild and domestic cat [6], but many paleontological sites do not have sufficient remains for definitive recognition. Europe has several Pleistocene sites with small cat remains (1). These remains are considered to be wildcats because they are found among other non-domesticated carnivore remains. Teeth were found in Jericho, dated to ~9000 BC and ~4000 BC in the Indus Valley (1) but distinctions between wild or domestic cat could not be made. More recent archaeological material has been recovered from sites where suggestions of domestication are already apparent. Intact remains of a cat were identified at a Neolithic human burial site in Cyprus, which was dated at approximately 9,000 years ago, 5,000 years prior to the rise of cats in Egyptian culture. The discovery of skeletal remains consistent with the size of *F. sylvestris*, dating from ~7,000 BC on Cyprus, implies that cats were traveling on ships with humans. Furthermore, the ritualistic positioning of the cat next to a human burial suggests possible cultural or religious importance of cats to the people inhabiting Cyprus at that time.

Egyptian feline remains are abundant and well documented [7]. The earliest cat representations and mummies date to the 12th Dynasty (1991 - 1778 BC). Cats became a very popular entombment companion in the Ptolemaic Dynasty, ~320 BC, and were specifically bred for mummification.

Artwork and writings show strong evidence that the cat began to have a role in many societies, perhaps independently, throughout the Old World (1, 20). Sanskrit writings from India describe small cats as early

as ~3000 BC. Chinese cultural works present cats at ~2000 BC with clear domestication circa 400 AD. Domestic cats are considered to have entered Japan at ~1000 AD from China. In Greece, the appearance of cats in artwork is evident at 1500 BC. Thus, Egyptians may have domesticated cats and/or domesticated cats may have been brought to Egypt via trade routes.

Contemporary Domestication: Development of Modern Breeds

Historical information of modern breed development identifies several once geographically-isolated populations that represent the earliest divergences of the domestic cat species, *F. catus*, and/or possible introgressive hybrids between *F. catus* and *F. sylvestris.* Darwin noted in 1868 that cats from South America and India were different from those found in England (21). Abyssinians were disputed to have been Ethiopian- or British-derived; and Persians were putatively described as having Pallas cat progenitors, *F. manul* (22). The first cat show was held at the Crystal Palace in England in 1871. At this time, British, Persian, Manx, Siamese, Angora and Russian Blue breeds were highly recognizable and distinctive. These early breeds were recognized prior to establishment of the National Cat Club in 1887, which initiated strong and formal breeding practices, thus they represent geographical distinctions. Along with the Maine Coon, an American foundation breed, these breeds were the first cats to be presented at shows in the United States. As the cat fancy has increased in popularity over the past century, the number of defined breeds has also increased (22). Therefore, some breeds that have been recognized over the past 100 years may truly represent geographical isolates while others do not. Some breeds are clearly new mutations that have occurred in recognized populations. Many recently designated breeds have been produced by creative husbandry practices. There are over forty recognized cat breeds, approximately 50% of these can be identified as potential geographical isolates, useful for estimating ancient migration patterns and gene flow. These isolated breeds may reflect several different domestication events, or they may trace to only one event from a single small wildcat species.

Mitochondrial DNA

Due to its non-recombining nature and fast mutation rate mitochondrial DNA is the perfect molecule for studying genealogies of recently diverged populations, such as the domestic cat breeds. We have successfully amplified a 474 bp fragment of the 5' portion of the mtDNA control region in domestic breeds and random bred populations. To date, 149 individuals from 17 breeds and 90 random bred cats have been sequenced. Thirty different haplotypes have been identified, twelve haplotypes were unique to a breed and not found in the random bred population (**Table 1**). However, the random bred population represents cats from only southern California. Nine haplotypes were unique to the random bred cats, although this number is likely to be reduced as more pure bred cats are sampled. Three breeds had more than one unique haplotype. Overall, the preliminary data shows a large amount of diversity in both random bred cats and pure bred cats. The Abyssinian breed, however, seems to have low diversity despite its reputed ancient origins and relatively large population.

Table 1. Mitochondrial DNA haplotypes of cats breeds and random breds.

Breed	N	Haplotypes	Breed	N	Haplotypes
Abysinnian	13	26, 29	Norwegian Forest Cat	16	6, 17, 26, 29
American Shorthair	10	**9, 25**, 26	Persian	10	6, **7**, 17, 26
Birman	10	29	Russian Blue	5	6, 26, 29
British Shorthair	6	26	Siamese	14	5, 6, **15, 16**, 26, 29
Chartreux	4	6, 29	Scottish Fold	2	1
Egyptian Mau	2	29	Selkirk Rex	1	10
Japanese Bobtail	11	**8,** 20, 26, 29	Turkish Angora	2	**11**, 20
Korat	9	**2**, 29	Turkish Van	4	**13**
Maine Coon	25	**14, 22**, 26, **27**, 29			
Random Bred	90	**3**, **4**, 5, 6, 10, **12**, **18**, **19**, 20, **21**, **24**, 26, **28**, 29, **30**			

Haplotypes in **bold** are unique to that breed/population.

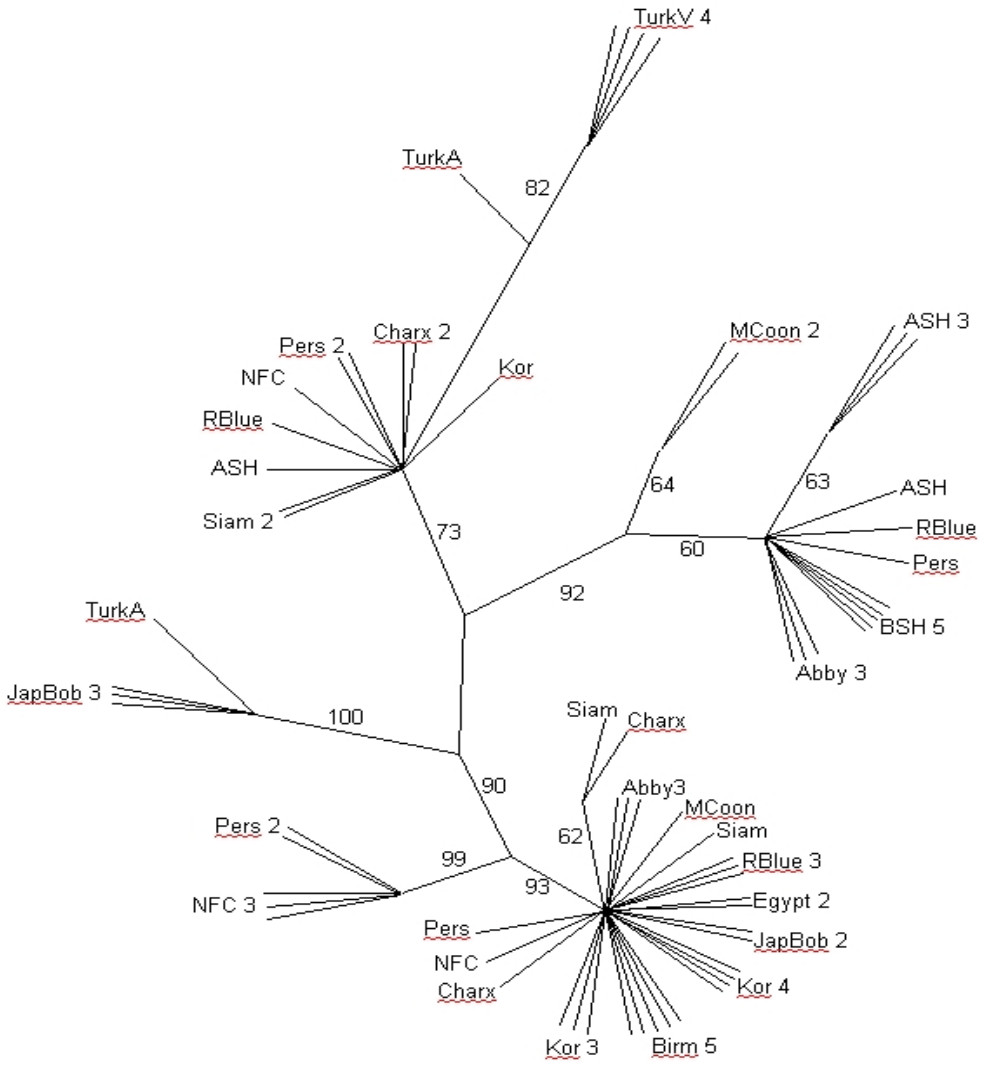

Figure 1. Unrooted mitochondrial neighbor-joining tree of 65 individuals representing 15 breeds. Bootstrap values greater than 60 are represented on internal branches. Values following names indicate the numbers of a specific breed in that cluster.

A neighbor-joining tree (**Figure 1**) was constructed using a subset of the samples. As expected, individuals from some of the older foundation breeds, such as the Persian and Russian Blue, are present in more than one cluster thus exhibiting more genetic diversity. Another observation is that all of the Turkish Angoras examined to date have the same, apparently unique, haplotype. This observation agrees with the historical account of the breed as being a small, geographically isolated population. More interesting insights are expected to emerge as more individuals, especially the different subspecies of wildcats, are added to the analysis.

Microsatellites

Microsatellite markers have long been used in population based studies and have recently proven to be useful in distinguishing between breeds [8]. These markers mutate at very high rates and therefore are useful when studying recent diversification events. Surveying 20 breeds and several random bred populations, we have found there to be high levels of genetic variation. The PIC values range from 0.5 – 0.86, and private alleles are present in many populations (data not shown).

Phylogenetic analysis on the microsatellite data has shown some interesting trends as pertaining to cats of African origin. ANeighbor-Joining tree constructed using Nei's genetic distance groups the feral cat population from the islands of Faza and Lamu (off the coast of Kenya) with both the African and European Wildcats, supporting the theory of the ancient origins of these island cats. A subsequent analysis using a Bayesian clustering algorithm as implemented in the software Structure was performed [9]. Here the Lamu cats were analyzed separately from the cats on the neighboring island of Faza. Seven of the ten Lamu cats analyzed formed a strong cluster along with a single Sokoke cat. Historically the Sokoke breed is said to have originated from feral Kenyan cats and our results support the theory that at least some of the cats in this breed show strong association with feral African cats.

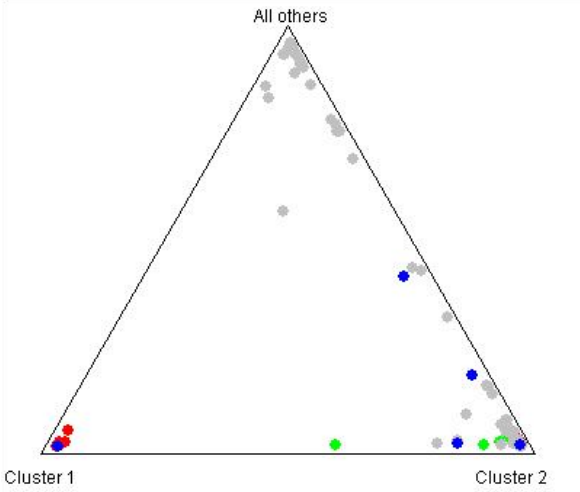

Figure 2. Triangle plot. Lamu cats are in red, Sokoke in blue, and Faza in green. All other cats are in grey.

Conclusion

Our preliminary results clearly show the usefulness of both mitochondrial DNA and microsatellite markers as tools for elucidating the genetic structure and genealogy of such recently diverged groups as domestic cat breeds. Our future efforts are focused on expanding our samples and genetic markers. One genetic marker system of interest is the Y chromosome. Our exploration of the genetic information contained on the cat Y chromosome has been slowed by the poor understanding of the Y chromosome in most companion animals.

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Inherited Kidney Diseases in Dogs and Cats

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Overview of the Issue

Inherited kidney diseases have been recognized in several breeds or kindreds of dogs and cats, and more examples of such conditions are likely to be identified as renal diseases that occur in a familial pattern are investigated thoroughly. In affected breeds/kindreds, accurate diagnosis of familial nephropathies is especially important because of the potential health and selective breeding implications for related animals. Additionally, because juvenile onset of chronic renal failure is the most common consequence of familial renal disease, discovery of kidney disease in a young dog or cat often evokes concern about whether the condition is congenital or acquired, and if it is congenital, whether or not it is inherited.

More familial renal diseases have been described in dogs than in cats, but polycystic kidney disease in cats likely is the single most common inherited nephropathy that occurs in these companion animal species worldwide. The main categories of familial nephropathies are listed in Table 1. To date, the pathogenesis and causative gene defect have been determined for only a few inherited kidney diseases in dogs and cats. However, the pace of progress in this field is accelerating rapidly because of advancing technology and increasing availability of genetic information regarding these species.

Most familial renal diseases are progressive and ultimately fatal, although the rate of progression often varies considerably among individuals with the same disorder. Therapeutic efforts generally are focused on combating complications (e.g., hypertension, urinary tract infection) as they arise and using conventional strategies for medical management of chronic renal failure to minimize disease progression and uremia.

Clinical findings

The clinical syndrome produced by most familial nephropathies is chronic renal failure, which often develops while the animals are adolescents or young adults. In dogs with renal dysplasia and some primary glomerulopathies, onset of renal failure usually occurs at 3 months to 3 years of age, with peak occurrence at about 1 year of age. However, many familial kidney diseases often produce renal failure somewhat later in life. For polycystic kidney disease, some primary glomerulopathies, amyloidosis, and glomerulonephritis, onset of renal failure often is at 3-7 years of age, depending on the condition.

Reduced appetite or anorexia, stunted growth or weight loss, polyuria and polydipsia, and vomiting are the most common clinical signs in dogs and cats with renal failure due to a familial nephropathy. Other signs that are often reported include poor hair coat, halitosis, and diarrhea.

Physical examination findings often include thin body condition, dehydration, mucous membrane pallor, uremic breath odor, and oral ulceration. Fibrous osteodystrophy or rubber jaw is occasionally observed, mainly in dogs that develop renal failure before 6 months of age. The kidneys of animals, especially cats, with polycystic kidney disease often are palpably enlarged. Otherwise, the kidneys usually are normal or reduced in size. Dogs with severe renal dysplasia often have especially small kidneys.

Laboratory testing most often reveals the expected abnormalities associated with chronic renal failure, especially impaired urine concentrating ability, azotemia, hyperphosphatemia and nonregenerative anemia. These findings usually reflect the severity of the animal's renal failure independent of its cause. Urinalysis findings, however, frequently help discriminate among the common causes of juvenile or familial nephropathy. Dogs with primary glomerulopathies and familial glomerulonephritis consistently have persistent renal proteinuria that emerges early in the course of disease and typically is of high magnitude (UPC ≥ 2). Dogs with renal dysplasia and dogs and cats with polycystic kidney disease usually exhibit little or no proteinuria, while proteinuria is an inconsistent finding that depends on the extent of glomerular involvement in dogs and cats with familial amyloidosis. Renal glucosuria is a consistent feature of the Fanconi syndrome in Basenji dogs but occasionally is observed in dogs with renal dysplasia or primary glomerulopathies. Bacterial urinary tract infection also sometimes develops as a secondary complication in dogs and cats with juvenile or familial nephropathies.

Diagnostic renal imaging is most helpful for animals with polycystic kidney disease in which a definitive diagnosis can be made by finding multiple cysts distributed in both kidneys using ultrasonography. In dogs with renal dysplasia, ultrasound can demonstrate abnormal size, shape, and sonic architecture of the kidneys, but it cannot distinguish renal dysplasia from other possible causes of small, fibrotic end-stage kidneys in affected dogs.

Diagnosis

For specific kidney diseases that are known or suspected to be inherited in particular breeds, diagnosis of the condition generally rests on recognition of the expected clinical features, exclusion of other conditions that might produce similar signs, and ultimately upon identification of characteristic renal lesions.

The exclusion of other disorders (especially those that are potentially treatable) is a key step because a variety of acquired diseases may occur in the same breeds and age-groups of animals that might have familial nephropathies. Careful interpretation of the results obtained from a thorough clinical investigation (history, physical exam, blood pressure determination, complete urinalysis, urine culture, and appropriate diagnostic imaging) often is adequate for presumptive diagnosis of a familial nephropathy. Even when the diagnosis remains uncertain, however, such an investigation generally is sufficient to properly guide the animal's medical care. Nonetheless, definitive diagnosis of many familial nephropathies ultimately rests upon identification of characteristic lesions in kidney specimens obtained at necropsy or by biopsy.

Light microscopic examinations are sufficient for many disorders, but especially for glomerular diseases, transmission electron microscopic and immunopathologic studies often are needed as well. Prior planning generally is needed to assure that specimens that will be suitable for these specialized evaluations are obtained when the tissue is collected because special materials and procedures are required. Centers that perform such studies should be contacted for guidance.

In breeds or families known to be at risk for certain familial nephropathies, apparently healthy animals can screened with tests that will aid early identification of affected individuals. The foremost examples of such screening are the use of ultrasonography to identify polycystic kidney disease and urinalyses to detect persistent renal proteinuria in animals that are at risk for glomerular disorders.

When a juvenile nephropathy is identified, questions about inheritance of the condition frequently arise. For breeds in which the specific nephropathy that has been diagnosed is known to be inherited, genetic counseling can be provided. In most other circumstances, heritability of the condition remains unknown unless or until studies of related animals show a familial pattern of disease occurrence.

Selected specific disorders

Renal dysplasia

Renal dysplasia is defined as disorganized development of renal parenchyma that is due to abnormal differentiation. For definitive diagnosis of this category of conditions, microscopic observation of structures in the kidney that are inappropriate for the stage of development of the animal is required. The presence of immature glomeruli and tubules usually within radial bands adjacent to more normally developed tissue (*i.e.*, asynchronous differentiation of nephrons) is the most consistent feature. Other findings indicative of renal dysplasia include persistent immature mesenchyme, persistent metanephric ducts, atypical tubular epithelial proliferation, and (albeit rare in dogs) dysontogenic metaplasia. Secondary changes that are commonly observed include compensatory hypertrophy and hyperplasia of glomerular tufts and tubules, interstitial fibrosis, tubulointerstititial nephritis, pyelonephritis, dystrophic mineralization, cystic glomerular atrophy, microcystic tubules, retention cysts, and glomerular lipidosis.

Renal dysplasia is most extensively reported and presumed to be familial in Lhasa Apso and Shih Tzu dogs. Other breeds in which published reports have suggested that renal dysplasia occurs in a familial pattern are listed in Table 1. Additionally, juvenile nephropathies with microscopic features of renal dysplasia have been reported in one or more unrelated dogs of so many different breeds that it seems likely that the disorder occurs at least sporadically in all breeds. The causes and pathogenesis of canine renal dysplasia are unknown. Renal dysplasia is widely accepted to be the same disease entity in both Lhasa Apso and Shih Tzu dogs, but the whether the other familial or sporadic forms of renal dysplasia are fundamentally the same disease or different diseases having similar adverse effects on development of the kidneys in affected dogs is uncertain. To date, data documenting the validity of genetic testing for renal dysplasia have not been published for any breed.

Primary glomerulopathies

A number of primary glomerulopathies, including several of the most well characterized inherited renal diseases of dogs, have been described. Conditions in which an abnormality of the type IV collagen in the glomerular basement membrane (GBM) is known or suspected to cause the disease lead this category. All basement membranes contain collagen IV, but in the GBM, a special collagen network containing the α3, α4, and α5 chains of type IV collagen is crucial for long-term maintenance of normal structure and function of the glomerular capillary wall. When this α3-α4-α5(IV) network is not formed properly, the GBM deteriorates and initiates a process of progressive renal injury that leads to chronic renal failure. These conditions are analogous to the kidney disease that occurs in human Alport syndrome, which is a genetically and clinically heterogenous group of diseases. In dogs as in people, the mode of inheritance can be X-linked, autosomal recessive, or autosomal dominant.

X-linked hereditary nephropathies caused by mutations in the gene (*COL4A5*) encoding the α5(IV) collagen chain have been described in two canine families. The mutations that cause these diseases have been identified, but each is unique within its kindred. An autosomal recessive hereditary glomerulopathy also occurs in Cocker Spaniels (known in North America as English Cocker Spaniels) worldwide. These dogs presumably have a COL4A3 or COL4A4 mutation, but the causative mutation has not yet been identified. These disorders are characterized by abnormal composition of the type IV collagen in renal basement membranes, which can be demonstrated by special immunostaining techniques, and by distinctive ultrastructural GBM changes, which can be detected only using transmission electron microscopy.

Onset of persistent proteinuria is the first clinical sign and usually occurs at 4-8 months of age. Thereafter, renal function progressively deteriorates, usually causing azotemia by 6-12 months of age and death from renal failure at 9-18 months of age. The light microscopic features of the nephropathy are nonspecific, having the morphologic features of a membranoproliferative glomerulonephropathy accompanied by secondary tubulointerstitial changes.

Autosomal dominant inherited glomerulopathies have been described in Bull Terriers and Dalmatians, mainly from Australia. The disorders also are characterized by ultrastructural GBM abnormalities that can be identified only with transmission electron microscopy; the changes are similar to those of the X-linked and autosomal recessive conditions described above. In contrast, however, immunostaining of kidney from affected Bull Terriers and Dalmatians shows a normal pattern of type IV collagen α-chain expression in their basement membranes. The gene mutations that cause these nephropathies in Bull Terriers and Dalmatians have not been identified. Clinical expression of autosomal dominant glomerulopathies is variable. Affected dogs have proteinuria (UPC ≥ 0.3), but the onset of renal failure occurs at 11 months to 8 years of age in Bull Terriers and at 8 months to 7 years of age in Dalmatians.

Polycystic kidney disease

Autosomal dominant polycystic kidney disease is prevalent in Persian and Persian-cross cats, affecting approximately 38% of Persian cats worldwide. Recently, a single mutation in the feline *PKD1* gene has been incriminated as the cause of this disorder in many if not all affected cats. A stop mutation caused by a single nucleotide transversion in exon 29 (of 46) was found in the heterozygous state in each of 48 affected cats (41 Persians and one cat in each of seven other breeds) from the United States. Because the mutation is likely to be identical by descent within the breed, a DNA test is now possible to identify Persian and Persian-cross cats that have or will develop polycystic kidney disease, although the expectation that this single mutation causes the disease worldwide remains to be verified by further studies. In affected cats, multiple cysts form in both kidneys and occasionally in the liver. Renal cysts arise from tubules and occur in both the cortex and medulla. They form early in life and gradually become more numerous and larger in size as the cat ages. Detection of multiple cysts distributed in both kidneys using ultrasonography is diagnostic. Cysts sometimes can be detected in kittens as young as 6-8 weeks of age; however, because the number and size of cysts increase with time, sensitivity of ultrasound as a diagnostic test for polycystic kidney disease increased from 75% at 16 weeks of age to 91% at 36 weeks of age in one study. Cyst growth eventually causes renomegaly, which can be an incidental finding during physical examination of seemingly healthy cats, and renal failure ensues later in adult life (at 3-10, average 7, years of age).

Autosomal dominant polycystic kidney disease also has been described in Bull Terriers, mainly in Australia. Affected dogs are identified by ultrasonography when multiple (≥ 3) cysts distributed in both

kidneys are detected in dogs with a family history of the disease. The gene mutation that causes this disease has not been identified, but dogs at risk for the disease can be screened with ultrasonography prior to breeding to minimize production of additional affected animals.

Amyloidosis

In Shar Pei dogs, familial amyloidosis usually causes renal failure in dogs that are 1 to 6 (average, 4) years of age. Some dogs have a history of previous episodes of high fever and joint swelling, and this disease in Shar Pei dogs may be analogous to familial Mediterranean fever in humans. Some evidence suggests that amyloidosis in Shar Pei dogs is inherited in an autosomal recessive fashion. Affected Shar Peis invariably have moderate to severe medullary interstitial amyloid deposits, but only two-thirds have glomerular deposits. Proteinuria and other elements of the nephrotic syndrome, which reflect the severity of glomerular involvement, occur in some dogs. Amyloid frequently is also deposited in many other organs. Severe amyloid deposition in the liver may cause hepatomegaly, jaundice, or hepatic rupture.

In Abyssinian cats, familial amyloidosis probably is inherited as an autosomal dominant trait with variable penetrance. Renal amyloid deposits first appear between 9 and 24 months of age mainly in the medullary interstitium. Glomerular deposits usually are mild but occasionally are severe, so proteinuria is a variable feature of renal amyloidosis in cats. Affected cats usually develop chronic renal failure at 1 to 5 (average, 3) years of age, but cats with mild deposits can live to be much older. Additionally, cats with severe medullary involvement sometimes develop papillary necrosis. Amyloid deposits often are also found in other organs, but in Abyssinian cats, extra-renal amyloid deposition usually is of little clinical consequence. In Siamese and Oriental cats with familial amyloidosis, however, severe amyloid deposition occurs predominantly in the liver and mainly causes intra-abdominal hemorrhage from hepatic rupture. Nonetheless, amyloid deposition also occurs in the kidneys and leads to renal failure in some affected cats.

Immune-mediated glomerulonephritis

A familial disorder that causes protein-losing enteropathy, protein-losing nephropathy, or both has been described in Soft-Coated Wheaten Terriers. Although pathogenesis of the disorder is incompletely understood, evidence suggests that food hypersensitivity and altered intestinal permeability develop first and that immune complex glomerulonephritis develops subsequently. The mode of inheritance has not been defined, but the disorder is common among Soft-Coated Wheaten Terriers, particularly in the United States where the condition is estimated to affect as many as 10-15% of the dogs of this breed. Females are affected slightly more often than males, and the average age when renal disease is diagnosed in affected dogs is 6 years of age. Clinical signs associated with the nephropathy include polyuria, polydipia, vomiting, and weight loss. Laboratory findings include proteinuria, hypoalbuminemia, and hypercholesterolemia, often associated with abnormalities attributable to renal failure (azotemia, hyperphosphatemia, and non-regenerative anemia). The disease is complicated by thromboembolism in about 12% of cases, and hypertension occurs occasionally. By light microscopy, renal lesions are those of a membranous to membranoproliferative glomerulonephritis progressing to glomerular sclerosis accompanied by periglomerular fibrosis and secondary tubulointerstitial changes. Evidence of mesangial deposition of immunoglobulin A (IgA), IgM, and complement has been detected in the glomeruli of affected dogs using immunofluorescent labeling and electron microscopy.

Additional Detail

Table 1 – Familial nephropathies in dogs and cats

- Dogs
  - Renal dysplasia
    - Lhasa Apso
    - Shih Tzu
    - Standard Poodle
    - Soft-Coated Wheaten Terrier
    - Chow Chow
    - Alaskan Malamute
    - Miniature Schnauzer
    - Dutch Kooiker (Dutch Decoy) Dog
  - Primary glomerulopathies
    - Samoyed kindred and Navasota kindred (X-linked)
    - English Cocker Spaniel (autosomal recessive)
    - Bull Terrier (autosomal dominant)
    - Dalmatian (autosomal dominant)
    - Doberman Pinscher
    - Bullmastiff
    - Newfoundland
    - Rottweiler
    - Pembroke Welsh Corgi
    - Beagle
  - Polycystic kidney disease
    - Bull Terrier (autosomal dominant)
    - Carin Terrier and West Highland White Terrier (autosomal recessive)
  - Amyloidosis
    - Shar Pei
    - English Foxhound
    - Beagle
  - Immune-mediated glomerulonephritis
    - Soft-Coated Wheaton Terrier
    - Burnese Mountain Dog (autosomal recessive, suspected)
    - Brittany Spaniel (autosomal recessive)
  - Miscellaneous
    - Basenji - Fanconi syndrome
    - German Shepherd - multifocal cystadenocarcinoma (autosomal dominant)
    - Pembroke Welsh Corgi - telangiectasia
- Cats
  - Polycystic kidney disease
    - Persian (autosomal dominant)
  - Amyloidosis
    - Abyssinian (autosomal dominant with incomplete penetrance, suspected)
    - Siamese and Oriental

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Pedigree Analysis

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Overview of the Issue

The term pedigree is an old word which is derived from the French "pie de grue", meaning crane's foot. The drawn pedigree was first used in the breeding of cattle and other domestic livestock. Now after more than six centuries, the tradition of using it as a primary breeding tool continues. Over time breeders learned the important uses of a pedigree were to identify carriers along with the strengths and weaknesses of each ancestor. Thus, when the frequency of a trait or disease occurred among the ancestors it should serve as a signal that something is likely to be heritable.

The Traditional Pedigree is the most popular of the pedigrees used by breeders. Unfortunately, the Traditional Pedigree, as a breeding tool has many shortcomings. Most notable is the importance it places on memory and knowing the names and titles of the ancestors which are not heritable. The custom has been for breeders to recognize and associate names and titles with what could be remembered about the traits and characteristics of each ancestor. This approach lacked reliability and it did not capture the information needed to plan a breeding. Another problem associated with the Traditional Pedigree occurred when litters were evaluated. When something worked, credit was given to the pedigree and the breeder. When it didn't, there was no record or source of information to be reviewed. This made it ineffective as a breeding tool. Perhaps its major criticism was that it did not lend itself to collecting the right kinds of information in sufficient detail to be useful to plan a breeding. A review of how most Traditional Pedigrees are used show that scribbled notes around the edges and in the margins typically serve as the record system. Words such as "beautiful coat", "wonderful type", a title or the name of a famous offspring becomes the information a breed has to use. This approach fails to collect what is relevant or specific to making improvements. In short, breeders had no way to learn from their mistakes.

Two other pedigrees were developed to compensate for the limitations of the Traditional Pedigree. The first was called the Stick Dog Color Chart Pedigree. It's focus is on the traits of conformation. The other is called the Symbols Pedigree. It is used to track and analyze health, performance and other special traits of interest.

FIGURE 1 TRADITIONAL PEDIGREE

CERTIFICATE OF PEDIGREE

Am/Can GV Ch WeLove DuChein's R-Man ROM OFA
BIS Am/Can Sel. Ch. Kismet's Sight For Sore Eyes ROM, OFA DNA certified
Kismet's Sweetheart Deal ROM OFA
2003 Fut. Ch O Danny Boy of Heinerburg, OFA Prelim DNA certified
Ch Schokrest San Deigo OFA
Magic Moment of Heinerburg
Cartel's Amber v Heinerburg (litter sister to Ch Levi OFA)

PUPPY "E" LITTER

BIS Am/Can Sel. Ch Kismet's Sight For Sore Eyes ROM OFA DNA certified
Ch Tindrock-Kaleef Thyme
Ken-Delaine's Katrina OFA ROM
Van Cleve's Cassandra v Kaleef OFA, DNA certified
Ch Brier Hill's Storm Buddy
Ch Kaleef's Blondie HS
Ch Hollow Hill's Sierra v Cherpa OFA

Notice that the Traditional pedigree in Figure 1 is easy to read, but it does not display the kinds of information needed to make decisions. For example, no information is collected about the carriers, normal or affected ancestors. It is not clear which ancestor(s) has the desirable and undesirable traits. The Traditional pedigree as a record, forces breeders to relay on titles, certificates and winning records all of which must be remembered.

STICK DOG COLOR CHART PEDIGREE

The Stick Dog Color Chart Pedigree was originally developed for research and computer analysis. Later, it was adapted to meet the needs of breeders.

CODING TRAITS

The Stick Dog Color Pedigree allows breeders to see the strengths and weaknesses of each ancestor based on the breed standard. The logic underlying this pedigree is that each ancestor is represented as a stick figure of coded information. Rather than a name and or title, each ancestor is drawn as a stick figure with seven structural parts: ears, head, neck, front, back, rear and tail. Each part is coded for its quality using four mutually exclusive colors. Each color is used to signify the rank or quality of the trait based on the breed Standard.

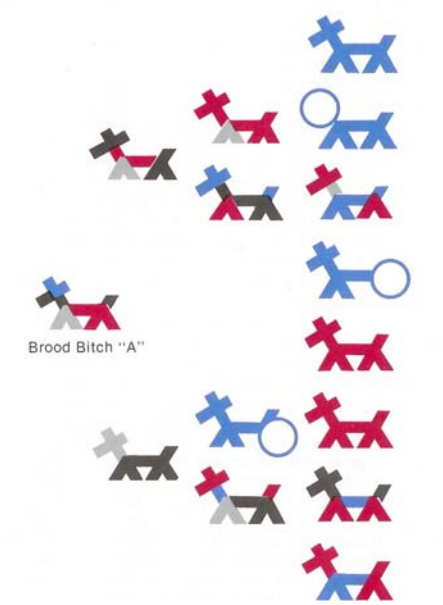
The Stick Dog Color Chart Pedigree helps breeders to identify and rank the traits of conformation of each ancestor using the seven key traits of conformation. Each trait is assigned a quality (rank) using a color-code. Notes are added to each stick figure to supplement and clarify the color codes.

USE OF COLOR TO CODE TRIATS

RIBBONS	CODES	RANK FOR QUALITY
Blue	Blue	- First place quality
Red	Red	- Second place quality
Yellow	Yellow	- Third place quality
White	Black	- Fourth place quality

The rules used to code the quality of a trait or the lack there of, is straight forward. When a trait is coded with a first place color (blue), it is viewed to be correct or ideal based on the breed standard. For example, if the ears on a sire were coded blue and those on the dam were coded black, the breeder would know that the sire's ears were correct but the ears on the dam were not correct and lacking in some way. Thus, the color-coding of each ancestor identifies their qualities along with their specific strengths and weaknesses. The color codes also show if there are trends or problems and whether they are on the sire or dams side of the pedigree.

FIGURE 2. STICK DOG PEDIGREE



Notice that brood bitch “A” has a fourth place front as does her father, grandfather, and her grandmother on her mother’s side. Thus, in the first two generations, three out of six ancestors or 50% of her ancestors all have the same fourth place front. This suggests that she inherits her faulty front legitimately from her ancestors. It should also be noticed that poor fronts occur on both sides of her pedigree. This is useful information when searching for the right stud dog and traits he is expected to improve.

Searching the Genotypes
Researchers and breeders often use the term phenotype and genotype. Phenotype refers to the characteristics that can be seen, meaning their external appearance. Hence, a dog that is observed to be black (phenotype) may or may not produce only black puppies. It could have a genetic make-up (genotype) that includes the genes for other colors. Since genotypes can not be seen directly, indirect methods must be used to learn about them. Indirect methods are not estimates or guessing games. Instead, they require the collection of detailed information about each ancestor and each of their littermates, usually for three generations. Those who do not collect and code information about the ancestors and their littermates usually rely on "type" breeding. This means they select sires and dams based on their appearance rather than on the traits observed in their offspring or the relationship that exists between them. Many times "type" breeding simply means breeding the winners to the winners. In practice, these breedings fail to take advantage of what the science of genetics has taught us about inheritance. Studying a pedigree for its genotypes means focusing attention on the occurrence of traits found among the ancestors and their littermates. While this approach requires more time, it is far superior to the Traditional Pedigree, which relies on learning names and titles.

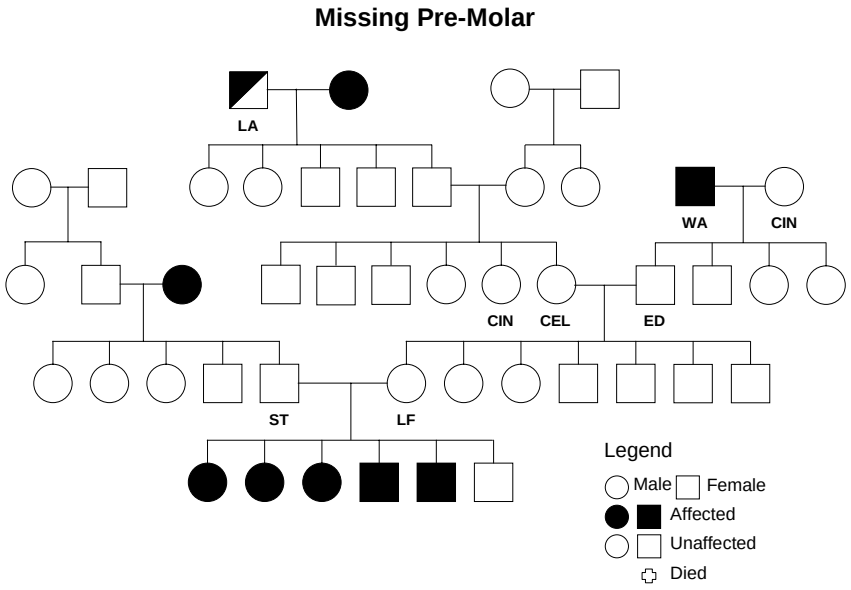
SYMBOLS PEDIGREE
Breeders interested in health, temperament or special traits needed a third kind of pedigree. One that was able to capture and display the strengths and weaknesses of each ancestor and all of their littermates. Breeders needed a way to the carriers of certain undesirable health problems or some special trait of interest on either the sire or dams side of the pedigree. Knowing where and how often these problems occurred increases the probability that the carriers could be controlled or eliminated.

What every breeder wanted was an inside look at the genes carried by the ancestors. Since no single method can look directly into the genotype, breeders had to rely on the information they are able to collect. The best pedigree for this purpose is called the Symbols Pedigree. It focuses on the breadth of a pedigree, meaning the littermates. The Symbols Pedigree relies on the logic that a pedigree can be understood by learning about the traits and characteristics observed among the littermates of each ancestor. It is especially effective for making improvements in the core elements: health, performance, temperament and other specific traits of interest.

The Symbols Pedigree gets its name because symbols rather than names are used to identify each ancestor. The inclusion of littermates further distinguishes this pedigree from the others. Its great advantage is that it produces a record of information that can be used to make improvements and eliminate problems.

CODING
The Symbols Pedigree is a powerful tool because of the amount of information that can be coded and quickly recognized. Squares are used to represent the males and circles to represent the females. The littermates for each ancestor are also represented as either a circle or a square. As information is collected about each individual it is coded using designated colors that represent specific traits or diseases. Because breeders are interested in many traits and diseases they will use several colors to code this pedigree. Key words and phrases are also added to clarify and further explain the characteristics, conditions, test results etc. for each ancestor. The repetition of a color, key word or phrase usually signals that a genetic trend or pattern may be present.

FIGURE 3 THE SYMBOLS PEDIGREE



Notice in Figure 3, that the sire of the litter had three sisters and one brother and that the dam had four brothers and two sisters. This pedigree shows a litter of six pups (3-3). Five of these six pups had missing teeth.

A comparison between what the Traditional Pedigree and the Symbols Pedigree and Stick Dog Color Chart Pedigree should be convincing evidence that pedigree analysis can be improved by using these new breeding tools.

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Carmen L Battaglia holds a Ph.D. and Masters Degree from Florida State University. As an AKC judge, researcher and writer, he has been a leader in promotion of breeding better dogs and has written many articles and several books.

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Early Neurological Stimulation of Puppies

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Overview of the Issue

Surprising as it may seem, it isn't capacity that explains the differences that exist between individuals because most seem to have far more capacity than they will ever use. The differences that exist between individuals seem to be related to something else. The ones who achieve and out perform others seem to have within themselves the ability to use hidden resources. In other words, it's what they are able to do with what they have that makes the difference.

In many animal-breeding programs the entire process of selection and management is founded on the belief that performance is inherited. Attempts to analyze the genetics of performance in a systematic way have involved some distinguished names such as Charles Darwin and Francis Galton. But it has only been in recent decades that good estimates of heritability of performance have been based on adequate data. Cunningham (1991) in his study of horses found that only by using Timeform data, and measuring groups of half brothers and half sisters could good estimates of performance be determined. His data shows that performance for speed is about 35% heritable. In other words only about 35% of all the variation that is observed in track performance is controlled by heritable factors, the remaining 65% are attributable to other influences, such as training, management and nutrition. Cunningham's work while limited to horses provides a good basis for understanding how much breeders can attribute to the genetics and the pedigrees.

Researchers have studied this phenomena and have looked for new ways to stimulate individuals in order to improve their natural abilities. Some of the methods discovered have produced life long lasting effects. Today, many of the differences between individuals can now be explained by the use of early stimulation methods.

Introduction

Man for centuries has tried various methods to improve performance. Some of the methods have stood the test of time, others have not. Those who first conducted research on this topic believed that the period of early age was a most important time for stimulation because of its rapid growth and development. Today, we know that early life is a time when the physical immaturity of an organism is susceptible and responsive to a restricted but important class of stimuli. Because of its importance many studies have focused their efforts on the first few months of life.

Newborn pups are uniquely different than adults in several respects. When born their eyes are closed and their digestive system has a limited capacity requiring periodic stimulation by their dam who routinely licks them in order to promote digestion. At this age they are only able to smell, suck, and crawl. Body temperature is maintained by snuggling close to their mother or by crawling into piles with other littermates. During these first few weeks of immobility researchers noted that these immature and under-developed canines are sensitive to a restricted class of stimuli which includes thermal, and tactile stimulation, motion and locomotion.

Other mammals such as mice and rats are also born with limitations and they also have been found to demonstrate a similar sensitivity to the effects of early stimulation. Studies show that removing them from their nest for three minutes each day during the first five to ten days of life causes body temperatures to fall below normal. This mild form of stress is sufficient to stimulate hormonal, adrenal and pituitary systems. When tested later as adults, these same animals were better able to withstand stress than littermates who were not exposed to the same early stress exercises. As adults, they responded to stress in "a graded" fashion, while their non-stressed littermates responded in an "all or nothing way."

Data involving laboratory mice and rats also shows that stress in small amounts can produce adults who respond maximally. On the other hand, the results gathered from non-stressed littermate show that they become easily exhausted and would near death if exposed to intense prolonged stress. When tied down so they were unable to move for twenty-four hours, rats developed severe stomach ulcers, but litter mates exposed to early stress handling were found to be more resistant to stress tests and did not show evidence of ulcers. A secondary affect was also noticed.

Sexual maturity was attained sooner in the littermates given early stress exercises. When tested for

differences in health and disease, the stressed animals were found to be more resistant to certain forms of cancer and infectious diseases and could withstand terminal starvation and exposure to cold for longer periods than their non-stressed littermates.
Other studies involving early stimulation exercises have been successfully performed on both cats and dogs. In these studies, the Electrical Encephalogram (EEG) was found to be ideal for measuring the electrical activity in the brain because of its extreme sensitivity to changes in excitement, emotional stress, muscle tension, changes in oxygen and breathing. EEG measures show that pups and kittens when given early stimulation exercises mature at faster rates and perform better in certain problem solving tests than non-stimulated mates.
In the higher level animals the effect of early stimulation exercises have also been studied. The use of surrogate mothers and familiar objects were tested by both of the Kelloggs' and Dr. Yearkes using young chimpanzees. Their pioneer research shows that the more primates were deprived of stimulation and interaction during early development, the less able they were to cope, adjust and later adapt to situations as adults.
While experiments have not yet produced specific information about the optimal amounts of stress needed to make young animals psychologically or physiologically superior, researches agree that stress has value. What also is known is that a certain amount of stress for one may be too intense for another, and that **too much stress can retard development**. The results show that early stimulation exercises can have positive results but must be used with caution. In other words, too much stress can cause pathological adversities rather than physical or psychological superiority.

Methods of Stimulation

The U.S. Military in their canine program developed a method that still serves as a guide to what works. In an effort to improve the performance of dogs used for military purposes, a program called "Bio Sensor" was developed. Later, it became known to the public as the "Super Dog" Program. Based on years of research, the military learned that early neurological stimulation exercises could have important and lasting effects. Their studies confirmed that there are specific time periods early in life when neurological stimulation has optimum results. The first period involves a window of time that begins at the third day of life and lasts until the sixteenth day. It is believed that because this interval of time is a period of rapid neurological growth and development, and therefore is of great importance to the individual.

The "Bio Sensor" program was also concerned with early neurological stimulation in order to give the dog a superior advantage. Its development utilized six exercises which were designed to stimulate the neurological system. Each workout involved handling puppies once each day. The workouts required handling them one at a time while performing a series of five exercises. Listed in order of preference the handler starts with one pup and stimulates it using each of the five exercises. The handler completes the series from beginning to end before starting with the next pup. The handling of each pup once per day involves the following exercises:

1. Tactical stimulation (between toes)
2. Head held erect
3. Head pointed down
4. Supine position
5. Thermal stimulation.

Tactile stimulation

1. **Tactile stimulation** - holding the pup in one hand, the handler gently stimulates (tickles) the pup between the toes on any one foot using a Q-tip. It is not necessary to see that the pup is feeling the tickle. Time of stimulation 3 - 5 seconds. (Figure 1)
2. **Head held erect** - using both hands, the pup is held perpendicular to the ground, (straight up), so that its head is directly above its tail. This is an upwards position. Time of stimulation 3 - 5 seconds (Figure 2).
3. **Head pointed down** - holding the pup firmly with both hands the head is reversed and is pointed downward so that it is pointing towards the ground. Time of stimulation 3 - 5 seconds (Figure 3).

4. **Supine position** - hold the pup so that its back is resting in the palm of both hands with its muzzle facing the ceiling. The pup while on its back is allowed to sleep struggle. Time of stimulation 3-5 seconds. (Figure 4)

5. **Thermal stimulation**—use a damp towel that has been cooled in a refrigerator for at least five minutes. Place the pup on the towel, feet down. Do not restrain it from moving. Time of stimulation 3-5 seconds. (figure 5)
These five exercises will produce neurological stimulations, none of which naturally occur during this early period of life. Experience shows that sometimes pups will resist these exercises, others will appear unconcerned. In either case a caution is offered to those who plan to use them. Do not repeat them more than once per day and do not extend the time beyond that recommended for each exercise. Over stimulation of the neurological system can have adverse and detrimental results. These exercises impact the neurological system by kicking it into action earlier than would be normally expected. The result being an increased capacity that later will help to make the difference in its performance. Those who play with their pups and routinely handle them should continue to do so because the neurological exercises are not substitutions for routine handling, play socialization or bonding.

Benefits of Stimulation

Five benefits have been observed in canines that were exposed to the Bio Sensor stimulation exercises. The benefits noted were:

1. Improved cardio vascular performance (heart rate)
2. Stronger heart beats,
3. Stronger adrenal glands,
4. More tolerance to stress, and
5. Greater resistance to disease.

In tests of learning, stimulated pups were found to be more active and were more exploratory than their non- stimulated littermates over which they were dominant in competitive situations.

Secondary effects were also noted regarding test performance. In simple problem solving tests using detours in a maze, the non-stimulated pups became extremely aroused, wined a great deal, and made many errors. Their stimulated littermates were less disturbed or upset by test conditions and when comparisons were made, the stimulated littermates were more calm in the test environment, made fewer errors and gave only an occasional distress when stressed.

Socialization

As each animal grows and develops three kinds of stimulation have been identified that impact and influence how it will develop and be shaped as an individual. The first stage is called early neurological stimulation, and the second stage is called socialization. The first two (early neurological stimulation and socialization) have in common a window of limited time. When Lorenz, (1935) first wrote about the importance of the stimulation process he wrote about imprinting during early life and its influence on the later development of the individual. He states that it was different from conditioning in that it occurred early in life and took place very rapidly producing results which seemed to be permanent. One of the first and perhaps the most noted research efforts involving the larger animals was achieved by Kellogg & Kellogg (1933). As a student of Dr. Kellogg's I found him and his wife to have an uncanny interest in children and young animals and the changes and the differences that occurred during early development. Their history making study involved raising their own new born child with a new born primate. Both infants were raised together as if they were twins. This study like others that would follow attempted to demonstrate that among the mammals there are great differences in their speed of physical and mental development. Some are born relatively mature and quickly capable of motion and locomotion, while others are very immature, immobile and slow to develop. For example, the Rhesus monkey shows rapid and precocious development at birth, while the chimpanzee and the other "great apes" take much longer. Last and slowest is the human infant.

One of the earliest efforts to investigate and look for the existence of socialization in canines was undertaken by Scott-Fuller (1965). In their early studies they were able to demonstrate that the basic technique for testing the existence of socialization was to show how readily adult animals would foster young animals, or accept one from another species. They observed that with the higher level animals it is easiest done by hand rearing. When the foster animal transfers its social relationships to the new species, researchers conclude that socialization has taken place. Most researchers agree that among all species, a lack of adequate socialization generally results in unacceptable behavior and often times produces undesirable aggression, excessiveness, fearfulness, sexual inadequacy, and indifference toward partners.

Socialization studies confirm that the critical periods for humans (infant) to be stimulated are generally between three weeks and twelve months of age. For canines the period is shorter, between the fourth and sixteenth week of age. During these critical time periods two things can go wrong. First, insufficient social contact can interfere with proper emotional development which can adversely affected the development of the human bond. The lack of adequate social stimulation, such as handling, mothering and contact with others, adversely affects social and psychological development.

Second, over mothering can prevent sufficient exposure to other individuals, and situations that have an important influence on growth and development. The literature shows that humans and animals respond in similar ways when denied minimal amounts of stimulation. In humans, the absence of love and cuddling increases the risk of an aloof, distant, asocial or sociopathic individual. Over mothering can also have its detrimental effects. It occurs when a patient insulates the child from outside contacts, or keeps the apron strings tight, thus limiting opportunities to explore and interact. In the end, over mothering generally produces a dependent, socially maladjusted and sometimes emotionally disturbed individual.

The absence of outside social interactions for both children and pups usually results in a lack of adequate learning and social adjustment. Protected youngsters who grow up in an insulated environment often times become sickly, despondent, lacking in flexibility and unable to make simple social adjustments. Generally, they are unable to function productively or to interact successfully then they become adults.

Owners who have busy life styles with long and tiring work and social schedules often times cause pets to be neglected. Left to themselves with only an occasional trip out of the house or off of the property they seldom see other canines or strangers and generally suffer from poor stimulation and socialization. For many, the side effects of loneliness and boredom set-in. The resulting behavior manifests itself in the form of chewing, digging, and hard to control behavior (Battaglia).
It seems clear that small amounts of stress followed by early socialization can produce beneficial results. The danger seems to be in not knowing where the thresholds are for over and under stimulation. Many improperly socialized youngsters develop into older individuals unprepared for adult life, unable to cope with its challenges, and interactions. Attempts to re-socialize them when adults have only produced small gains. These failures confirm the notion that the window of time open for early neurological and social stimulation only comes once. After it passes, little or nothing can be done to overcome the negative effects of too much or too little stimulation.

The third and final stage in the process of growth and development is called enrichment. Unlike the first two stages it has no time limit and by comparison covers a very long period of time. Enrichment is a term which has come to mean the positive sum of experiences, which have a cumulative effect upon the individual. Enrichment experiences typically involve exposure to a wide variety of interesting, novel, and exciting experiences with regular opportunities to freely investigate, manipulate, and interact with them. When measured in later life, the results show that those reared in an enriched environment tend to be more inquisitive and are more able to perform difficult tasks. The educational TV program called Sesame Street is perhaps the best known example of a children's enrichment program. The results show that when tested, children who regularly watched this program performed better than playmates who did not. Follow up studies show that those who regularly watched Sesame Street tend to seek a college education and when enrolled, performed better than playmates who were not regular watchers of the Sesame Street Program.

There are numerous children studies that show the benefits of enrichment techniques and programs. Most focus on improving self-esteem and self-talk. Follow up studies show that the enriched Sesame

Street students when later tested were brighter and scored above average and most often were found to be the products of environments that contributed to their superior test scores. On the other hand, those whose test scores were generally below average, (labeled as dull) and the products of underprivileged or non- enriched environments often times had little or only small amounts of stimulation during early childhood and only minimal amounts of enrichment during their developmental and formative years. Many were characterized as children who grew up with little interaction with others, poor parenting, few toys, no books and a steady diet of TV soap operas.

A similar analogy can be found among canines. All the time they are growing they are learning because their nervous systems are developing and storing information that may be of inestimable use at a later date. Studies by Scott and Fuller confirm that non-enriched pups when given free choice preferred to stay in their kennels. Other litter mates who were given only small amounts of outside stimulation between five and eight weeks of age were found to be very inquisitive and very active. When kennel doors were left open, the enriched pups would come bounding out while littermates who were not exposed to enrichment would remain behind. The non-stimulated pups would typically be fearful of unfamiliar objects and generally preferred to withdraw rather than investigate. Even well bred pups of superior pedigrees would not explore or leave their kennels and many were found difficult to train as adults. These pups in many respects were similar to the deprived children. They acted as if they had become institutionalized, preferring the routine and safe environment of their kennel to the stimulating world outside their immediate place of residence.
Regular trips to the park, shopping centers and obedience and agility classes serve as good examples of enrichment activities. Chasing and retrieving a ball on the surface seems to be enriching because it provides exercise and includes rewards. While repeated attempts to retrieve a ball provide much physical activity, it should not be confused with enrichment exercises. Such playful activities should be used for exercise and play or as a reward after returning from a trip or training session. Road work and chasing balls are not substitutes for trips to the shopping mall, outings or obedience classes most of which provide many opportunities for interaction and investigation.

Finally it seems clear that stress early in life can produce beneficial results. The danger seems to be in not knowing where the thresholds are for over and under stimulation. However, the absence or the lack of adequate amounts of stimulation generally will produce negative and undesirable results. Based on the above it is fair to say that the performance of most individuals can be improved including the techniques described above. Each contributes in a cumulative way and supports the next stage of development.

Conclusion

Breeders can now take advantage of the information available to improve and enhance performance. Generally, genetics account of about 35% of the performance but the remaining 65% (management, training, nutrition) can make the difference. In the management category it has been shown that breeders should be guided by the rule that it is generally considered prudent to guard against under and over stimulation. Short of ignoring pups during their first two months of life, a conservative approach would be to expose them to children, people, toys and other animals on a regular basis. Handling and touching all parts of their anatomy is also necessary to learn as early as the third day of life. Pups that are handled early and on a regular basis, generally do not become hand shy as adults.

Because of the risks involved in under stimulation a conservative approach to using the benefits of the three stages has been suggested based primarily on the works of Arskeusky, Kellogg, Yearkes and the "Bio Sensor" program (later known as the "Super Dog Program").

Both experience and research have dominated the beneficial effects that can be achieved via early neurological stimulation, socialization and enrichment experiences. Each has been used to improve performance and to explain the differences that occur between individuals, their trainability, health and potential. The cumulative effects of the three stages have been well documented. They best serve the interests of owners who seek high levels of performance when properly used. Each has a cumulative effect and contributes to the development and the potential for individual performance.

Additional Detail

Early Stimulation Exercises

Figure # 1 Tactical stimulation

Figure # 2 Head held erect

Figure # 3 Head pointed down

Figure # 4 Figure Supine position

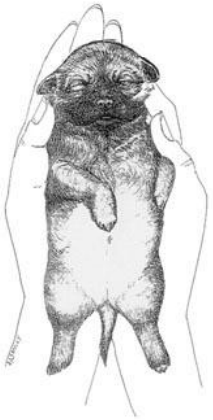
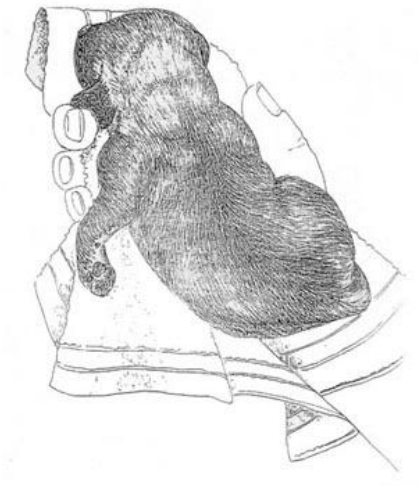


Figure # 5 Thermal stimulation



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ABOUT THE AUTHOR

Carmen L Battaglia holds a Ph.D. and Masters Degree from Florida State University. As an AKC judge, researcher and writer, he has been a leader in promotion of breeding better dogs and has written many articles and several books.

Dr. Battaglia is also a popular TV and radio talk show speaker. His seminars on breeding dogs, selecting sires and choosing puppies have been well received by the breed clubs all over the country. Those interested in learning more about his seminars should contact him directly. Visit his website at http://www.breedingbetterdogs.com

Selected Heritable Skin Disorders

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Overview of the Issue
There are hundreds of conditions that are considered heritable in dogs and cats, and certainly many dermatologic conditions for which breed predispositions are recognized. However, there are relatively few dermatologic disorders for which actual genetic documentation is available.

Additional Detail

Sebaceous Adenitis

Sebaceous adenitis is a periappendageal inflammatory process directed against hair follicles and glands. It is presumed autosomal recessive in Standard poodles but the condition in other breeds may be quite different. An immune-mediated process is suspected which, in turn, is influenced by other conditions such as atopy, food allergy, vaccination, and season (estrus). Clinical signs include non-inflammatory hair loss and scaling. Most dogs are young adults when first affected. There is extreme variability between breeds suggesting that this is a common end-result of potentially very different processes.

The diagnosis is confirmed with biopsies for histopathology, which should be taken from normal, mildly-affected and severely-affected skin. Carriers can sometimes be predicted by early biopsy.

The condition responds to anti-inflammatory therapies, and the best approach is frequent anti-seborrheic shampoos (including dishwashing liquid) and emollients (e.g., baby oil, propylene glycol, urea, lactic acid) and oral supplementation with high-dose marine oil. In rare cases, dogs can go into spontaneous (if only transitory) remission.

Dermatomyositis

Dermatomyositis has a definite hereditary component (autosomal dominant with variable expressivity), but is also believed to be affected by infections (viral particles recovered) and an immune-mediated process. The list of breeds affected continues to grow.

Clinical signs tend to become evident by 12 weeks of age and consist of erosions and scarring on face, elbows, ears and hocks; muscle wasting is seen in some cases. Some dogs have difficulty eating, drinking and swallowing and megaesophagus may result in aspiration pneumonia. The diagnosis can be confirmed with histopathology and electromyography.

Some cases spontaneously improve so all therapies must be based on individual assessment. Vitamin E is recommended to help decrease scarring. Corticosteroids are of little benefit. Other therapies include pentoxifylline and avoiding sun exposure.

Demodicosis

Demodex mites are obligatory parasites. The whole life cycle takes place in the skin (in the hair follicle and sebaceous glands or on the skin surface, depending on species). The mite is typically acquired from the dam during nursing in the first few days of life, but propagation of the mite probably occurs throughout the animal's life.

Demodicosis is usually arbitrarily designated as localized demodicosis, juvenile-onset generalized demodicosis, adult-onset demodicosis and demodectic pododermatitis. Juvenile-onset generalized demodicosis may be the result of heritable or non-heritable immune dysfunction. Adult-onset cases are often associated with underlying disease or immunosuppressive drug therapy, but in about 30-40% of cases, no underlying disease or drug is identified.

Demodicosis is usually diagnosed by deep skin scrapings, but superficial scrapings are often needed to find the short-bodied variety that resides on the skin surface. There are many treatment options for

demodicosis. For very mild cases, using a follicle-flushing shampoo (e.g., benzoyl peroxide shampoo), a 3-4 week course of bactericidal antibiotic and a nutritious diet rich in antioxidants (or supplemented with vitamin E) is sufficient to achieve resolution. For more persistent cases, those often referred to as generalized demodicosis, specific miticidal therapy is often needed, in addition to searching for underlying causes of immune suppression. Therapies of most use in this regard are topical amitraz (250 ppm), or macrocyclic lactones such as ivermectin, milbemycin or moxidectin.

Ivermectin toxicity

Ivermectin belongs to the avermectin class of antiparasitic agents and is certainly not a new parasite-control treatment. In most mammals, the blood-brain barrier prevents access of ivermectin to the central nervous system. However, there are some animals that have genetic mutations of the mdr1 (ABCB1) gene, making them particularly sensitive to the neurological effects of ivermectin. Affected breeds include collie, Shetland sheepdog, Australian shepherd, Old English sheepdog, white German shepherd dog, and longhaired whippets. Homozygotes for the deletion seem to show toxicity to ivermectin at 100 mcg/kg, while heterozygotes are relatively tolerant of doses up to 600 mcg/kg. Animals that are sensitive to ivermectin may also have adverse reactions to a variety of medications, including loperamide (Imodium®), cyclosporine, digoxin, vinca alkaloids, and doxorubicin. Fortunately, a genetic test is now available to detect susceptibility to ivermectin toxicity.

References/Suggested Reading

- Ackerman, L: The Genetic Connection: A Guide to Health Problems in Purebred Dogs. AAHA Press, 2000.
- Nesbitt, G; Ackerman, L: Canine & Feline Dermatology, Veterinary Learning Systems, 1998, 498pp.

THE WINN FELINE FOUNDATION

For the Health and Well-Being of Cats

September 29, 2005

Dear Participant:

Welcome to the Tuft's Canine and Feline Breeding and Genetics Conference. The Winn Feline Foundation is proud to be a sponsor. Winn has been a strong believer in the importance of learning more about feline genetics with the aim of improving feline health. We have sponsored other genetics conferences such as the 2nd International Feline Genetic Diseases Conference held at the University of California at Davis (2000) and the Advances in Canine and Feline Genomics Conference held in St. Louis, MO (2002).

The Winn Feline Foundation, formed by The Cat Fanciers' Association (CFA) in 1968, has been funding feline health studies for over 30 years. Our first studies focused on infectious disease such as feline leukemia and feline peritonitis. As needs in the veterinary community changed, more studies were funded in areas such as diagnostic imaging, cardiology, and feline urinary tract disease.

Our involvement in genetics and the investigation of genetically based diseases and disorders has strengthened over the past 15 years as a natural extension of our funding efforts. Studies funded by the Foundation have explored the genetic components of renal amyloidosis, feline blood types, and neonatal isoerythrolysis, and investigations of luxation and hip dysplasia. Our support has helped identify the genes causing polycystic kidney disease (PKD) in Persians and related breeds and hypertrophic cardiomyopathy in the Maine Coon Cat. We have also funded investigation of the genetic diversity of the Havana Brown breed as a prototype breed study.

Cat breeders have become increasingly aware that genetics can help them design breeding programs that produce healthier kittens destined to be cherished pets or valuable breeding animals. In recent surveys, breeders expressed significant interest in genetics. Articles about genetic issues written by breeders and for breeders are regularly appearing in breed and cat fancy publications. Speakers at the annual Winn Foundation Feline Symposium have addressed issues in this area as well. At the CFA International Cat Show and many other CFA shows around the United States, breeders have participated in genetic sampling programs for researchers. When a problem is identified within a breed, breeders are taking the initiative and are working with geneticists to understand the components of the disease and the mechanism for eliminating it from their breeding stock.

This conference is another major milestone in bringing together scientists, veterinarians, and breeders that are interested in both feline and canine genetics. It is our hope that this event will spark an expanding interest in feline genetics and lead to new collaborative efforts among scientists, breeders, and veterinarians. Please visit our website: www.WinnFelineHealth.org for more information.

We are sure this conference will be productive and interesting for everyone.

Susan Little DVM

Susan Little, DVM, DABVP (Feline)
President, The Winn Feline Foundation

Tufts' Canine and Feline
Breeding and Genetics Conference

ARTICLES

Title of Article:	Name:
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Canine Molecular Genetic Diseases	John C. Duffendack, MBA, MS, Ulreh V. Mostoskey, DVM, George A. Padgett, DVM, Al W. Stinson, DVM, George J. Brewer, MD
The Genetic Test for Persian-related PKD: Will it be constructive or destructive?	Bell, Jerold S
Examining Elbow Dysplasia	Orthopedic Foundation For Animals
Popular-Sire Syndrome: Keeping watch over health and quality issues in purebreds	Bell, Jerold S
Removing The Stigma of Genetic Disease	Bell, Jerold S

A List of Available Canine and Feline Genetic Tests

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Jerold.Bell@tufts.edu

Disorder	Breeds	Type of Test	Test Facility
Canine Leukocyte Adhesion Deficiency (CLAD)	Irish Red & White Setter Irish Setter	Direct	Optigen
Coat Color and Nose Color Variation	Australian Shepherd Border Collie Brittany Belgian Shepherd Cardigan Welsh Corgi Collie (Rough, Smooth) Cocker Spaniel Curly-Coated Retriever Dachshund Dalmatian Doberman Pinscher English Cocker Spaniel English Setter English Springer Spaniel Field Spaniel Flat-coated Retriever French Bulldog German Shepherd German Longhaired Pointer German Shorthaired Pointer German Wirehaired Pointer Great Dane Greyhound Groenendael Labrador Retriever Laekenois Large Munsterlander Lowchen Malinois Newfoundland Pointer Pomeranian Poodle Portuguese Water Dog Pudelpointer Shetland Sheepdog Staffordshire Bull Terrier Tervuren Whippet Wirehaired Pointing Griffon	Direct	HealthGene
Coat Color Gene Variations	Alaskan Klee Kai American Cocker Spaniel Australian Cattle Dog Border Collie Curly Coated Retriever Dalmatian Doberman Pinscher English Cocker Spaniel	Direct	VetGen

Disorder	Breeds	Type of Test	Test Facility
	English Springer Spaniel Flat Coated Retriever Gordon Setter Labrador Retriever Newfoundland Pointer Poodle Schipperke Scottish Terrier Stumpy Tail Cattle Dog		
Cobalamin Malabsorption **(**Methylmalonic Aciduria)	Australian Shepherd Giant Schnauzer	Direct	PennGen
Cobalamin Malabsorption **(**Methylmalonic Aciduria)	Beagle Border Collie DSH Shar Pei	Phenotypic	PennGen
Collie Eye Anomaly (Choroidal Hypoplasia)	Australian Shepherd Border Collie Lancashire Heeler Rough Coated Collie Shetland Sheepdog Smooth Coated Collie	Direct	Optigen
Cone (Retinal) Degeneration	German Shorthaired Pointer	Direct	Optigen
Congenital Hypothyroidism with Goiter (CHG)	Toy Fox Terrier	Direct	Michigan State University – Fyfe Lab.
Congenital Stationary Night Blindness (RPE65-CSNB)	Briard	Direct	Optigen
Copper Toxicosis	Bedlington Terrier	Linkage	VetGen
Cyclic Neutropenia (Grey Collie Syndrome)	Smooth Coated Collie Rough Coated Collie	Direct	HealthGene
Cystinuria	Newfoundland Labrador Retriever	Direct	Optigen (Newf only) PennGen VetGen (Newf only)
Fanconi Syndrome	Basenji Norwegian Elkhound	Phenotypic	PennGen
Factor VII Deficiency	Beagle	Direct	PennGen
Fucosidosis	English Springer Spaniel	Direct	PennGen
Glanzmann's Thrombasthenia (Type I)	Great Pyrenees Otterhound	Direct	Auburn Univ. – Boudreaux Lab.
Globoid cell leukodystrophy	Cairn terrier West Highland White Terrier	Direct	Jefferson Medical Coll.
Glycogenosis (GSD) Type IV	Norwegian Forest Cat	Direct	PennGen
GM1-Gangliosidosis	Portuguese Water Dog	Direct	New York University Neurogenetics Lab
Hemophilia B	Airdale Terrier Bull Terrier German Wirehaired Pointer Labrador Retreiver Lhasa Apso	Direct	Cornell Univ. Comparative Coag. Lab (GWP) HealthGene (Others)
Ivermectin Sensitivity (MDR1)	Australian Shepherd Collie Old English Sheepdog Shetland Sheepdog	Direct	Washington State University

Disorder	Breeds	Type of Test	Test Facility
	Other breeds		
Mannosidosis	DSH Persian	Direct	PennGen
Methylmalonic Aciduria - Cobolamin Malabsorbtion	Beagle Border Collie Giant Schnauzer Shar Pei	Phenotypic	PennGen
Mucolipidosis II (I-Cell Disease)	DSH	Direct	PennGen
Mucopolysaccharidosis (MPS)	DSH German Shepherd Dog Miniature Pinscher Miniature Schnauzer Schipperke Siamese	Direct	PennGen
Myotonia Congenita	Miniature Schnauzer	Direct	Optigen PennGen
Narcolepsy	Dachshund Doberman Pinscher Labrador Retriever	Direct	Optigen
Phosphofructokinase Deficiency (PFK)	American Cocker Spaniel English Springer Spaniel	Direct	Optigen PennGen VetGen
Polycystic Kidney Disease (PKD)	American Shorthair Himalayan Persian Scottish Fold	Direct	UC-Davis – Lyons Lab.
Progressive Retinal Atrophy - Dominant	Bullmastiff (English) Mastiff	Direct	Optigen
Progressive Retinal Atrophy – Type A	Miniature Schnauzer	Direct	Optigen
Progressive Retinal Atrophy – X-Linked	Samoyed Siberian Husky	Direct	Optigen
Progressive Retinal Atrophy (prcd)	American Eskimo Dog Australian Cattle Dog Chesapeake Bay Retriever Chinese Crested English Cocker Spaniel Entelbucher Mountain Dog Labrador Retriever Nova Scotia Duck Trolling Retrievers Poodle; Miniature & Toy Portuguese Water Dog Stumpy Tail Cattle Dog	Linkage	Optigen
Progressive Retinal Atrophy (rcd1)	Irish Red & White Setter Irish Setter Sloughi	Direct	Optigen VetGen (Irish Setter)
Progressive Retinal Atrophy (rcd3)	Cardigan Welsh Corgi	Direct	Michigan State Univ. - Peterson-Jones Lab. Optigen VetGen
Pyruvate Kinase Deficiency (PK)	Abyssinian American Eskimo Dog Basenji	Direct	Optigen (Basenji) PennGen (All)

Disorder	Breeds	Type of Test	Test Facility
	Beagle Cairn Terrier Chihuahua Dachshund DSH Somali West Highland White Terrier		VetGen (Basenji)
Renal Dysplasia	Lhasa Apso Shih Tzu Soft Coated Wheaton Terrier	Linkage	VetGen
Severe Combined Immunodeficiency (SCID)	Bassett Hound Cardigan Welsh Corgi Pembroke Welsh Corgi	Direct	PennGen
Von Willibrand's Disease	Bernese Mountain Dog Doberman Pinscher Drentsche Patrijshound German Pinscher Kerry Blue Terrier Manchester Terrier Papillion Pembroke Welsh Corgi Poodle Scottish Terrier Shetland Sheepdog	Direct	VetGen

Auburn Univ. – Boudreaux Lab: http://www.vetmed.auburn.edu/index.pl/boudreaux_mk (334-844-2692)
Cornell Univ. Comparative Coagulation Lab: http://www.diaglab.vet.cornell.edu/coag/test/hemopwh.asp (607-275-0622)
HealthGene: www.healthgene.com (877-371-1551)
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: http://www.pwdca.org/GM1app.html (212 263-2943)
Optigen: www.optigen.com (607-257-0301)
PennGen: www.vet.upenn.edu/penngen (215-898-8894)
VetGen: www.vetgen.com (800-483-8436)
UC-Davis – Lyons Lab.: http://www.vgl.ucdavis.edu/service/cat/PKD.html (530-752-2211)
Washington State University: http://www.vetmed.wsu.edu/announcements/ivermectin/ownerInfo.asp (509-335-3745)

Collecting and utilizing phenotypic data to minimize disease: A breeder's practical guide

Rhonda Hovan

Since its inception in 1966, the OFA has been providing information intended to help breeders reduce the incidence of genetic disease in dogs. Most breeders have found this service to be very helpful, and have seen important improvements in the health of their dogs through the diligent use of OFA data.

Yet there remains a widespread lack of understanding regarding the optimal use of phenotypic information in breeding programs. As a result, many breeders have not taken full advantage of the information available, slowing their progress toward minimizing disease. The methods of collecting and analyzing phenotypic data presented here offer breeders the opportunity to dramatically decrease the incidence of genetic disease in their breeding programs to a level often significantly below that reported as a breed average by OFA and other statistical databases.

A Comparison of Genotypic Tests to Phenotypic Tests

In recent years, DNA tests for numerous canine diseases have been developed, and this progress is expected to continue. Where available, such tests offer breeders direct information about the genes that an individual tested dog can contribute to his or her offspring. DNA tests are an example of genotypic tests, and are the gold standard as tests for disease causing genes. Breeders can be confident that DNA tests will provide them with very accurate information, leaving little room for an unexpected appearance of the gene in offspring.

full siblings are, on average, equally genetically similar to each other as they are to each of their parents.

However, DNA tests are not available for the majority of common canine diseases. Most tests intended to offer breeders health information about a dog's suitability for breeding, rely instead on an evaluation of the dog's physical status at the time of examination. These are called phenotypic tests, and include evaluations for hip and elbow dysplasia, many eye and cardiac diseases, patella and thyroid disease, and most current canine disease evaluations.

Fortunately for the dogs – but unfortunately for breeders attempting to reduce the incidence of disease – many harmful genes do not manifest as detectable disease during the prime breeding age of the dog, if ever. These dogs may appear normal, yet carry genes capable of causing disease. A number of types of gene actions can contribute to this confusion, for example: recessive genes, incomplete penetrance of the gene, variable expressivity of the gene, multiple genes involved in the disease, and even environmental influence on expression of the trait. Further, diseases that have a late age of onset such as certain eye and cardiac abnormalities, can result in normal phenotypes for a period of time, even when the disease gene is present.

In an attempt to compensate for these inherent flaws with common phenotypic tests, many breeders have long realized the importance of gathering test information on more than just the prospective sire and dam of a litter. Because standard pedigrees include only direct ancestors such as parents, grandparents, great-grandparents and so on, these are the relatives on which breeders usually focus when seeking additional health information. It is not uncommon for

reprinted from the orthopedic foundation for animals website, www.offa.org.

conscientious breeders to build pedigrees which are described as "three generations clear" for a disease, meaning that the sire and dam, the 4 grandparents, and the 8 great-grandparents have all tested phenotypically normal. Yet such breedings may produce less than satisfying results, as the disease genes may still be present, and affected offspring may still be produced.

Fortunately, there is an additional way of utilizing phenotypic test data which improves the likelihood of producing predictable results. It involves a different method of building pedigrees.

Vertical Pedigrees

Traditional pedigrees expand horizontally; that is, they are read from left to right with relatively few dogs appearing at the far left and increasing to the predominant number of ancestors listed to the right. Although the dogs to the left (the sire, dam, and grandparents) most directly impact the resulting offspring, there are only six of these contributing data on this type of pedigree. That is a small sampling of the relevant information that may actually be available. While many additional dogs are named on the right side of the page, these more distantly related dogs are less significant genetically than are those on the left.

A pedigree can also be constructed vertically, most easily using a three column format. A vertical pedigree of "Dog A" begins page left with Dog A and all of his full siblings (from one or more litters). The central column lists his sire and dam, and their full siblings; with the right column doing the same with the four grandparents. Clearly, vertical pedigrees can include many more first and second generation relatives than do traditional horizontal pedigrees.

The value of vertical pedigrees can be most fully appreciated through understanding an essential genetic principle that should correctly be the foundation of most complex breeding decisions. This principle is that full siblings are, on average, equally genetically similar to each other as they are to each of their parents. All of the littermates taken as a group represent various combinations of their parents' genes, and are good indicators of the *range of possibilities* that are likely to be passed on from any one of them. Likewise, phenotypic information about the aunts and uncles of a given dog, is equally as important as is that of the grandparents. Thus, dogs who do not even appear on traditional horizontal pedigrees, may be more significant genetically than are the more distant relatives who do. By overlooking these siblings, aunts, uncles, great-aunts, and great-uncles, the pertinent data base may be reduced by as much as four fold or more (the number of littermates for whom data might be available).

The broad data base that is accessible using vertical pedigree analysis gives breeders accurate information about any trait that cannot be tracked in a direct manner. Whenever multiple genes and/or other complex modes of inheritance are involved, a larger sampling will be more likely to contain enough individuals to indicate a pattern. Accuracy then, is dependent upon accumulating phenotypic information on as many of these direct and indirect relatives as possible.

A simple example should help illustrate how this works. Suppose that a breeder would like to compare two potential stud dogs, "A" and "B," with regard to their likelihood of producing normal hips. Written in standard horizontal form, their pedigrees with OFA hip status noted* are as follows:

		Paternal Gr-Sire "Fair"
	Sire "Good"	
		Paternal Gr-Dam "Good"
Stud Dog A "Fair"		
		Maternal Gr-Sire "Good"
	Dam "Good"	
		Maternal Gr-Dam "Good"

		Paternal Gr-Sire "Fair"
	Sire "Good"	
		Paternal Gr-Dam "Good"
Stud Dog B "Good"		
		Maternal Gr-Sire "Good"
	Dam "Good"	
		Maternal Gr-Dam "Good"

OFA Fair is in **Blue** type; OFA Good is in **Black** type, OFA Excellent is in **Green** type; Dysplastic dogs are in {red} type with brackets.

On the surface, both of these pedigrees appear to be making progress toward reducing the incidence of hip dysplasia. All other factors being equal, the investigating breeder might be persuaded by his more favorable hip rating to choose Stud Dog B. Imagine the frustration, then, if several of the resulting pups develop hip dysplasia.

Now expand the two pedigrees vertically, and compare the data that is available in this format:

1st Generation	2nd Generation (Parents, Aunts, Uncles)	3rd Generation (Gr-parents, Gr-Aunts, Gr-Uncles)
Stud Dog A "Fair" Sibs (7): **Fair** **Fair** **Good** **Good** **Good** **Excellent**	**Sire "Good"** Sibs (8): **Fair** **Fair** **Good** **Good**	**Paternal Grandsire "Fair"** Sibs (6): **Good** **Good**
		Paternal Granddam "Good" Sibs (8): **Good** {dysplastic}
	Dam "Good" Sibs (10): **Fair** **Fair** **Fair** **Good** **Excellent** {dysplastic}	**Maternal Grandsire "Good"** Sibs (?)
		Maternal Granddam "Good" Sibs (9): **Good**
Stud Dog B "Good" Sibs (9): **Fair** **Fair** **Good** {dysplastic} {dysplastic} {dysplastic} {dysplastic}	**Sire "Good"** Sibs (7): **Fair** **Good** **Good** **Good** {dysplastic}	**Paternal Grandsire "Fair"** Sibs (7): **Fair** **Fair** **Good** **Good**
		Paternal Granddam "Good" Sibs (?)
	Dam "Good" Sibs (6): **Fair** **Fair** {dysplastic} {dysplastic}	**Maternal Grandsire "Good"** Sibs (10) Fair {dysplastic} {dysplastic} {dysplastic}
		Maternal Granddam "Good" Sibs (6): **Fair** **Good** **Excellent**

An evaluation of the vertical pedigree reveals that Stud Dog A comes from a litter with predominantly normal hips, and this is consistent also with his sire, dam, and their siblings. Thus, the r*ange of possibilities* in his genetic package heavily favors normal hips.

In contrast, Stud Dog B dog comes from a litter in which half of the dogs are normal, and the other half are dysplastic (with one unknown). Furthermore, this is a pattern which can be traced back through his dam and maternal grandsire. Thus, despite his own "good" rating, Stud Dog B's *range of possibilities* may include a higher likelihood of transmitting hip dysplasia. This pedigree is not demonstrating progress toward reducing the incidence of affected dogs.

Additional Factors to Consider In Evaluating Vertical Pedigrees

Of course, not all vertical pedigrees will be as clear-cut as in the previous example. Further, diseases other than hip dysplasia may require a different process of analysis. Two of the most important variables to examine are:

1) the frequency of the disease in the vertical pedigree as compared to the frequency of the disease in the breed population, and
2) the location of the affected individuals on the pedigree.

Frequency of the disease in the vertical pedigree as compared to the frequency of the disease in the breed population

Because of the larger number of individuals represented on vertical pedigrees, sometimes very few pedigrees will appear completely free of affected dogs, as might be found using the traditional format. As illustrated with the example of Stud Dog A, even respectable and desirable pedigrees often contain an occasional affected dog. Therefore, a realistic goal for breeders is to use those pedigrees which demonstrate a significantly lower rate of disease as compared to the general breed population.

Since the incidence of any given disease varies from one disease to another, and from one breed to another, such factors should be taken into consideration when evaluating pedigrees. For example, with less common diseases, using completely disease-free vertical pedigrees may be the only way to maintain a low rate of disease in the breed. Diseases with a moderate or high breed frequency are most effectively managed by selecting individuals whose vertical pedigrees contain a large amount of data, and few affected dogs.

The location of the affected individuals on the pedigree

Of course, in addition to how many affected dogs appear on a vertical pedigree, their location on the page must also be taken into consideration. Clearly, dogs appearing farther to the left on the page have a more direct genetic impact than do those appearing toward the right. Further, multiple affected dogs within a single location may be an important factor. Thus, Stud Dog A's pedigree, with 2 dysplastic relatives scattered among 28 total dogs, would generally be considered to be a strongly normal pedigree. Yet even if nothing else were known about Stud Dog B except that he has 4 dysplastic siblings, this pedigree should be excluded from most breeding programs. With four affected dogs focused in the column farthest to the left, this pedigree has disease genes concentrated in a very influential position.

Collecting Data for Vertical Pedigrees

In many breeds and for many diseases, it is recognized that the availability of vertical pedigree data currently may be limited. This is partially due to the historical lack of understanding of the importance of such information. Now, through its commitment to breeder education, OFA hopes to stimulate a new and steady improvement in results, using this method of building and analyzing vertical pedigrees. If breed clubs also begin to emphasize the value of such pedigrees to their membership, it is reasonable to anticipate much wider availability of this data in the near future.

However, all breeders are encouraged not to wait for future change within their breed, but rather to create that change within their own breeding programs. Significant improvements are possible even in a relatively brief period of time, using very achievable methods of collecting data.

Building A Personal Data Base

Both long term breeders, and new hobbyists, can build a useable data base in as little as one or two generations. The most immediate information can be collected retrospectively by seeking examination results on the siblings of dogs currently being considered for breeding. Where available, results on those dogs' aunts and uncles can also be gathered. While such data may be spotty, it is worth making the effort to contact owners and breeders of those siblings, aunts, and uncles. OFA's online database is also searchable by siblings, and may provide some additional information.

Perhaps more effective, however, is to begin building a data base of all future puppies that a breeding program produces. There are two very effective strategies which can be used in combination to facilitate this process.

Strategy 1

Beginning prior to the sale of a puppy, the breeder typically discusses with prospective buyers, health examinations that have been performed on the sire, dam, and other relatives. This is the ideal time to explain to the buyer that his new puppy will also have valuable information to contribute to the next generation of the breeding program. Just as the current buyer appreciates and trusts a breeder who is conscientious about health issues, so the buyer will understand that he has a role in helping the breeder make good decisions about future litters. The breeder should explain clearly what examinations are expected, at what ages these are performed, typical costs, and other factors such as convenience (local health screening clinics if available; or if any traveling may be required).

In breeds for which there are numerous, expensive, or very inconvenient genetic screening tests, breeders may need to compromise to keep those burdens at a level which would be acceptable to most reasonable buyers. In such cases, the breeder may choose to focus efforts toward the most common or most debilitating diseases in the breed, or those which have the greatest impact on the individual breeding program. Because of practical considerations, it may be necessary to request only certain specific health screening examinations; and have the flexibility to forego results on diseases of lower priority.

Nonetheless, while agreeable at the time of sale, some buyers are still reluctant to follow through when the time comes. These buyers can often be coaxed to participate by offers of assistance, such as the breeder taking the dog for the exam, or the breeder offering to groom the dog prior to the examination. For greater assurance of compliance, many breeders find it effective to take a refundable deposit at the time of sale. This may be set approximately equal to or slightly higher than the costs of the health examinations, so that the buyer has incentive to complete the testing. It is also helpful to include the request for health screening examinations in the written sales contract or guarantee.

Strategy 2

The factors which take the greatest toll on buyer compliance are elapsed time, and lack of contact with the breeder. In diseases such as hip dysplasia, for which final OFA clearances are not available until the dog is two years old, it may have been 18 months or more since the breeder last spoke with the owner of the puppy. Especially if all is going well with the pup, the buyer may no longer be as concerned with health issues as he was prior to the purchase. Further, buyers are more reluctant to leave a refundable deposit when the term of the deposit extends out nearly two years.

To overcome all of those issues, breeders are encouraged to take advantage of early preliminary examinations in diseases for which preliminary evaluations have a high percentage of accuracy. For example, OFA preliminary hip evaluations

done on dogs at 3 to 6 months of age, have an overall reliability of 89.6% for dogs graded as normal, and 80.4% for dogs graded as dysplastic.[1] That is, nearly 90% of dogs graded normal at 3-6 months of age on OFA preliminary evaluations, remained normal at 2 year final evaluations; and about 80% of those diagnosed as dysplastic between 3-6 months of age, remained dysplastic as adults. These percentages are even higher for dogs who were graded as "Good" or "Excellent", and "Moderate" or "Severe." Most of the dogs whose 2 year evaluations differed between normal and abnormal as compared to their 3-6 month evaluations, were those who were graded "Fair," "Mild," or "Borderline" on their preliminary report. The status of such dogs can be determined with greater accuracy with increasing age, and when possible, even non-breeding dogs in those categories should be resubmitted as adults.

A number of other factors also contribute to the concept that obtaining early preliminary hip x-rays is a nearly ideal plan for breeding programs.

This high degree of reliability means that OFA preliminary hip evaluations can have great value for breeders constructing vertical pedigrees. Because of the probability that most of the puppies a breeder produces will never have final hip x-rays submitted for certification, the possibility of obtaining much of that otherwise lost data on preliminary examination is very attractive.

A number of other factors also contribute to the concept that obtaining early preliminary hip x-rays is a nearly ideal plan for breeding programs. First, current vaccine protocol recommends the last puppy vaccination at approximately 3-1/2 to 4 months of age. Since presumably all pups will be going to the veterinarian for this vaccination, it is usually a very convenient time to schedule the preliminary hip x-ray. Further, most pups of this age do not need sedation or anesthetic to obtain good positioning, thereby reducing cost and perceived risk to the dog. This time frame is also well within the period that the breeder usually has the most contact and influence with the buyer. All of these considerations combine to produce excellent compliance, and thus build a much larger data base than most breeders are able to obtain with 2 year hip evaluations. This greatly increased volume of data more than compensates for the slightly decreased rate of accuracy of preliminary OFA hip evaluations.

Breeders using this recommended early preliminary method of data collection may also choose to follow certain other associated procedures. The breeder may request that each puppy owner send the x-rays to the breeder, rather than directly to OFA. This provides the breeder with the opportunity to evaluate the films for correct positioning, and the breeder can then take advantage of OFA's reduced price for preliminary hip evaluations of littermates submitted together. OFA's preliminary evaluation service provides the important advantages of known accuracy and consistency of preliminary evaluations; and of a written report that is widely accepted among breeders nationwide. Please note, however, that even when littermates' x-rays are submitted together, the OFA reports will be released only to the owners or co-owners as represented on the accompanying information cards.

An additional and highly useful advantage for breeders obtaining early preliminary data, is that the information gathered from a relatively recent litter may be taken into consideration as part of the decision making process prior to breeding subsequent litters with the same or similar parentage. Particularly in the case of a bitch, genetic information about her offspring that is not available until they are two years of age, sometimes comes too late in her breeding career to influence decisions appropriately. Preliminary genetic screening permits breeders to use the information in a much more timely manner, amplifying its immediate value to the breeding program.

Beyond Disease

As breeders become familiar with evaluating dogs or potential breedings using vertical pedigrees, they will find that its principles can be applied equally well to many genetic characteristics other than disease. Any trait for which there

is a complex mode of inheritance can be examined more successfully using vertical pedigrees, than using the traditional horizontal format. And in fact, most conformation and performance characteristics are ideal candidates for vertical pedigree analysis; because achieving correct balance, breed type, movement, and desired temperament clearly is an extremely complicated task!

Vertical pedigrees can assist breeders in identifying "families" that have a strong likelihood of producing highly desirable characteristics, or those families in which less desirable traits may predominate. Careful examination of vertical pedigree data can help breeders avoid one of the most common mistakes of many breeding programs. This is that breeders frequently select an individual dog who manifests excellence in certain characteristics, and bring that dog into a breeding program hoping to add or strengthen those characteristics in their lines. Although they may have given consideration to that dog's sire and dam, they often overlook the fact that the desired traits are weak or absent in the littermates. Unfortunately, a single "star" littermate is likely to produce exactly that: litters that may contain a promising individual, but among more ordinary, possibly disappointing siblings.

Consistent, predictable qualities are typically produced only when vertical pedigrees demonstrate those qualities consistently.

Consistent, predictable qualities are typically produced only when vertical pedigrees demonstrate those qualities consistently. In the majority of cases, dogs who are known as "prepotent" are those dogs whose vertical pedigrees show strong evidence of this consistency. Therefore, high quality dogs whose siblings and aunts and uncles are of similar high quality and desirability, are the dogs who will contribute that quality and desirability most reliably to their own offspring. Contrary to some common beliefs, it is not necessary for a dog to be line-bred to be prepotent, providing there is strong consistency within its first and second generation vertical pedigree. Using vertical pedigree data to achieve consistency without linebreeding also provides breeders with the opportunity to maintain a richer and more vigorous gene pool within their breeding program.

A Dependable Breeding Philosophy

The concepts advocated herein are based on sound genetic principles, and are designed to help breeders manage many types of complicated real world breeding decisions. The OFA recognizes that most hobby and competition breeders have admirable intentions, but are faced with a challenging blend of art and science in which one of the most frustrating aspects is the seeming unpredictability of results. Vertical pedigree construction and analysis is a very powerful tool which can assist in reducing surprises and improving predictability. With this method, progress toward one's goals is usually more assured, and the risks of unexpected and potentially devastating disease is decreased. This technique can help breeders build a foundation which can become stronger and more dependable with every successive generation. By working closely together, OFA and the conscientious breeders who depend on its services, can continue to make significant strides toward protecting and advancing the health of dogs.

Rhonda Hovan is a breeder, exhibitor, and judge of Golden Retrievers, and serves on the Board of Directors of the OFA. As a canine health and genetics writer, she has won the Veterinary Information Network Health Education Award.

1 Corley EA, Keller GC, Lattimer JC, et al. Reliability of early radiographic evaluations for canine hip dysplasia obtained from the standard ventrodorsal radiographic projection. J Am Vet Med Assoc 1997; Vol 211, No. 9; 1142-1146.

Canine Molecular Genetic Diseases

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Focal Point

- The explosion in DNA-related technology offers a unique opportunity to greatly reduce the frequency of serious genetic diseases in dogs.

Key Facts

- The molecular genetics revolution makes detecting carriers of genetic diseases feasible.
- Genetic diseases are a major health problem in purebred dogs, but reducing the occurrence of these primarily recessive diseases is difficult.
- Once a DNA test to detect carriers of a particular disease gene is available, that disease can almost be eliminated through appropriate breeding.
- DNA tests are now available for approximately 15 canine disease genes.
- Genetic testing and counseling for genetic diseases are likely to become standard in veterinary medicine.

ABSTRACT:

The field of molecular genetics has generated substantial information about how genetic diseases are inherited. Discovery of the causative gene for a particular disease can allow a DNA test to be developed to identify carriers of that gene. Selective breeding can then be used to reduce the frequency of that disease in the general population. This article reviews basic genetic principles and discusses how DNA tests are developed. Information about DNA tests currently available to detect inherited disease in several canine breeds is also provided.

A molecular genetics revolution is being fueled by funding for the Human Genome Project and the hope that gene therapy can revolutionize the prevention and treatment of human diseases. *Molecular genetics* refers to the DNA- and RNA-based technologies undergoing explosive growth in such areas as disease gene discovery, genetic engineering, paternity testing, and other forensic uses.

The impact of molecular genetics may initially be more profound in the veterinary field than in human medicine. For example, discovery of the causative gene for a disease often leads to a DNA test to detect disease gene carriers. The frequency of the disease gene, and thus the frequency of the disease, can then be greatly reduced through selective breeding. However, when a DNA test to detect carriers of a human genetic disease becomes available, the disease gene frequency in the population is not reduced as rapidly because humans rarely practice selective breeding.

GENETICS PRIMER

Veterinarians do not need to be professional geneticists to develop a fundamental understanding of the effects of molecular genetics on veterinary medicine. However, knowledge of basic genetic principles is required; readers who are familiar with these principles may proceed to the section on Establishing a DNA Test for a Genetic Disease.

Dominant versus Recessive Diseases

With the exception of genes on the X and Y chromosomes (i.e., the sex chromosomes), genes come in pairs. Non-sex chromosomes are called *autosomes*, and the paired genes on them are termed *autosomal genes*. Diseases caused by mutations in autosomal genes are classified according to whether one or two copies of the mutant gene are needed to produce disease. If only one copy of a mutant gene is needed to produce the disease and the other copy of the gene is normal, the resultant disease is called *autosomal dominant*. If both copies of the gene must be mutant to cause disease, the term *autosomal recessive* is used.

Dominant diseases tend to be less troublesome to breeders than are recessive diseases: A dominant disease is often detected before an animal is bred, and thus that animal is not used for breeding. Dominant diseases can still be problematic, however, if they develop after breeding age is reached (late onset) or are incompletely penetrant. Examples of late-onset diseases in dogs include some forms of cataracts, epilepsy, and hip dysplasia, although whether these diseases are dominant is unknown. *Incomplete penetrance* refers to absence of disease despite presence of the dominant disease gene. For example, a dominant disease gene that causes disease 50% of the time is 50% penetrant. Thus absence of a disease in a dog's parents and grandparents does not indicate absence of an incompletely penetrant dominant disease gene; however, even an incompletely penetrant dominant disease gene should have caused the disease to surface somewhere in the animal's ancestry.

Autosomal recessive genes are entirely different. Identifying carrier animals usually is not possible until mating of two previously unknown carriers produces one or more affected offspring. By this time, the animal has already been bred. In addition, when undiagnosed carriers are mated to noncarriers, 50% of their offspring will carry the disease gene--and no one will know.

A classic example of the problems associated with recessive diseases is canine copper toxicosis (CT) in Bedlington terriers.(1) CT is an autosomal recessive disease that causes copper accumulation and resultant liver failure; affected animals become ill and, if untreated, die of liver disease at 2 to 5 years of age.(1) Because disease onset is at a relatively late age, affected animals are often bred before being diagnosed. Affected animals can be diagnosed at 1 year of age by measuring copper in a liver biopsy, but no test for carriers (other than test-mating) existed before the advent of DNA technology.(2) Because of the high disease gene frequency, CT has been a substantial problem for the breed: 25% of Bedlington terriers are affected, 50% are carriers, and only 25% are clear of the disease gene.

Test-Mating

One historic method to detect carriers is test-mating. The animal being assessed is bred to a known affected animal, and the progeny are evaluated for the presence of disease. If affected puppies are produced, the animal under evaluation is a carrier. If exactly five puppies are produced and none is affected, the odds are 31 of 32 (about 97%) that the dog in question is not a carrier. These odds are derived as follows: If the dog being tested is a carrier, each puppy has a 1 of 2 (50%) chance of being affected. If all five puppies are free of disease, the probability that the dog being tested is affected is (1 of 2)(5) (i.e., one half raised to the fifth power) or 1 of 32.

This approach has several disadvantages. If the animal being tested is a carrier, test-mating will produce affected animals, which must then be euthanized or placed with owners willing to treat them. With late-onset disease, such as CT, the progeny should be maintained by the breeder (or at least be under the breeder's control) until a liver biopsy can be performed to determine whether the puppies are affected. In

breeds that tend to have small litters, more than one test-mating may be needed to attain five progeny. As a result of all these disadvantages, only a handful of Bedlington terriers have been cleared of CT through progeny testing during the several decades that breeders have dealt with the disease. An excellent and complete discussion of the advantages and disadvantages of test-mating has recently been published.(3)

X-Linked Diseases

Females have two X chromosomes, whereas males have only one. Most X-linked disease genes are recessive; thus carrier females, which have one disease gene and one normal gene, do not have the disease. However, males that have the disease gene do exhibit the disease because a normal gene is not present. Hemophilia is an X-linked recessive disease that occurs in both humans and dogs. As with autosomal dominant genes, X-linked genes are less of a problem for breeders than are recessive genes because the male ancestry of potential breeding stock is likely to have exhibited the disease. (The Y chromosome is so small that Y-linked diseases are very rare.)

ESTABLISHING A DNA TEST FOR A GENETIC DISEASE

Developing a DNA test for a genetic disease is always complex, but the process is simplest when a disease has the same genetic cause in more than one species and the disease gene has been cloned in one species (in this context, *cloned* means that the causative gene has been identified and its DNA isolated). For example, von Willebrand's disease (vWD) is a bleeding disorder that affects humans and dogs. The human disease gene had already been cloned and the DNA sequence established. To develop the test for a canine breed (e.g., Scottish terrier), the gene was sequenced in an affected Scottie and compared with the normal sequence, the causative mutation found, and a DNA test developed to differentiate between normal and mutant DNA.(4) A different causative mutation was identified in Shetland sheepdogs(5) and another in Doberman pinschers.(6) The Doberman mutation is shared by Manchester terriers, poodles, and Pembroke Welsh corgis.

Although this is the most straightforward approach, it is not always simple. Sequencing the canine vWD gene was difficult and time-consuming. The vWD gene is very long and has many introns (noncoding regions interspersed among coding regions). Using messenger RNA material (i.e., material from which the introns have already been removed) to sequence a gene is therefore desirable. However, because messenger RNA for canine vWD is produced only in blood vessels, vWD messenger RNA material had to be isolated from tissue samples containing blood vessels.

Unlike the situation with vWD, the causative gene is unknown in most genetic diseases. However, geneticists can usually identify a number of potential causative genes (referred to as *candidate genes*). For example, a fairly large number of genes possess mutations that have produced cataracts in humans and mice; each of these genes is a candidate for causing cataracts in a canine breed. A candidate gene may prove to be causative for cataracts in one or a few breeds but not in others, in which case the remaining candidate genes must be evaluated.

The first step in evaluating whether a candidate gene is the actual causative gene is to establish genetic variation in a piece of DNA close to the candidate gene; these genetically varying pieces of DNA are usually microsatellites. DNA comprises a sequence of four nucleotide bases--adenine, guanine, cytosine, and thymine, which are identified using the first letter of each (A, G, C, and T, respectively). Approximately 1% of DNA is coding DNA (i.e., codes for a gene product); the remaining 99% is noncoding, and its purpose remains unclear. Perhaps it provides spacing for the genes themselves (the coding DNA). In noncoding regions, DNA may repeat itself 20 or so times. Repeats (or runs) composed of two nucleotide bases are termed *dinucleotide repeats*; repeats of three and four nucleotide bases are called *trinucleotide* and *tetranucleotide repeats*, respectively. These types of repeats in the DNA are also collectively referred to as *microsatellites*. (The term *satellite* DNA was previously used for much larger runs of repeat DNA.)

Microsatellites are useful because they show a large amount of genetic variation in the population. This variation is because chromosomes pair up at meiosis and mispairing can occur in an area of a repeat (Figure 1). If recombination occurs while the repeats are mispaired, two additional repeats of differing sizes are generated. In Figure 1, two $[CA]_{10}$ repeats mispaired and recombination generated $[CA]_{11}$ and $[CA]_{9}$. Through this process and over evolutionary time, microsatellites tend to accumulate size variants in the population.

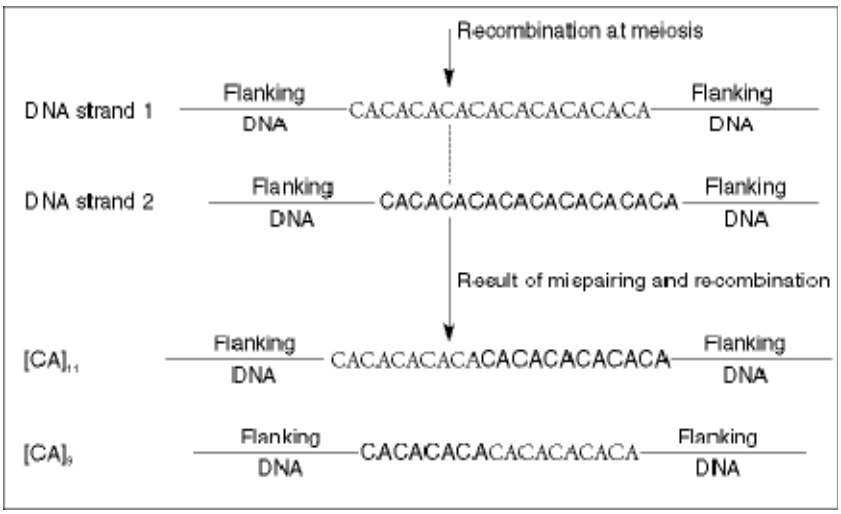

Figure 1—Illustration of microsatellite mispairing at meiosis, which generated a $[CA]_{11}$ and a $[CA]_9$ from two original $[CA]_{10}$.

Mammalian genomes, including that of dogs, have tens of thousands of microsatellites that can be used as a ready source of genetic variation. The size of microsatellite genetic markers can be detected by gel electrophoresis (Figure 2); the larger the DNA is, the slower it migrates in the gel. Each marker (or allele) is inherited like any other DNA. In Figure 2, dog 1 has alleles 24 and 22, dog 2 has alleles 24 and 20, and dog 3 has alleles 22 and 20.

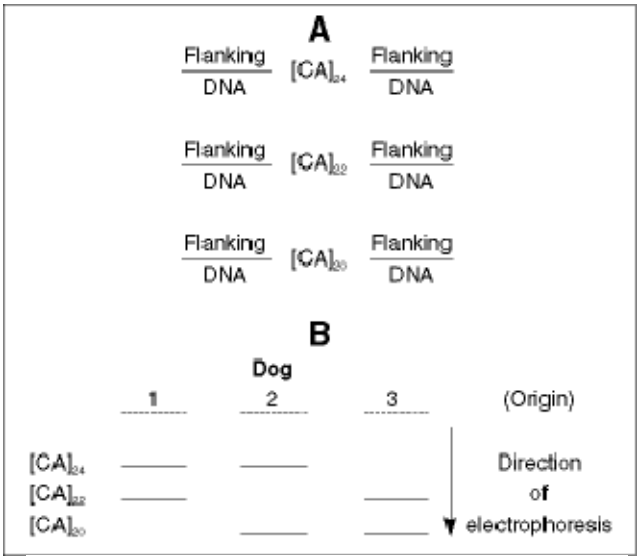

Figure 2—*(A)* The canine population being studied has three size variants (24, 22, and 20) of a particular CA repeat. *(B)* Results of a gel electrophoresis test to detect size variants. Each column represents a canine DNA sample placed at the *origin*; the DNA then undergoes electrophoresis. The different-sized CA repeats migrate different distances.

This article describes two uses of microsatellite markers. In the first use, a specific marker closely linked to a candidate gene is selected. A microsatellite marker provides the genetic variation needed to determine whether the candidate gene is being inherited concomitantly with the disease gene--a necessary event if the candidate gene is actually the disease gene. Alternatively, microsatellites can be used for a genome-wide scan.

Linkage studies are used to evaluate whether a candidate gene is actually the disease gene (because of the difficulties in sequencing studies, the linkage approach is more efficient than is wholesale gene

sequencing to detect causative mutations). To perform a linkage study, 10 to 20 pedigrees (each containing a minimum of two affected dogs) are collected from the breed to be studied, and a microsatellite associated with the first candidate gene is established. It is necessary to have a detectable genetic variation associated with the candidate gene to test whether that particular genetic variation is co-inherited with the disease gene in the pedigree.

Figure 3 illustrates this method. For this example, it is assumed that the microsatellite used is closely associated with the candidate gene and that either allele (1 or 2) can be inherited in the breed being studied. The pedigrees are tested to determine whether alleles at the disease gene and candidate gene are inherited together (a process referred to as *co-segregation*). If the candidate gene does not co-segregate with the disease gene (i.e., if it is not genetically linked), that candidate gene is excluded as a disease gene and subsequent candidate genes are then similarly tested until one is found to be closely linked to the disease gene. When one is found, DNA in the vacinity is sequenced to search for a causative mutation, which, in turn, leads to a DNA test.

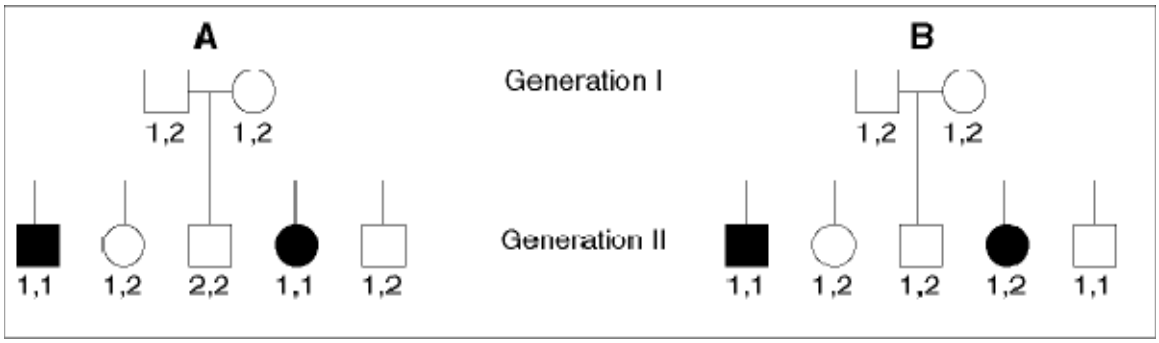

Figure 3—An example of *(A)* co-segregation and *(B)* lack of co-segregation of a candidate gene–associated microsatellite with a disease gene. The numbers below each symbol indicate the microsatellite typing for that animal. In both *A* and *B*, the parents *(Generation I)* are heterozygous for the two alleles (i.e., they each have alleles 1 and 2). *(A)* Both affected offspring *(Generation II)* have the same type (1,1), and none of the unaffected progeny has that type. These data are consistent with co-segregation of the disease gene and candidate gene, suggesting that the disease gene is linked to marker type 1 in both parents. However, this pedigree could behave in this manner through chance, even if the genes are unlinked; thus to establish linkage, another 10 to 20 pedigrees would need to be similarly studied to show a statistically high probability of linkage. *(B)* Close linkage is excluded because the affected siblings have different allele types (called *genotypes*), and unaffected siblings share the genotypes of affected siblings. (□ = males; ○ = females; ■ = affected male offspring; ● = affected female offspring)

Although it was successfully used to identify the causative gene for progressive retinal atrophy (PRA) in Irish setters,(7,8) the candidate gene approach is not always productive. If the causative gene has not been discovered in any species, it cannot be included on the candidate gene list. If a candidate gene search is unsuccessful, a genome-wide scan (i.e., the second use of microsatellite markers) is performed.

A large number of DNA markers (microsatellites) have been established for dogs. These microsatellites are not known to be associated with any particular genes but are merely distributed, more or less randomly, across the canine genome. Sufficient DNA markers have been developed to saturate the canine genome--regardless of where a disease gene is located on a chromosome, one or more established DNA microsatellite markers are nearby.

The same type of pedigrees used to screen candidate genes are used to perform a genome-wide scan. The DNA microsatellite markers are individually examined to detect co-segregation with the disease gene. Linkage is usually found by the time 200 markers have been examined, although scrutiny of 400 to 500 markers may be needed to find a close linkage.

Once a linkage between a microsatellite marker and a disease gene is found, it can be used to develop a pedigree linkage test (Figure 4). The test can then be used to counsel breeders about likely disease genotypes in some of their dogs. Because a DNA marker of this type is always somewhat distant from the disease gene, a small chance of error is possible because of genetic recombination (a DNA event that can separate the two alleles). Thus counseling is done in terms of probabilities (e.g., a 95% probability that a given dog is clear, a 95% probability that the dog is a carrier). Another disadvantage of this approach is that the pedigree used to establish a linkage phase must include an affected dog; thus testing of individual dogs is impossible. Consequently, dogs in many pedigrees cannot be evaluated because

their pedigree does not include an affected animal. Because multiple dogs must be tested, this approach is more expensive.

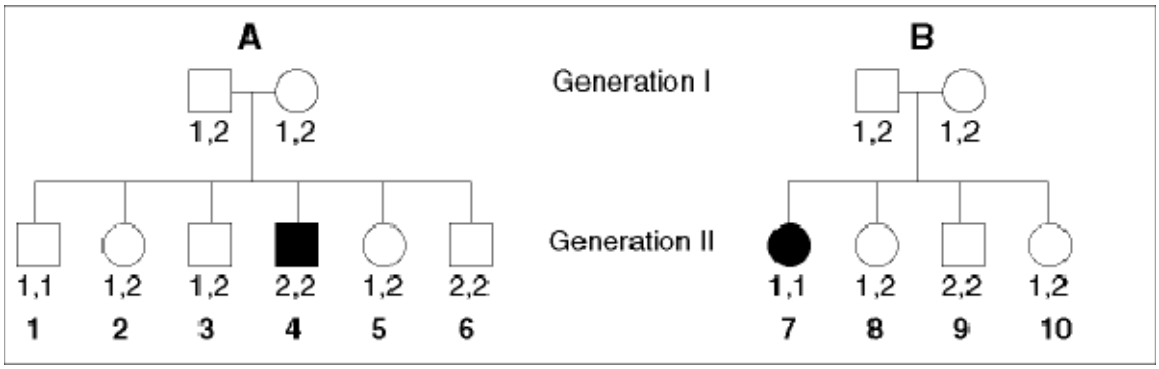

Figure 4—A pedigree linkage test. It is assumed that the microsatellite being studied, with alleles 1 and 2, has previously been shown via a genome-wide scan to be linked to the disease gene. The marker types are shown below each animal. In *Generation II*, the animals are numbered for easy reference. *(A)* Animal 4 establishes the linkage phase for the entire pedigree. It is affected and is type 2,2, which indicates that the disease allele is on the chromosome with marker 2 and was received from both parents. This allows the following conclusions to be made about the other siblings: 1 is clear; 2, 3, and 5 are carriers; and 6 can be expected to have the disease (such conclusions always have a slight potential to be erroneous because of occasional genetic recombination). *(B)* The same disease gene, breed, and marker are present, but the linkage phase is different. In this example, the disease alleles travel on chromosomes with marker 1. Animal 7 reveals this; it is affected and type 1,1. Furthermore, animal 9 is clear, whereas it would have been classified as affected in the pedigree in example *A*; this illustrates the need to have a typed, affected animal in the pedigree to establish linkage phase when using a pedigree linkage test. (□ = males; ○ = females; ■ = affected male offspring; ● = affected female offspring)

If the linked marker is sufficiently close to the disease gene, linkage disequilibrium may be present and can lead to a superior DNA test than can be created using the pedigree linkage test. Linkage disequilibrium occurs when a particular marker allele is associated with the disease allele in a disproportionately high frequency while another marker allele is highly associated with the normal gene (Figure 5). Linkage disequilibrium allows a pedigree linkage test to be converted to a linked marker test and permits testing of individual dogs rather than pedigrees. As with a pedigree linkage test, probabilities must be used when counseling breeders.

	Disease Phenotype	
Marker Genotype	*Affected*	*Unaffected*
2,2	25	4
1,2	2	151
1,1	1	240

Figure 5—Linkage disequilibrium. Dogs diagnosed as affected predominantly have a 2,2 marker genotype, even though the 1 marker allele is much more common in the population. Thus there is a strong disequilibrium: The 2 marker allele is predominantly on chromosomes linked to the disease allele, whereas the 1 marker allele is predominantly on chromosomes linked with the normal allele. The unaffected column includes both disease gene carriers (which are predominantly 1,2 genotype) and clear animals (which are predominantly 1,1 genotype). With these data, veterinarians can counsel breeders with better than 95% confidence that 1,1 animals are clear, 1,2 animals are carriers, and 2,2 animals are affected.

SOME CURRENTLY AVAILABLE CANINE DNA TESTS

Copper Toxicosis in Bedlington Terriers

As discussed, CT has been a problem in Bedlington terriers. However, a linked microsatellite marker to the CT gene was found, and a pedigree linkage test was developed.(9) Strong linkage disequilibrium was

later observed--the 2 marker allele was more than 95% associated with the disease allele, and the 1 marker allele was more than 95% associated with the normal allele--and led to the development of a linked marker test.(10) At least half the breeding population of Bedlington terriers has been tested,(10) and the disease frequency should be dropping rapidly. Because of the late onset of CT, this test is also useful to identify potentially affected animals, whether pets or breeding stock, so that they can be definitively diagnosed (via liver biopsy) and treated.

von Willebrand's Disease

Scottish Terriers

von Willebrand's disease is severe in affected Scotties and usually fatal in puppyhood. The vWD gene was sequenced, the causative mutation discovered, and a DNA test developed.(2) Carrier frequency is about 10%.

Shetland Sheep Dogs

Like in Scotties, von Willebrand's disease is severe in affected Shelties and usually fatal in puppyhood. The mutation differs from that in Scotties, and a different DNA test is required.(5) The carrier frequency in this breed is also about 10%.

Doberman Pinschers, Manchester Terriers, Poodles, and Pembroke Welsh Corgis

In Dobermans, vWD has been confusing. The disease is mild, and spontaneous bleeding is unusual; however, dogs undergoing surgery or suffering trauma are at risk for serious bleeding. Results of the vWD factor (protein) assay have also been very confusing. Low factor levels are very common in the Doberman population--up to 75% of animals have abnormal values(11)--but levels also vary widely over time. The mild nature of the disease made identifying affected dogs truly difficult. Various genetic hypotheses, including dominant inheritance, abounded.

The disease gene was sequenced in an affected Doberman, the causative mutation discovered, and a DNA test developed.(6) This work immediately clarified much about vWD in Dobermans. In Dobermans, as in Scotties and Shelties, vWD is a simple autosomal recessive disease. Unlike the situation in Scotties and Shelties, in which the mutation completely disables the gene, the Doberman gene is only partially disabled. About 10% of the normal level of von Willebrand's factor is produced in affected animals, providing some protection against bleeding and making the disease much milder. Also, the disease gene frequency is very high in Dobermans: Approximately 30% of Dobermans are affected, and 50% are carriers, leaving only 20% of Dobermans completely clear of the disease gene.

Using only Dobermans that are clear of the disease gene for breeding is ill advised because it unnecessarily narrows the gene pool; it also places an undue hardship on breeders who have developed good lines of animals only to discover that all or most are affected with vWD or are carriers of the vWD gene. In addition to breeding clear animals to clear animals, a recommended breeding strategy is to breed carrier dogs to clear dogs. Litters produced by carrier–clear matings are half carriers and half clear animals; no affected animals are produced. This strategy further reduces the disease gene frequency and, in subsequent generations, eventually eliminates the gene. Even affected stud dogs with particularly favorable characteristics can be bred to clear bitches. (Affected bitches probably should not be bred because of the risk of bleeding during delivery or cesarean section.)

DNA testing for vWD is also useful in pets, especially Dobermans, to identify affected animals, which are at increased risk of bleeding during surgery. A forewarned veterinarian can take appropriate precautions.

The same disease-causing mutation that is present in the Doberman vWD gene is present in Manchester terriers, poodles, and Pembroke Welsh corgis, albeit at a lower frequency. As in Dobermans, vWD is mild in these breeds. Frequencies are listed in Table I.

Renal Dysplasia in Shih Tzus, Lhasa Apsos, and Soft-Coated Wheaten Terriers

Renal dysplasia is a disease of inadequate kidney development that leads to kidney failure, which is common in these and other breeds. Although the genetic situation is not completely clear, a current favorite hypothesis is that defects in two different genes are required to produce the disease.

A linked marker to at least one of the genes has been found in these three breeds.(12) The marker is in linkage disequilibrium in all three breeds--one marker allele is associated with the disease allele approximately 80% of the time--a finding that led to the development of a linked marker test for these three breeds.

Pyruvic Kinase Deficiency in Basenjis
A defect in the gene coding for pyruvic kinase of erythrocytes causes an autosomal recessive hemolytic anemia in the Basenji breed.(13) The causative gene has been identified.

Phosphofructokinase Deficiency in English Springer Spaniels
A defect in the gene coding for phosphofructokinase leads to an autosomal recessive disease in this breed. The enzyme defect causes hemolytic anemia and muscular weakness. The causative mutation has been identified.(14)

Progressive Retinal Atrophy
Irish Setters
The gene causing the type of PRA leading to retinal disease and blindness in Irish setters has been identified.(7,8)

Cardigan Welsh Corgis
The gene causing PRA in Cardigan Welsh corgis has been identified.(15)

Portuguese Water Dogs, Chesapeake Bay Retrievers, and English Cocker Spaniels
A linked marker test for PRA has been developed for Portuguese water dogs, Chesapeake Bay retrievers, and English cocker spaniels.

Globoid Leukodystrophy in Cairn and West Highland White Terriers
The gene causing globoid leukodystrophy in Cairn and West Highland white terriers is the same as that causing a similar disease in humans. The disease is caused by a missing enzyme, galactocerebrosidase, which is required for production of stable and healthy myelin (the insulation around nerves in the central and peripheral nervous systems). No effective therapy is available for animals affected by this fatal neurologic disease. The mutation in these breeds has been identified.(16)

Congenital Stationary Night Blindness in Briards
Briards are affected with a recessively inherited retinal disorder characterized by congenital night blindness with various degrees of visual impairment under photopic illumination. Day vision in affected dogs ranges from normal to profound blindness.(17) The disease was initially described in Swedish dogs as a stationary disorder analogous to human congenital stationary night blindness. It is now believed to have a progressive component and has been termed *hereditary retinal dystrophy*.

Cystinuria
Cystinuria is an autosomal recessive disease caused by a defective kidney transporter of cystine and other amino acids. The cystine precipitates in acid urine and forms crystals and calculi (stones). The disease is characterized by difficulty in urination, blood-tinged urine, crystals and calculi in urine, or complete inability to urinate (especially in male dogs).

THE ROLE OF VETERINARIANS

It is appropriate for veterinarians to stay well-informed about DNA testing for genetic diseases and to advise breeders and pet owners about how such tests might be used. For example, breeders and owners of Dobermans should be made aware of the vWD problem in this breed and that a DNA test is available. Most breeders of breeds affected by vWD are confused about the differences between the new DNA testing and the old factor assay, which had notoriously variable results. Veterinarians can help educate breed fanciers about the differences between a DNA test (which provides life-long genotyping, even for carriers) and such phenotype assays as vWD factor assays.

Veterinarians should also offer breeding advice to help eliminate the causative gene(s) without unduly narrowing the gene pool. Doberman owners should be advised about the potential usefulness of DNA testing for vWD in case surgery is required later. Conversely, offering vWD testing to an owner of a Scottie who has no plans to breed the animal would be impractical--if the dog was affected, hemorrhagic disease would have been obvious. Knowing the carrier status of a dog that is not going to be bred is irrelevant.

Approximately 73% of canine patients seen in private veterinary practices in the United States are breed identifiable (purebred).(18) Veterinarians need to recognize the potential effect of genetic diseases on their practices. Many genetic diseases directly affect diagnosis, short-term treatment, and long-term care in addition to breeding practices.

THE FUTURE

Intensive research is underway on a number of canine genetic diseases, including hip dysplasia, progressive rod–cone degeneration (a type of PRA), cataracts, epilepsy, cardiomyopathy, and deafness. Simple DNA diagnostic tests will eventually be available to detect most canine genetic diseases. Economic incentives will spur progress toward common disorders affecting popular breeds. However, DNA tests for rarer diseases affecting breeds with fewer dogs will also be developed, fueled by the molecular genetics activity in human genetics.

Veterinarians must recognize that their practices are likely to change rather dramatically in terms of genetic diseases. The change will shift the focus from diagnosis and management to prevention using genetic testing. Veterinarians should keep abreast of advances in molecular genetics so that they can advise their clients about DNA testing for genetic diseases and counsel them on breeding choices. The diseases for which DNA tests are currently available represent only the beginning.

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The Genetic Test for Persian-related PKD: Will it be constructive or destructive?

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A genetic test is the hope for every disorder. Once one is found, it is a double-edged sword: Its use can enable breeders to improve a breed or devastate it. Cat breeds have closed gene pools; in other words, the diversity of genes in a given breed is fixed. However, once a genetic test is developed that allows breeders to positively determine if a cat is affected or a carrier of a defective gene, many owners are likely to remove all of these cats from their breeding stock. It is important to carry on lines. A test that should be used to help maintain breed diversity should not result in limiting it.

The wide scale elimination of 38% of the breed in a short period of time would put a significant negative pressure on the gene pool – even one as large as the Persian breed.

The Dangers

It is important that breeders and owners are educated on how genetic tests should be properly interpreted and used. History in other domestic animal species has shown that breeders can be successful in reducing breed-wide genetic disease through testing and making informed breeding choices. You should remember, however, that there are also examples of breeds that have actually experienced more problems as a result of excessive culling and restriction of their gene pools.

Concurrently preserving the diversity of the gene pool over the next few generations while at the same time eliminating the defective gene is the most practical and desirable way to manage the disorder.

Polycystic Kidney Disease (PKD)

PKD is an autosomal dominant genetic disorder in Persian and Persian-related cats. Affected cats inherited the defective gene from one of their parents, who was also affected, and can pass the gene to approximately half of their offspring. Of Persians in the United States, 38% carry the defective gene, and therefore are affected with polycystic kidney disease.

Up until now, the only diagnosis of PKD was through abdominal ultrasound of the kidneys. At ten months of age, 98% of affected cats can be diagnosed by ultrasound. Now, with the (cheek swab) DNA test for PKD that has been developed in Dr. Leslie Lyon's lab at UC-Davis, affected cats and kittens can be identified at any age.

The standard recommendation to manage autosomal dominant genetic disorders is to not produce, and therefore not breed affected cats. To produce the next generation of a line, a normal full sibling of an affected cat, the normal parent, or a normal prior-born offspring can be used to replace the affected cat in the breeding program. By replacing, but not eliminating breeding lines, genetic diversity can be maintained.

The only difference with the introduction of the DNA test for PKD is the age of diagnosis. The genetic test now allows breeders to determine which kittens should be placed in breeding homes.

The availability of the genetic test creates an important opportunity for breeding decisions regarding PKD. It is obvious that breeders do not want to produce additional affected cats, and this test can guarantee against this. However, the wide scale elimination of 38% of the breed in a short period of time would put a significant negative pressure on the gene pool – even one as large as the Persian breed. The amount of quality genes and quality cats that can be lost forever from such selection could be devastating.

PKD shows variable expressivity, which means that the age when affected cats develop kidney failure varies. Most affected cats will develop kidney failure between 3 to 10 years of age, with an average of 7 years of age. Some affected cats can develop small cysts in their kidneys, but not progress to kidney failure.

Knowledge of the size of the kidney cysts (but not necessarily the number of cysts) is instrumental in identifying those cats that will progress to early kidney failure: The larger the cysts, the more severe the disease. However, only the disease itself, and not cyst size or disease severity is passed on from an affected parent. A mildly (late-onset kidney failure) affected cat can produce a severely affected (early onset kidney failure) cat and vis versa.

Breeders must make decisions that benefit their breeding program and the breed as a whole. If a quality PKD-affected cat can be replaced in a breeding program by a quality sibling, non-affected parent, or non-affected prior-born offspring, then a breeding line can be continued. This is the recommended method for reducing the frequency of the defective gene and maintaining breed diversity. However, if a superior PKD affected cat does not have quality relatives with whom it can be replaced, it is acceptable to breed the cat and attempt to produce a quality, normal-testing offspring for replacement in the breeding program. Affected offspring should be selected against and not placed in breeding homes.

Hopefully breeders will not institute widespread euthanasia as a result of early PKD testing of kittens. While approximately 50% of offspring from an affected parent can be affected, the majority of these cats will live seven or more years before developing kidney failure. Concurrently preserving the diversity of the gene pool over the next few generations while at the same time eliminating the defective gene is the most practical and desirable way to manage the disorder.

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.

Examining Elbow Dysplasia

*Prepared by the **Orthopedic Foundation for Animals***

Elbow dysplasia has been found in 78 breeds evaluated by the Orthopedic Foundation for Animals, which opened its ED database in 1990. The incidence of elbow dysplasia in these breeds ranged from 1.2 to 47.9 percent of the evaluated dogs.

Elbow dysplasia can lead to lameness or abnormal gait, but a number of affected dogs show no obvious clinical manifestations. Three factors produce elbow dysplasia, either singularly or in any combination.

Elbow dysplasia can be extremely debilitating, but there is no satisfactory medical protocol or surgical procedure that can significantly alter the progression of the disorder or cure it. This makes it increasingly important to reduce the incidence of the disease through selective breeding, which has been shown to reduce its incidence.

The terminology of elbow dysplasia (ED) was introduced in 1961 to describe a generalized osteoarthrosis (OA) of the elbow reported in association with an ununited anconeal process (UAP). A report followed that revealed the same OA without the UAP. Over time, fragmentation of the medial coronoid process (FCP) of the ulna and osteochondrosis dissecans (OCD) of the humeral condyle also were described with this generalized elbow arthritis. These three components are the currently-accepted entities comprising elbow dysplasia.

UAP, FCP and OCD may be present singularly or in any combination. It has been reported that FCP with OCD can occur with a frequency as high as 37 percent . With such a high frequency the question arises whether the second lesion is a result of the first lesion in contrast to the two lesions being present as separate entities. Certain breeds tend to be affected with a particular entity more frequently than the other components, which lends support to the heritability of the disorder.

Etiology

The exact mechanism of these abnormalities has not been clearly defined.

There are two different theories for the resulting lesions. The first theory, proposed by Olsson, was that all three disorders are manifestations of osteochondrosis. Osteochondrosis is a disturbance of endochondral ossification, which is the formation of bone through the ossification of cartilage. In the area of the abnormality, there is a thickening of the cartilage due to deprivation of nutrients supplied to the chondrocytes by diffusion from the synovial fluid. The cells at the bottom of the thickened area do not receive adequate nutrition and become necrotic, hence the cartilage in this area will not be attached to the underlying bone. Movement of the bones in the joint provide the forces necessary to break this thickened area free, forming a cartilage flap or a complete full-thickness cartilagenous defect.

The movement of the bony or cartilaginous fragments prevents healing of the exposed subchondral bone. Pain resulting in lameness, due to inflamed nerve endings in the subchondral bone, may persist or may diminish with time. The

Elbow dysplasia can be an extremely debilitating disorder for which there are no satisfactory treatments. Prevention, through selective breeding, is the best means of management.

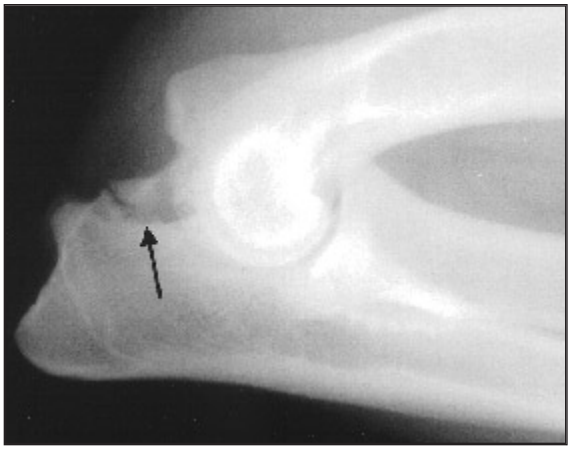


Figure 1. Extreme flexed lateral radiographic projection of the elbow joint. The ununited anconeal process is completely separated from the ulna by the line at the point of the arrow.

fragments serve as a constant irritant, exacerbating lameness and the progression of arthritic changes. This theory was supported by the different components of elbow dysplasia present in the elbows of the same animal.

The second theory, proposed by Wind, is of joint incongruity as a primary cause of the fragmentation or resulting lack of fusion (ununited). Congruency is important in the elbow joint because three bones must fit together smoothly to allow for a gliding movement in flexion and extension as well as internal and external rotation.

The entire ulnar trochlear notch is in close contact with the articular cartilage of the olecranon fossa and the humeral trochlea which articulates with both the radius and the ulna. Any asynchronous growth of the radius and ulna or insufficient development of the ulnar trochlear notch would result in loading forces in the area of the anconeal process and or the coronoid process. It has been proposed, in two different studies, that these separate components are entirely different disease entities since inheritance is independent.

Presentation

A number of affected dogs show no obvious clinical manifestations of the disorder. Some affected dogs may be clinically lame or have an abnormal gait. In dogs that are clinically lame, varying degrees of lameness maybe exhibited and exacerbation with activity is seen. Gait abnormality usually is present in dogs with bilateral disease, unless one elbow is worse than the other, making a unilateral lameness evident.

Affected limbs are usually rotated inward with elbows rotated outward. Manipulation of the elbow will reveal a decrease in range of motion. Crepitation, joint effusion, joint capsule thickening and muscle atrophy are variable. This variability is the result of the tremendous range of the abnormality even within a single entity.

The radiographic signs of ED and the clinical presentation do not necessarily correlate directly. A dog may have significant radiographic changes and not be clinically lame. In one study, dogs were evaluated when an acute lameness was present, but no radiographic changes were seen at this time. These same dogs were evaluated at a later date when the clinical lameness had resolved, but radiographic signs of ED were now seen.

This resolution in lameness with a progression of degenerative changes can be attributed to pain in the acute phase of the disease. As healing occurred, the lameness resolved. The damage persisted and, being consistent with the chronic progressive nature of arthritis, resulted in the subsequent radiographic changes.

Diagnosis

In the young dog with lameness from elbow dysplasia, diagnosis is made from typical clinical signs and standard radiographic evaluation. For diagnosis of a clinically lame patient, views should include a lateral, a craniocaudal and a flexed lateral of the elbow joint. The radiographic appearance is characterized by incongruity and or degenerative changes.

The anconeal process may not fuse to the diaphysis of the ulna until 4 to 5 months of age, so a diagnosis of UAP prior to 4 to 5 months of age is premature. No studies have been conducted for the giant breeds to determine an age range; this age range maybe even older in the giant breeds. The usual age of presentation with clinical signs is 6 to 12 months, but affected dogs may not be clinically lame until much older.

UAP is bilateral in 20 to 35 percent of affected dogs. Males are more frequently affected than females, potentially because of a more rapid growth rate and a greater over-all size.

The most significant radiographic sign of UAP is the appearance of a radiolucent line of separation between the olecranon and the anconeal process (***Fig.1***). This line may be present in varying degrees since the anconeal process can actually be partially fused (***Fig. 2***).

It is important to obtain a lateral radiograph of the elbow in extreme flexion. If not flexed, the distal, caudal aspect of the medial epicondyle of the humerus is superimposed on

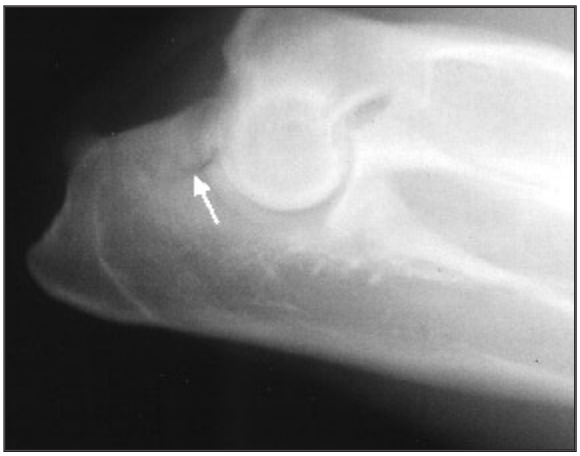


Figure 2. Extreme flexed lateral radiographic projection of the elbow joint. A partially fused anconeal process is evident. The anconeal process is fused caudally and the line at the point of the arrow is the area where fusion failed to occur.

the anconeal process, which can be confused with a separation line.

As the disease progresses, sclerosis can be seen along the margins of separation. Degenerative changes subsequently appear at 7 to 8 months of age.

The fragmented coronoid process occurs at 4 to 10 months of age. Unlike a UAP, an FCP may not be visible radiographically (***Fig. 3)***. Fissures in the cartilage or slightly irregular articular margins would be the least abnormal (***Figs. 4 & 5***). These changes are not evident radiographically, but the resulting degenerative changes would eventually become evident radiographically.

Definitive radiographic identification of FCP often is not possible because of super-imposition of the radial head over the medial coronoid process. In addition, the central radiographic beam rarely intersects the cleavage line in a parallel manner.

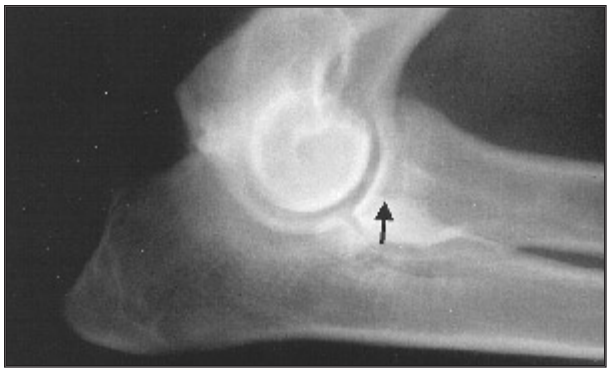


Figure 3*. Extreme flexed lateral radiographic projection of the elbow joint. Although not typically evident via radiographic examination, the arrow marks the line of separation of a fragmented medial coronoid process. A small, triangular, radiodense fragment can be seen adjacent to the line of separation.*

The diagnosis of the FCP is typically from the secondary degenerative changes that result from the abnormality (***Figs. 9& 10, page 5***). These are seen as early as 7 to 8 months of age but may not be evident until maturity. These are osteophytes located on the proximal and lateral aspects of the anconeal process. Similar changes on the medial humeral epicondyle and medial aspects of the joint develop as the disorder progresses. Sclerosis between the proximal radius and ulna and/or an increased humeroradial joint space maybe seen on a lateral projection.

The primary radiographic sign of OCD is common to all locations. There is evidence of a subchondral bone defect that causes a flattening or concavity of the articular surface. In the elbow OCD is diagnosed by visualization of either a subchondral bone defect or cartilage flap on the medial humeral condyle. This defect may have a sclerotic margin.

The cartilage flap may become calcified or contain a piece of subchondral bone facilitating recognition radiographically as a "joint mouse."

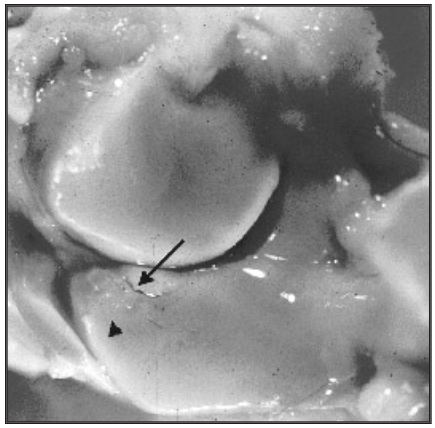


Figure 4 *A gross tissue specimen of the articular surface of the radius (right) and ulna (left). The arrow points to a fissure in the cartilage. The arrowhead points to an area of roughened articular cartilage. These lesions would not be evident radiographically but could result in changes consistent with Grade 1 elbow dysplasia.*

A calcified cartilaginous flap maybe seen radiographically as a thin, linear, mineral opacity covering the defect. The disease occurs between 4 to 10 months of age. OCD can affect multiple joints (elbow, shoulder, stifle, hock) and is commonly bilateral. If a dog is lame and elbow dysplasia is a suspected diagnosis, then the contralateral elbow should be radiographed as well. A craniocaudal projection or a slightly oblique lateral craniocaudal projection is necessary to visualize a lesion in the elbow (***Fig. 6, page 4***).

In general, the amount of arthritic changes that result from elbow dysplasia parallels the amount of joint instability created by the abnormality. Since the UAP is typically the most unstable, it is likewise associated with the most degenerative changes and subsequently the worse prognosis in reference to debilitation. The FCP is less unstable.

The osteochondrosis lesions may not create gross instability but are associated with incongruency and inflammation that would result in degenerative changes.

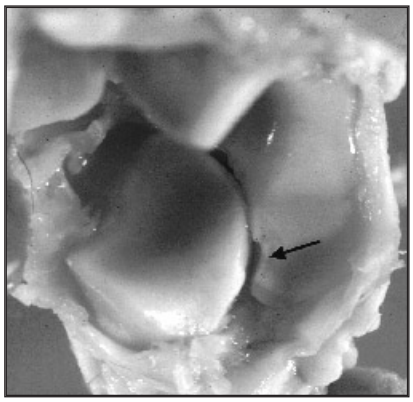


Figure 5*. A gross tissue specimen of the articular surface of the radius (right) and ulna (left). The arrow points to the irregular articular margin between the radius and ulna. It appears that a portion of the ulna is missing (in the proximity of the coronoid process). This lesion would not be evident radiographically, but could result in changes consistent with Grade 1 elbow dysplasia.*

At this time there have been no scientific studies that have correlated the amount of degenerative change with the amount of dysfunction or prognosis. With the chronic progressive nature of arthritis, it is generally a matter of time before the animal becomes clinically lame from the disorder.

Therapy

Treatment for elbow dysplasia will vary according to the individual case. Factors to be considered are the present amount of degenerative joint disease, age of the patient and degree of lameness. Typically all immature dogs showing lameness referable to FCP, OCD or a UAP are surgical candidates. Mature dogs with mild to moderate arthritic changes and a component of instability also may be considered for surgery.

Removal of the unstable component may provide for some decrease in pain. Dogs with pain from severe degenerative changes, but in which the joint is stable, are not considered surgical candidates because surgery may disrupt stability and aggravate the problem. Surgery is recommended to facilitate healing of the cartilagenous defect and to remove the loose fragments.

Depending on the component of elbow dysplasia and the amount of abnormality present at the time of surgery, owners should be aware that arthritis is a progressive disorder and improvement may be seen, but normality probably will not be achieved. Medical and surgical management is often unrewarding. Fewer than 50 percent of the dogs treated medically and fewer than 60 percent of the dogs treated surgically (for FCP) had a satisfactory long-term recovery.

The rate and extent of arthritic changes are variable. Elbow dysplasia can be a crippling disorder. The degree of osteoarthritis may be severe enough that by the age of 2 years clinical signs are evident and the use of analgesics are indicated for pain relief.

Often the best results (for future use and minimal arthritic changes) are achieved with early intervention. Therefore, a more aggressive surgical approach is advocated. Although some animals do recover spontaneously, this can happen only if the flap breaks loose and is absorbed in the joint cavity. This process may take 9 to 12 months and bilaterally affected animals (incidence of 27 percent and 68 percent) are unlikely to recover to the point of clinical soundness.

When an animal is markedly lame in one leg, it is difficult to assess lameness in the contralateral leg. Both limbs should be radiographed even if only one limb is clinically lame. An additional danger is that the loose cartilage flap may survive within the joint to become a large ossicle (joint mouse) that will cause severe inflammatory changes and degenerative joint disease. If the flap never breaks free, a similar deterioration of the joint occurs. Partially attached flaps have been removed in 3-year-old dogs.

The best method for surgery would be through arthroscopy by a surgeon who has a significant amount of experience performing arthroscopies, since there is a learning curve associated with this method.

Genetic Databases

Multiple studies support the theory that the various components of ED are heritable. The heritability index and incidence varies by the breed, component and population studied. It appears that the disorder is inherited polygenically with development being multifactorial. Both environmental factors and the additive effect of many genes contribute to expression.

The International Elbow Working Group (IEWG), a consortium of experts from around the world, was founded in 1989 to lower the incidence and prevalence of elbow dysplasia by coordinating worldwide efforts. These efforts include research, dissemination of information, formulation of guidelines for national registries and provision of education about elbow arthrosis.

It was necessary to develop a protocol for screening elbows that would be acceptable to the scientific community and breeders. It was agreed that ED was the manifestation of inherited FCP, UAP, OCD, articular cartilage anomaly and/or joint incongruity that resulted in elbow arthrosis. Until a DNA test is available for the detection of animals genetically predisposed for ED, genotype can only be estimated by knowledge of the evaluations of the extended family. The

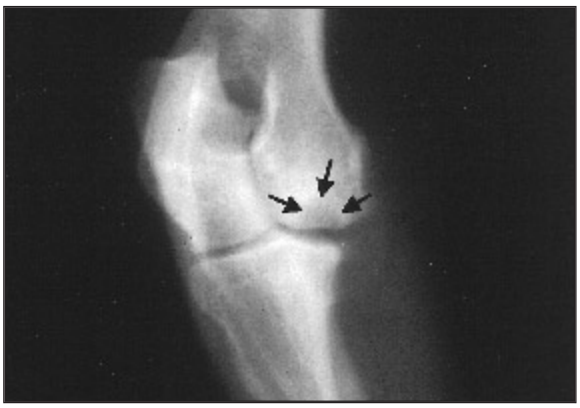


Figure 6*. Slightly oblique craniocaudal radiographic project of the elbow joint. The arrows emphasize a radiolucent subchondral bone defect from osteochondrosis.*

IEWG has continued to meet periodically to provide international discussions for the purpose of exchanging information and reviewing the elbow evaluation protocol.

The OFA started its elbow database in 1990 using a modified protocol of the IEWG. Initially the database was semi-closed, but since July 1, 2000, owners have had the choice of an open database as well. When establishing criteria for a protocol for screening breeding animals, several aspects need to be considered. To encourage submission of data from multiple-dog owners (i.e., breeders) the cost must be reasonable. This should take into consideration the cost to the submitters of obtaining such data prior to the entry into the database.

The minimum age for certification must allow for detection of a significant number of affected dogs by that age and still allow for entry of an individual into the breeding program in a timely manner. Therefore, the OFA requires one view of

each elbow in extreme flexion. Certification can be obtained at 24 months of age. The age of 2 years is well past the active stage of the disorders and arthritic changes should be evident on the lateral projection, regardless of the magnitude of the abnormality.

The OFA reports elbows as normal (***Fig. 7***) or dysplastic. The abnormals are graded as grades 1 (***Figs. 8 & 9*** through 3 (***Fig. 10***), with grade 1 being the least abnormal. The grades are differentiated by the relative amount of osteophyte formation on the anconeal process. The grades of dysplasia will facilitate the calculation of heritability.

Summary

Elbow dysplasia can be extremely debilitating. The onset is early and patients are bilaterally affected in a significant number of cases. Despite the ability to treat affected dogs, there is no satisfactory medical protocol or surgical procedure to significantly alter the progression or cure the disorder. This poor response to medical and surgical management is substantiated with force plate gait analysis pre- and post-therapy. This makes it increasingly important to reduce the incidence of the disease through selective breeding. Selectively breeding phenotypically normal individuals has been shown to reduce the incidence of the disorder.

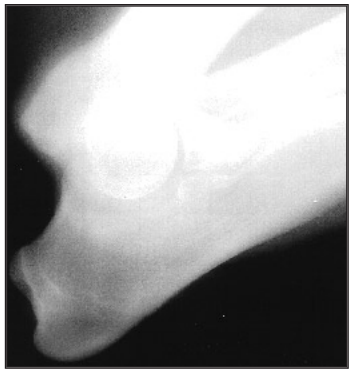


Figure 7. *Extreme flexed lateral radiographic projection of a normal elbow joint.*

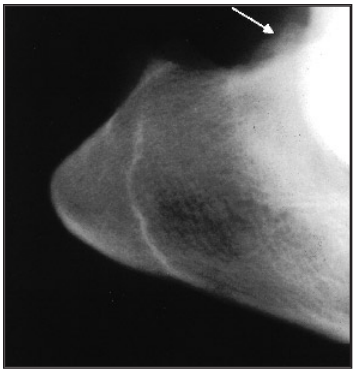


Figure 8. *Extreme lateral radiographic projection of the elbow joint. The arrow shows the osteophyte development on the anconeal process. The elbow is an example of Grade 1 elbow dysplasia.*

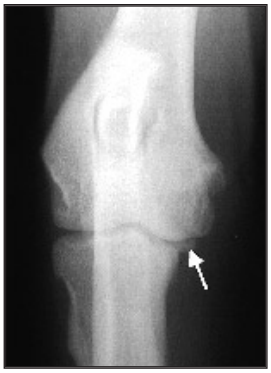


Figure 9. Craniocaudal radiographic projection of the elbow joint. The arrow shows an osteophyte on the proximal ulna. This is an example of Grade 1 elbow dysplasia, but is not a required view for the OFA.

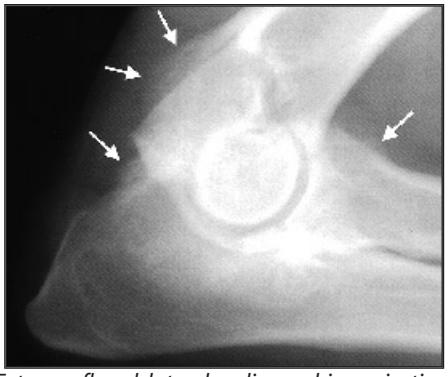
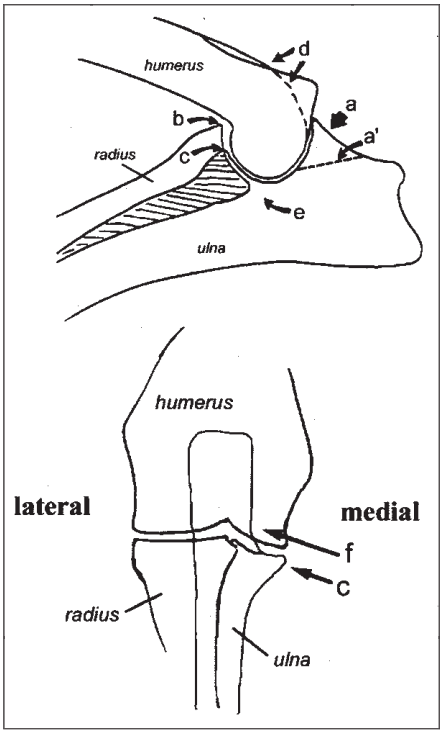


Figure 10. Extreme flexed lateral radiographic projection of the elbow joint. The arrows show osteophyte development in the area of the anconeal process on the ulna, the caudal aspect of the humerus and the cranial margin of the radius. This is an example of a Grade 3 elbow dysplasia.

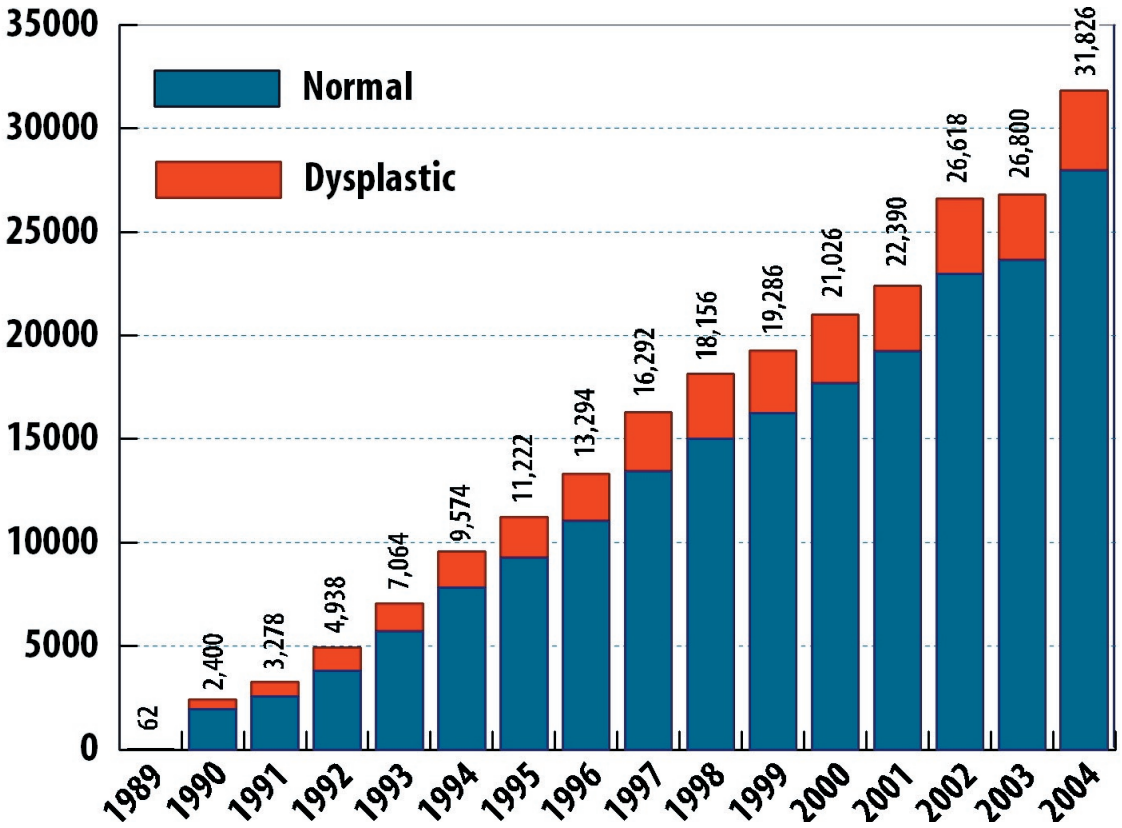
Next page: Elbow dysplasia statistics and the Canine Elbow illustrated.

The Canine Elbow

a) Anconeal process, site of osteophyte development
a') Line of separation for UAP
b) Site of osteophyte development
c) Medial coronoid process
d) Site of osteophyte development
e) Trochlear notch
f) Site of osteochondrosis lesion



Normal versus Dysplastic Elbows, 1989 – 2004



OFA's Elbow Dysplasia Database

From 1989 through 2004, 235,048 dogs were submitted to the OFA elbow database for evaluation. 234,958 were found to be normal. 35,790 dogs (15.23%) were found to be dysplastic. 732 dogs had equivocal results (not represented in this chart). In 2004, 3,854 of the 31,848 dogs tested were found to be dysplastic, 12.10%.

Popular-Sire Syndrome: Keeping watch over health and quality issues in purebreds

Jerold S Bell, DVM, Tufts Cummings School of Veterinary Medicine
(Adapted from an article that originally appeared in the "Healthy Dog" section of the August, 2004 *AKC Gazette*)

An important issue in breeding is the popular-sire syndrome. This occurs when a stud or tom is used extensively for breeding, spreading his genes quickly throughout the gene pool. There are two problems caused by the popular-sire syndrome. One is that any detrimental genes which the sire carries will significantly increase in frequency – possibly establishing new breed-related genetic disorders. Second, as there are only a certain number of bitches or queens bred each year, overuse of a popular sire excludes the use of other quality males, thus narrowing the diversity of the gene pool.

The popular-sire syndrome is not limited to breeds with small populations. Some of the most populous breeds have had problems with this syndrome. Compounding this, there are several instances where a popular sire is replaced with a son, and even later a grandson. This creates a genetic bottleneck in the breeding population, narrowing the variety of genes available.

Every breed has its prominent individuals in the genetic background of the breed. But most of these become influential based on several significant offspring that spread different combinations of the ancestor's genes over several generations. The desirable and undesirable characteristics of the ancestor were passed on, expressed, evaluated by breeders, and determined if they were worthy of continuing in future generations.

The Challenges

The problem with the popular-sire syndrome is that the individual's genes are spread widely and quickly - without evaluation of the long-term effects of his genetic contribution. By the time his genetic attributes can be evaluated through offspring and grand-offspring, his genes have already been distributed widely, and his effect on the gene pool may not be easily changed.

In almost all instances, popular sires are show dog and cat champions. They obviously have phenotypic qualities that are desirable, and as everyone sees these winning individuals, they are considered desirable mates for breeding. What breeders and especially stud-dog and tom-cat owners must consider is the effect of their mating selection on the gene pool. At what point does the cumulative genetic contribution of a popular sire outweigh its positive attributes? A popular sire may only produce a small proportion of the total number of litters registered. However, if the litters are all out of top-quality, winning females, then his influence and the loss of influence of other quality males may have a significant narrowing effect on the gene pool.

In some European countries, dog-breeding legislation is being considered that limits the lifetime number of litters a dog can sire or produce. If, however, certain matings produce only pet-quality dogs, but no quality breeding prospects, should the dog be restricted from siring a litter from a different line? The popular sire's effect on the gene pool is on the number of offspring that are used for breeding in the next generation, and how extensively they are being used. This cannot be legislated.

At what point does a popular sire owner determine that their dog or cat has been bred enough? It can be difficult to deny breeding requests when asked, but the genetic effect of a popular sire on the whole breed must be considered. If everyone is breeding to a certain male, the intelligent decision may be to wait and see what is produced from these matings. If you still desire what the male produces, it is possible that you can find an offspring who has those positive attributes, and also a genetic contribution from its dam that you may find desirable. If a popular sire deserves to make a significant genetic contribution to the breed, doing so through multiple offspring, and therefore getting a mixed compliment of his genes, is better than focusing on a single offspring.

Wait-and-See Approach

All breeding males should be health tested for the conditions seen in the breed. If a dog breed has enrolled in the AKC-Canine Health Foundation/Orthopedic Foundation for Animals CHIC program (www.caninehealthinfo.org), prospective breeding dogs and bitches should complete the recommended

breed-specific health testing prior to breeding. These may include hip radiographs, CERF eye examinations, or specific genetic tests.

It is important to monitor the positive and negative characteristics being produced by popular sires. While it is satisfying to own a popular male, a true measure of a breeder's dedication is how negative health information in the offspring is made available. All dogs and cats carry some undesirable traits. Based on the variety of pedigree background of females who are usually brought to popular sires, there is a greater chance that some undesirable traits could be expressed in the offspring. It is up to the sire owner to keep in touch with dam owners, and check on the characteristics that are being produced.

Some breeders will argue that the strength of a breed is in its females, but the fact remains that the males potentially have the greatest cumulative influence on the gene pool. There will always be popular sires, and that is not necessarily bad for a breed. But a male's influence on a breed should be gradual, and based on proven production and health testing. Maintaining surveillance of health and quality issues in breeding individuals and their offspring, and preserving the genetic diversity of the gene pool, should allow a sound future for purebred dogs and cats.

Removing the stigma of genetic disease

Jerold S Bell, DVM, Tufts Cummings School of Veterinary Medicine, N. Grafton, MA
(Adapted from an article that originally appeared in the "Healthy Dog" section of the October, 2003 *AKC Gazette*)

An inevitable consequence of breeding is the occurrence of genetic problems. No one wants to produce affected dogs or cats, yet some breeders and owners are quick to assign blame. There are no perfect individuals, and all dogs and cats carry some detrimental genes.

The emotional reaction to producing an individual with a genetic disorder often follows what is called the grief cycle:
* Denial: This isn't genetic. It was caused by something else.
* Anger: This isn't right! Why is this happening to my cats?
* Bargaining: My dog sired more than 100 other dogs that are healthy. So this one doesn't really count, right?
* Depression: My kennel or cattery name is ruined. No one will breed to my animals.
* And, finally, acceptance: My dog or cat was dealt a bad genetic hand. There are ways to manage genetic disorders, breed away from this, and work toward a healthier breed.

Getting beyond denial

Unfortunately, many breeders can't get beyond the denial stage. Some will hold to increasingly improbable excuses, rather than accept that a condition is genetic. They will falsely blame relatively rare disorders on common viruses, bacteria, or medications. The fact that these organisms or drugs are common to millions of dogs and cats annually that do *not* have these disorders is not considered.

Some owners state that their veterinarian recommended not sending in a hip radiograph because the dog would probably not get certified. Then these owners lull themselves into believing that since the dog wasn't evaluated, it does not have hip dysplasia. The fact that a dog does not have an official diagnosis does not mean the dog is normal or "not affected."

It is important to confirm diagnoses of genetic disorders with blood tests, radiographs, or pathology specimens. However, the primary concern should always be for the individual animal. If an affected individual is not suffering, it *should not* be euthanized simply to obtain a pathological diagnosis. The increased availability of non-invasive techniques has made diagnoses easier to obtain.

Once confirmation of a genetic disorder is made, denial sometimes becomes deception, which is not acceptable. There are breeders who actively seek to prevent diagnoses and later necropsies, but who eventually realize that their actions are detrimental to their breed, and in the long run to themselves.

Working together to improve our breeds

Reducing the stigma of genetic disease involves raising the level of conversation from gossip to constructive communication. Dealing with genetic disorders is a community effort. Each breeder and owner will have a different level of risk or involvement for a disorder. We do not get to choose the problems we have to deal with. Breeders should be supportive of others who are making a conscientious effort to continue breeding their animals while decreasing the risk of passing on defective genes.

Breeders ought to follow up on the puppies and kittens they have placed. They should periodically contact their buyers and ask about the health of the offspring. Some breeders fear they will be castigated if an offspring they placed develops a problem. However, the vast majority of owners of affected individuals are *pleased* that their breeder is interested in their dog or cat, and in improving the health of the breed so that other affected individuals are not produced. A

breeder cannot predict or prevent every health problem. If an owner's dog or cat is discovered to have a problem, show your concern.

Breeders and breed clubs should be cooperative and supportive of researchers studying genetic disorders in their breed. Through research funded by breed clubs, the AKC Canine Health Foundation (CHF), the Winn Feline Foundation, and the Morris Animal Foundation, new genetic tests for carriers of defective genes are continually being developed.

The Canine Health Information Center (www.caninehealthinfo.org) was established by the CHF and the Orthopedic Foundation for Animals. CHIC is an online registry that works with the breed parent clubs to establish a panel of testable genetic disorders that should be screened for in each breed. The beauty of the CHIC concept is that dogs achieve CHIC certification by completing the health-checks. Passing each health test is not a requirement for certification. CHIC is about being health conscious, not about being faultless.

My hope for each dog and cat breed is that there will eventually be so many testable defective genes that it will not be possible for any dog or cat to be considered "perfect." Then we can put emotions aside and all work together on improving our breeds.

Breeders must lead the way to remove the stigma of genetic disorders. The applications for both the OFA and CHIC health registries include options that allow for open disclosure of all health-test results or semi-open disclosure listing only normal results. It is up to breeders to show that we are ready to move genetic disorders out of the shadows and check off the boxes for full disclosure.

More national clubs are having health seminars and screening clinics at their specialties. It was thought these events would scare away potential owners. We now know that without addressing the problems, in the long run, the breeds may not be there for the owners.

Tufts' Canine and Feline Breeding and Genetics Conference

POSTER ABSTRACTS

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A Practical Veterinary Cancer Registry	Steinberg, H. Steven
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Anatomy of a Breed Health Initiative	Bell, Jerold S
Canine and Feline Blood Types and Blood Compatibility Issues	E. Withnall, M.-C. Blais, N. Weinstein, L. Berman, K. Greiner, D.A. Oakley, U. Giger.
Comparative Gene Expression Analysis of Canine and Human Osteosarcoma	Melissa Paoloni, Sean Davis, Sue Lana, Steve Withrow, Paul Meltzer and Chand Khanna
Controlling Progressive Retinal Atrophy in a Labrador Retriever Breeding Colony	E.A. Leighton and D.H. Holle
Do Mild, Infrequent Seizures Constitute Inherited Idiopathic Epilepsy in Dogs?	Barbara Licht, Ph.D. , Linda Hyson, B. A., Shili Lin, Ph.D., Kathleen Harper, DVM, Ph.D. , Mark Licht, Ph.D., Yuqun Luo, Ph.D., Soledad Fernandez, Ph.D.
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Screening for Hereditary Diseases by the Josephine Deubler Genetic Disease Testing Laboratory (PennGen) at the University of Pennsylvania	A. Huff, A. Seng, P. Wang, L. D. Berman, A. Traas, M.L. Casal, M.B. Callan, E. Tcherneva, P.S. Henthorn, M.E. Haskins, U. Giger
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Use of Canine Genetic Testing in Breeding Programs	Felix, Jeanette S.

Tufts' Canine & Feline Breeding and Genetics Conference, September 29 - October 1, 2005
Poster Abstract

Anatomy of a Breed Health Initiative

Jerold S Bell, DVM, Tufts Cummings School of Veterinary Medicine

A breed health initiative is often a demanding process. However, once accomplished it offers the reward of improved health and vitality for the breed. Many dog and cat breeds have not carried out a breed health initiative, and may not know the steps to take, or planning that is involved. The following may serve as an anatomical template

BRAIN: What health issues exist in the breed?
There will always be "hot button" health issues that breeders will list as most important for the breed. These will not necessarily be the most frequent health issues, or issues that are significantly affecting breed health. They may just be health issues that breeders and owners are talking about at the time. Regardless of this fact, if a health issue is generating dialogue within a breed, then it presents an opportunity for action and progress.

The best means to establish a priority list for breed health initiatives is through a statistically valid breed health survey. Just like a census, a breed health survey should be conducted every 5-10 years, to document the number and frequency of conditions that are occurring. Several university-based veterinary epidemiologists conduct breed health surveys for parent clubs. Such scientific surveys can eliminate sampling errors and bias that can invalidate survey results.

Once a breed health issue is identified and established as important for the breed, the hard work and hard questions start: Is this health issue an inherited condition? Does it occur with increased frequency in your breed versus other breeds? Has an inherited basis and a mode of inheritance been established in your breed, in other breeds, or in other species?

To answer these questions, the breed would be best served by contacting a professional researcher with an interest in the condition. The AKC Canine Health Foundation, or Winn Feline Foundation may know of such researchers. Often times, a parent club member (in many instances a breeder or owner of an affected animal) will contact several veterinary colleges and scientists looking for someone with an established interest into the problem.

HEART: We love our breed, but are we willing to tackle our health problems?
A breed health initiative is an emotional issue that involves owners and breeders of affected individuals, club leaders and members. It is only through the love of the breed that all can join together and successfully address health issues for the overall benefit of the breed.

Once a list of reported disorders in a breed is established through a breed health survey, the parent club can determine where to prioritize research and funding efforts. Health issues with the highest priority may not be the most frequently occurring. Disorders that cause death or incurable debilitating illness may have a higher priority than those that are easily treatable or do not affect the general health of the animal. The late Dr. George Padgett of Michigan State University ranked health issues based on a "hierarchy of disagreeability."

Addressing a health issue requires research and therefore, funding. While the AKC Canine Health Foundation and Winn Feline Foundation regularly fund research, the breed club will need to support a breed-specific research effort. Many clubs have carried out novel fund-raising methods for health research. These can include proceeds from show entries and advertising, the purchase of health pins (kitty and dog "angels"), raffles, and silent auctions.

GUTS: Fortitude and perseverance.
There will be resistance within the breed to a health initiative. For some, it will be fear of negative publicity for the breed by openly addressing a breed-related disorder. For others, it will be a self-serving effort due to fear of uncovering health issues in their own animals.

Resistance to a breed health effort can have a polarizing effect on a breed and its parent club. Lawsuits may be threatened, though in my experience, no such lawsuits have ever benefited a breed, or in the long run any breeders that initiate them. Some club members may declare that if everything is not known about the disorder, then information about it should not be discussed or disseminated. However, discussion of breed health issues can not be limited to executive sessions. It is only through open discussion and dissemination of information that a breed health initiative can be successful.

Despite the rapid progress and success of the genome projects, the identification of defective genes, and establishment of genetic testing programs could still take several years. The breed club must continue to educate its members, pass on valid recommendations for breeding management, and maintain a positive environment for breeders and owners dealing with the health issue. No one wants to breed carriers or produce affected offspring. Each breeder will find themselves in a different situation concerning each identified health issue. If lucky with one health issue, breeders must recognize that they may be the one dealing with carrier breeding stock and affected offspring with the next health issue.

LEG WORK: Identification and commitment to a health issue is only the beginning.
Once a health issue has been identified, the data must be collected to allow the possibility for valid research. This requires the trust of breeders and owners. In most instances, a professional researcher can ensure confidentiality and work to confirm diagnoses and collect data.

For involved families, breeders will have to contact the owners of offspring for blood or cheek swab collection. Breeders may feel uncomfortable making these contacts, especially to pet owners. However, experience shows that owners are interested and willing to cooperate with health research, and are usually impressed with the breeder's interest. For cases where the breeder is uncomfortable making the contacts, the researcher can often do so on their behalf.

In most instances, research grants do not fund the collection DNA samples, so the collection and shipment to the researcher will be born by the owners. In most instances, this is not a problem. With researcher generated literature on the research and collection of samples, the primary veterinarian will often keep the cost for sample collection to a minimum. On an individual basis, the parent club may want to subsidize the overnight shipment of blood samples in hardship cases.

???: The end result.
If a breed health initiative is successful, a genetic test can be developed to assist breeders. A genetic test is a powerful tool, and can have either positive or devastatingly negative results on a breed. Breeders must recognize that a test for carriers only identifies one of the 30,000 to 40,000 genes in their animal.

With such a test, carriers can be bred to normal-testing individuals, and replaced in a breeding program with normal-testing offspring. With a genetic test, no affected individuals ever need to be produced. If an individual who was determined to be of breeding quality tests as a carrier, the worst thing you can do is remove that individual from breeding.

It is only through the breed's quality individuals that the breed can improve, become more diverse, and healthier. Removing all carriers from breeding will restrict the gene pool, reduce genetic diversity, and possibly promote future genetic disease through genetic bottlenecking.

Tufts' Canine & Feline Breeding and Genetics Conference, September 29 - October 1, 2005
Poster Abstract

Use of Canine Genetic Testing in Breeding Programs

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Opportunities to benefit from DNA-based genetic testing for canine inherited diseases are increasing steadily. Breeders and owners, with a basic understanding of genetics, can use these powerful tools for enhanced breeding strategies and health evaluations. They can stay up-to-date on the available technology, tests and approaches through internet resources. Many clubs offer breed-specific in-depth materials and/or provide links to informational resources.

OptiGen specializes in tests for inherited forms of eye disease, currently with 10 tests used, in total, for 29 breeds/varieties. Together with genetic tests for other conditions, ranging from rare metabolic diseases like ceroid lipofuscinosis to coat color selection, these demonstrate growth in the field of canine genetic testing. In 2005, OptiGen announced two advances: a mutation test for CEA and, replacing the previous marker test, a mutation test for prcd-PRA,.

CEA - Collie Eye Anomaly, also known as Choroidal Hypoplasia, is an autosomal recessive disease in the Border Collie, Rough and Smooth Collie, Australian Shepherd, Shetland Sheepdog, and Lancashire Heeler. With this condition, the choroid layer at the back of the eye does not develop normally, so the primary abnormality can be diagnosed clinically at a very young age. There is a huge range in severity of the disease, from extremely mild with no consequences to vision, to total blindness in a small percentage of cases. The mutation frequency is especially high in Rough and Smooth Collies.

The retinal disease family includes many conditions, both genetic and non-inherited. The list of eye conditions tracked by CERF (Canine Eye Registration Foundation) demonstrates this variety. OptiGen tests for Lebers Amaurosis (congenital stationary night blindness - Briards) and cone degeneration (German Shorthaired Pointers), as well as for multiple genetic forms of Progressive Retinal Atrophy. PRA is an "umbrella" term covering all inherited diseases that cause progressive degeneration of the retina. However, not all retinal disease is PRA, and not all PRA is caused by the same genetic defect. The inheritance pattern of a retinal disease in a specific breed might be dominant, recessive or X-linked – all modes of inheritance have been documented. OptiGen's tests include 7 types of PRA, each caused by a different gene, with each requiring a specific laboratory test.

The most prevalent type of PRA, progressive rod cone degeneration or *prcd*, has been identified in 13 breeds/varieties as an autosomal recessive condition. With a test based on the disease-causing mutation, a dog's status of Normal/Clear, Carrier or Affected can be determined with the highest degree of accuracy available in genetic testing. The current frequency of affected status ranges from 3-22%, and the carrier status from 28-48%. Clearly, the *prcd* test is important for prevention of this blinding disease. To date, these breeds have *prcd*: Amer. Cocker Spaniel, Amer. Eskimo, Aust. Cattle Dog, Aust. Stumpy Tail Cattle Dog, Chesapeake Bay Retriever, Chinese Crested, English Cocker Spaniel, Entlebucher, Labrador Retriever, Miniature and Toy Poodle, Nova Scotia Duck Tolling Retriever, and Portuguese Water Dog.

Other types of PRA can be diagnosed by direct detection of mutations: recessive early onset PRA in the Irish Setter and Sloughi; autosomal dominant PRA in the (Old English) Mastiff and Bullmastiff; X-linked (i.e., on the X chromosome) PRA in the Samoyed and Siberian Huskie; and Type A PRA in the Miniature Schnauzer.

Recently, OptiGen licensed the DNA-based test for the Border Collie neuronal ceroid lipofuscinosis disease, referred to as CL.. This recessive gene defect is identified in lines of Australian descent, with up to 3% Carriers. CL results in accumulation of lysosomal storage bodies, leading to progressive degeneration of brain and eye cells with severe neurological impairment and early death. Affected dogs exhibit symptoms early in life – around 1- 2 years.

OptiGen also exclusively offers the test for CLAD (canine leukocyte adhesion deficiency) in the Irish Setter and the Irish Red and White Setter, and a test for narcolepsy in the Dachshund, Doberman Pinscher and Labrador Retriever.

The breeder can use genetic test information to immediately avoid producing affected pups and to control the frequency and occurrence of the mutant gene in their lines over the long-term. For autosomal recessive disease, the breeder should always select one parent that tests clear. The other parent can be untested, carrier, or affected, because the offspring can be tested before planning the next generation. For autosomal dominant disease, the breeder should select only normal males and females, because all dogs with either one or two mutant genes will be affected. For X-linked disease, the breeder should select only normal females, but can select either normal or affected males because no affected offspring will be produced in these breedings.

Several useful conclusions can be drawn from OptiGen's experience with canine genetic testing:

- Veterinarians and breeders/owners generally are eager to make use of genetic testing.
- Veterinarians often are involved in testing recommendations.
- Clients expect to access information and genetic counseling through the testing lab
- Direct, confidential interaction with the testing lab is valued.
- Internet educational materials are key to teaching and learning more about genetics.
- Many breed clubs play a central role in educating members about genetics.
- Breed club participation in the processes of test implementation has been essential.
- Genetic registries and tracking of breed-specific disease frequency enhance test value.
- Accurate parentage, pedigree and registration information must be tied to registries.
- Breeders/owners need to plan for testing well in advance, not test at the "last minute".
- A commercial testing lab serves client needs most reliably and efficiently.

OptiGen is a private company originally organized to access Cornell University biotechnology. Its sole focus is DNA-based veterinary diagnostics with test offerings for a growing list of canine vision diseases. An international market accesses OptiGen's 16 disease tests for 39 breeds/varieties, DNA archives, individualized genetic counseling, and research and development. OptiGen maintains an information-dense website to educate and update clients (www.optigen.com).

Tufts' Canine & Feline Breeding and Genetics Conference, September 29 - October 1, 2005
Poster Abstract

Genetic Tests for the Domestic Cat

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Domestic cat breeds, as with most domesticated animals, are defined by variation in phenotype. This can manifest as dramatic differences in body style, such as the Siamese and Persian, coat color, as found in Persians and Himalayans and hair length variations of the Persians and Exotic shorthairs. Many of these traits are the result of variation in single genes and the underlying genetic cause has been identified. In addition to phenotype defining characteristics, cats also possess many inherited genetic disorders. Many of these are also defined by mutations in single genes resulting in diseases such as polycystic kidney disease (PKD), progressive retinal atrophy (PRA) and Duchenne's muscular dystrophy (DMD) to name a few. The genetic mutations resulting in Siamese points, Burmese points, brown or chocolate, cinnamon (Abyssinian red) and Agouti have been identified. Additionally, a genetic cause of autosomal dominant PKD in Persians and British shorthairs has been identified. All of these tests, as well as parentage determination, can be performed using DNA samples personally collected and submitted by cat breeders/owners. This project summarizes the current status of genetic tests available for the domestic cat.

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Screening for Hereditary Diseases by the Josephine Deubler Genetic Disease Testing Laboratory (PennGen) at the University of Pennsylvania

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The Josephine Deubler Genetic Disease Testing Laboratory was established by the University of Pennsylvania School of Veterinary Medicine. Together with the Pediatrics and Genetics Clinic, it is part of a service that encompasses genetic screening and counseling for hereditary diseases in companion animals in the School's Section of Medical Genetics. The testing laboratory is named after Dr. Josephine Deubler, the Veterinary School's first woman veterinary graduate and first woman to receive a PhD recipient from the Penn Veterinary School, an AKC judge, and active show chairman of the Bucks County and the Montgomery County Kennel Clubs.

Hereditary diseases of companion animals are an important problem for breeders and owners. More than 500 inherited disorders have been identified in the dog and over 190 in the cat. Over the past two decades the Section of Medical Genetics at the University of Pennsylvania discovered and characterized many hereditary disorders in dogs and cats from the clinical features to the molecular genetic defect. Once the mode of inheritance was determined, hematological, biochemical, and genetic tests were developed to identify affected and carrier animals. The Josephine Deubler Genetic Disease Testing Laboratory was established to offer these genetic tests to veterinarians, breeders, and pet owners to assist them in their effort to breed animals free of hereditary diseases that were known to occur in their breed.

Since most genetic diseases are recessively inherited, tests to identify carriers that are clinically asymptomatic but can pass on the defective (mutant) gene have been developed. Several DNA-based tests, that represent the most accurate screening tests, have been introduced by PennGen and are listed below. Recently established tests in 2005 include coagulation factor VII deficiency in Beagles that causes a mild to moderate bleeding tendency and appears to be prevalent in show and research Beagles (in collaboration with The Children's Hospital of Philadelphia), I-Cell disease in domestic shorthair cats, and cobalamin malabsorption in Australian Shepherds (in collaboration with John Fyfe at MSU).

Inherited metabolic diseases, which include all biochemical disorders caused by genetically determined defects, have gained notable interest. The mission of the Metabolic Screening Laboratory at the University of Pennsylvania is to characterize new diseases in companion animals with signs suspicious for genetic disorders. Genetic metabolic screening includes analyses of amino acids, organic acids, and carbohydrates (glycosaminoglycans and oligosaccharides) of urine and serum samples for various inborn errors of metabolism. These tests can detect a metabolic defect in any breed and thus can be applied to define defects in breeds where a disease has not been previously identified. Some of the typical clinical signs of inborn errors of metabolism include neonatal death, failure to thrive, growth retardation, corneal clouding, chronic vomiting or diarrhea, anorexia, neurological signs, hepatosplenomegaly, skeletal abnormalities and facial dysmorphia. Any animal showing these signs is a candidate for metabolic screening by submission of urine and serum/plasma samples to our laboratory.

The PennGen laboratory is a non-profit operation under the auspices of the School of Veterinary Medicine at the University of Pennsylvania, is supported by National Institutes of Health and donations from individuals and companion animal organizations, with modest service fees. Detailed information on testing and sample submission may be found at http://www.vet.upenn.edu/penngen.

Molecular Genetic Tests offered by PennGen (http://www.vet.upenn.edu/penngen for more info)

Hereditary Disease	Canine/ Feline	Breeds (in some cases breed specific mutations)	Test PennGen
Cystinuria type I	C	Newfoundland, Labrador	DNA
Cobalamin Malabsorption	C	Giant Schnauzer, Australian Shep.	DNA
Factor VII Deficiency	C	Beagle	DNA
α-Fucosidosis	C	English Springer Spaniel	DNA
Glycogenosis (GSD) Type IV	F	Norwegian Forest Cat	DNA
Mucolipidosis II (I-Cell Disease)	F	DSH	DNA
α-Mannosidosis	F	Persian, DSH	DNA
Mucopolysaccharidosis IIIB	C	Schipperke	DNA
Mucopolysaccharidosis VI	C F	Miniature Pinschers, M. Schnauzer Siamese, DSH	DNA DNA
Mucopolysaccharidosis VII	C F	Mixed Breed, German Shepherd DSH	DNA
Myotonia Congenita	C	Miniature Schnauzer	DNA
Phosphofructokinase (PFK) Deficiency	C	English Springer and American Cocker Spaniel, Mixed Breeds	DNA
Pyruvate Kinase (PK) Deficiency	C F	Basenji, Beagle, Eskimo, West Highland White & Cairn Terrier, Dachshund Abyssinians, Somali, DSH	DNA
X-Linked Severe Combined Immune-deficiency (SCID)	C	Basset Hound, Pembroke Welsh Corgi	DNA
Other tests offered			
Cystinuria	C	Any Breed (see also DNA test for Newfoundland and Labrador)	Urine Amino Acid
Fanconi Syndrome	C	Basenji, Norwegian Elkhound, Other Breeds	Urine Amino Acid, Glucose
Hereditary Blood Diseases	C/F	Any Breed	Varied
Methylmalonic Aciduria –Cobalamin malabsorption	C	Giant Schnauzer, Beagle, Shar pei, Border Collie, Other Breeds	Urine Organic Acid
Metabolic Screening for animals with suspected genetic disease	C/F	Any Breed (Detailed Clinical Information Required)	Metabolic Tests Urine & Serum
Mucopolysaccharidosis	C/F	Any Breed (See also mutation tests)	Urine, serum & EDTA blood

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CANINE AND FELINE BLOOD TYPES AND BLOOD COMPATIBILITY ISSUES

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Advances in veterinary medicine in recent years have dramatically increased the availability and use of canine and feline blood products. Understanding the role of blood types in dogs and cats and the inheritance of blood types is therefore important for veterinarians, breeders, and pet owners. Blood typing, in any species, is necessary to improve the safety and efficacy of transfusions. In cats, the knowledge which blood typing provides can also prevent fatal neonatal isoerythrolysis during the first days of life. Methods for blood typing and crossmatching have become available for use in the laboratory and in clinical practice.

BLOOD TYPES

Blood types represent genetically determined markers on the surface of red blood cells (erythrocytes) and are both species-specific and antigenic. Antigenicity refers to the likelihood that the immune system will react and make antibodies, known as alloantibodies or isoantibodies, against the foreign substance. These antibodies can be detected in the animal's plasma.

A blood group system is made up of a set of allelic blood types (i.e. two or more markers at the same gene locus). Both species-specific antisera and chemical reagents, directed against specific red blood cell antigens, are used in in-vitro blood-typing methods. A positive result occurs if there is clumping (hemagglutination) or rupture (hemolysis) of red blood cells exposed to the antiserum or typing reagent. Individuals who lack a particular red cell antigen may develop antibodies against the blood type containing that antigen if sensitized to it via a mismatched transfusion. These alloantibodies are responsible for incompatibility reactions.

CANINE BLOOD TYPES

Canine blood types are commonly referred to as Dog Erythrocyte Antigens (DEA), followed by a number. There are at least a dozen DEA types known, although currently we can only test for a few. In addition to the known DEA systems, the University of Pennsylvania has recently identified a novel canine blood group system known as Dal. Dal may be missing in an unknown proportion of Dalmatians.

A dog can either be positive or negative for each specific DEA. If positive, the antigen of that blood type is present on the red cell surface and if negative, the antigen is missing. The DEA 1 system appears to have several subtypes, namely DEA1.1, DEA1.2 and possibly DEA1.3. DEA 1.1 has garnered the most clinical attention as it is the most antigenic and is responsible for serious clinical transfusion reactions. Approximately 50% of all dogs are positive for the DEA 1.1 antigen.

In dogs without prior sensitization to cells bearing a foreign antigen, no clinically significant alloantibodies have been recognized against blood types different to the individual's own type. Sensitization will occur if DEA 1.1 positive blood is transfused into a DEA 1.1 negative dog. This results in the formation of strong alloantibodies against the DEA 1.1 antigen. A delayed transfusion reaction occurs as the body builds up these alloantibodies over time and destroys the transfused red blood cells still in circulation. Delayed transfusion reactions can be seen in as little as a week following the original mismatched transfusion. Subsequent transfusions with DEA 1.1 positive blood to an already sensitized DEA 1.1 negative dog are much more serious. In the time since the mismatched transfusion was administered, the body has formed alloantibodies. These antibodies are now available to bind to the foreign antigen on the DEA1.1 positive cells and destroy the red cells resulting in life-threatening hemolytic reactions. The role of DEA 1.1 blood typing is therefore crucial to ensure that donor and recipient have the same blood type. If the blood type of the recipient is unknown a DEA 1.1 negative donor is essential to avoid sensitizing the recipient in case they are DEA1.1 positive. Blood-typing cards are available for in-house use (DMS Laboratories, Flemington, NJ) and a novel gel test for laboratory use has recently been standardized.

Correctly typed and matched blood only takes into account the DEA 1.1 antigen. Thus, following a DEA 1.1 matched transfusion, alloantibodies may still develop against other known or unknown blood types. These alloantibodies may become responsible for incompatibility reactions with subsequent transfusions. Blood compatibility testing, known as crossmatching, is used to identify possible incompatibilities against any blood type. More specifically, a crossmatch indicates the serologic compatibility, or lack thereof, between the recipient and the intended donor.

FELINE BLOOD TYPES

Only one blood group system, the AB system, has been well defined in the cat. Three blood types make up the AB blood group system: type A, type B, and type AB. Cats must have one of these blood types, there is no type "O". The "a" allele is dominant over the "b" allele. Type AB is a rare third allele which appears to be recessive to "a", but codominant to "b". Type A cats are by far the most prevalent world wide in domestic cats, but in some pure breeds the frequency of type B cats can be greater than 50%. Although the percentage of type B cats does vary depending on the breed, some breeds are known to have higher type B frequencies. These breeds include the Devon and Cornish Rex, British and Exotic Shorthair, the Turkish Van and the Angora. The majority of domestic shorthair cats have blood type A, but higher percentages of blood type B can be seen in certain geographic regions relative to others. Although the clinical relevance is not yet known, our laboratory recently identified a new feline blood group called Mik.

In contrast to dogs, cats do possess naturally occurring alloantibodies against the blood type antigen they are lacking. Type B cats have strong anti-A antibodies, while type A cats have generally weak anti-B alloantibodies. Given the potentially fatal hemolytic transfusion reaction that will result from a mismatched transfusion, all feline blood donors, as well as recipients, should be blood typed.If blood typing is not available, the recipient and intended donor should be crossmatched to ensure blood type compatibility.

In addition, neonatal isoerythrolysis is caused by the naturally occurring anti-A alloantibodies present in a type B queen's colostrum. Since type B is a recessive blood type, if a type B queen is mated to a type A or AB tom, we expect from half to all of her kittens to be blood type A (or AB). These kittens will passively acquire strong anti-A antibodies via the colostrum and will likely succumb to neonatal isoerythrolysis. Although these anti-A alloantibodies are only absorbed during the first 16 hours of life, they cause lysis of the kittens' red blood cells resulting in anemia, jaundice, darkly-pigmented urine, anorexia or sudden death. Therefore, all cats used for breeding should be blood typed and compatible mates must be selected based on these results to avoid the loss of kittens from neonatal isoerythrolysis.

The inheritance pattern, natural occurrence of alloantibodies, and varied breed distribution are all of considerable importance to cat breeders as well as veterinarians.Blood typing cards for use in practice (DMS Laboratories, New Jersey,) are available. Furthermore, a novel gel test typing method has been introduced for feline typing.

The Transfusion Laboratory at the Ryan Veterinary Hospital of the University of Pennsylvania accepts samples for the assessment of feline and canine blood typing and resolving difficult compatibility issues (penngen@vet.upenn.edu; http://www.vet.upenn.edu/penngen).

Blood type A and B frequency in cats

Breed	Type A %	Type B %
Abyssinian*	84	16
American Shorthair	100	0
Birman*	82	18
British Shorthair*	64	36
Burmese	100	0
Cornish Rex	67	33
Devon Rex	59	41
Domestic Shorthair USA*	96-99	1-4
Exotic Shorthair	73	27
Himalayan	94	6
Japanese Bobtail*	84	16
Main Coon	97	3
Norwegian Forest*	93	7
Oriental Shorthair	100	0
Persian*	86	14
Scottish Fold*	81	19
Ragdoll	92	8
Russian Blue	100	0
Siamese	100	0
Somali*	82	18
Sphinx	83	17
Tonkinese	100	0
Turkish Angora	54	46
Turkish Van	40	60

***breeds in which the rare type AB has been found**

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Mucopolysaccharidosis in Dogs and Cats: Clinical Signs to DNA Tests

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The diagnosis, control and treatment of hereditary diseases play an important role in modern veterinary medicine. The Section of Medical Genetics at University of Pennsylvania has been involved in the discovery of hereditary disorders of companion animals for several decades. A particular area of research has been the study of lysosomal storage diseases (LSD) including Mucopolysaccharidosis (MPS). Several MPS disorders have been identified and the disease processes have been characterized. Furthermore, some disease-causing mutations have been identified and breed-specific DNA tests have been established for the diagnosis of affected animals and carrier detection. (Supported by National Institutes of Health, the National MPS Society, Inc., and the Canine Health Foundation)

MPS, a group of inherited metabolic disorders, is due to defects in a series of catabolic enzymes, which results in multisystemic accumulation of improperly degraded glycosaminoglycans (GAGs). The undegraded GAGs usually leak out of the cells and can be detected in the urine, which serves as a screening test. The common clinical signs include growth retardation, skeletal deformities, corneal cloudiness, facial dysmorphia and, in some cases, neurological signs. MPS disorders have been identified in both dogs and cats (see Table).

The first canine MPS disorder identified was MPS VII in a mixed breed dog at the University of Pennsylvania about 25 years ago. The study of this and other MPS disorders led to better understanding of the clinic pathologic process and also to the first successful attempt to treating a multisystemic disorder with gene therapy. The same mutation causing MPS VII in the original mixed breed dog has now been identified to cause MPS VII in German Shepherds; two separate families have recently been found in Georgia and Minnesota. We have also documented MPS VII in a Rat Terrier. MPS I, which was first recognized in the Plott Hound, has since also been identified in a Rottweiler. All MPS disorders are autosomal recessively inherited, except for MPS II repored in a Labrador Retriever, which is inherited as an x-linked trait. MPS III, also known as Sanfilippo syndrome, is unique in that this is the only MPS disorder with mostly neurologic signs such as ataxia and tremors. MPS IIIA occurs in Wirehaired Dachshunds and the New Zealand Huntaway dog. MPS IIIB has been found in Schipperkes (and also emus). Typically dogs with MPS IIIB do not exhibit clinical signs until they are two years of age and their condition is slowly progressive until they need to be euthanized before 5 years of age. The disease-causing mutation has been identified, and the results from screening >1000 Schipperkes would indicate that the mutant allele seems to be very prevalent in the breed (almost 20% of a somewhat biased sample were carriers). MPS VI was first seen in Miniature Pinschers with stunted growth and skeletal abnormalities mostly involving the hips, hence they were misdiagnosed as having hip dysplasia or femural head necrosis. The molecular defect in this breed has been identified and screening of Miniature Pinschers for carriers and affected dogs is now possible. Further studies are in progress to define the MPS VI mutation in affected Chesapeake Bay Retrievers and Miniature Schnauzers. MPS I, MPS VI, and MPS VII have also been observed in cats. In addition, mucolipidosis II, a unique and devastating storage disease, which is closely related to MPS, has recently been characterized in domestic shorthair cats from the clinical signs to the molecular defect.

MPS may be suspected based on clinical signs, white blood cell granules, and a positive urine MPS spot test. Further diagnostics includes dramatically reduced specific catabolic enzyme activity, and molecular analysis. While enzyme testing requires special handling of fresh blood samples with a control sample to be shipped on ice overnight, samples for DNA testing are stable and can be performed on small EDTA blood sample or cheek swab shipped by regular mail. For further information, contact the Josephine Deubler Genetic Disease Testing Laboratory at the University of Pennsylvania http://www.vet.upenn.edu/penngen, email at penngen@mail.vet.upenn.edu or fax at 215-573-2162.

Mucopolysaccharidoses and other Lysosomal Storage Diseases in Animals

Disease	Deficient Enzyme	Species/Breed
Mucopolysaccharidosis		
MPS I (Hurler & Scheie Syndrome)	α-L-iduronidase	DSH cat, Plott hound, Rottweiler
MPS II (Hunter Syndrome)	Iduronate-2-sulfate sulfatase	Labrador Retriever dog (X-linked)
MPS IIIA (Sanfilippo A)	Heparan N-sulfatase,	Wirehaired dachshund
MPS IIIB (Sanfilippo B)	Sulfamidase, α-N-acetyl-glucosaminidase	New Zealand huntaway dog Schipperke dog
MPS VI (Maroteaux-Lamy Syndrome)	N-Acetylglucosamine 4-sulfatase (Arylsulfatase B)	Siamese & DSH cats Miniature pinscher, Welsh corgi, Miniature schnauzer, Chesapeake Bay retriever
MPS VII (Sly disease)	β-glucuronidase	Mixed breed, German shepherd Rat terrier DSH cat
Oligosaccharidoses		
α-Fucosidosis	α-fucosidase	English springer spaniel dog
Glycogenosis II (Pompe Disease)	α-glucosidase	Lapland dog DSH cat
α-Mannosidosis	α-mannosidase	Persian, DSH & DLH cats
Lipidosis		
Galactosylceramide lipidosis (globoid cell leukodystrophy, Krabb disease)	Galactosylceramidase (β-D- galactocerebrosidase)	Cairn & West Highland White Terrier, Miniature poodle, Beagle, Irish setter & Blue tick hound DSH cat
Glucocerebrosidosis (Gaucher disease)	Acid -glucosidase (β-D glucocerebrosidase)	Sydney silky terrier dog
G_{M1}-gangliosidosis	β-D-galactosidase	Siamese, Korat & DSH cat Beagle mix, Springe spaniel, Portuguese water dog
G_{M2}-ganliosidosis (Sanhoff disease)	β-hexosaminidase A and B	DSH & Korat cats Japanese spaniel, German short-haired pointer
Mucolipidosis II (I-cell disease)	N-acetylglucosamine-1-phosphotransferase	DSH cat
Sphingomylenosis A and B (Niemar Pick A and B)	Acid sphingomyelinase, Cholesterol esterification deficiency (type C)	Balinese, Siamese & DSH cat Boxer, Miniature poodle

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Feline C-Kit Evaluation for White Spotting

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The presence or absence of white spots on a domestic cat can be a financially important trait for cat breeders, especially in certain breeds. For example, the Turkish Van breed requires that patches of color be present on the head and tail, while the rest of the body must be white. Curiously, this same marking pattern that defines the Turkish Van is also present in other breeds, including Persians and Japanese Bobtails, and can occur in offspring from non-"van"-patterned cats; indeed, some cat breeders have attempted to develop breeding strategies that increase the number of kittens born with "van" or other white spotting patterns. Previous research has linked the locus responsible for the white spotting patterns known as "and white" and "van" in the domestic cat to a region of the genome that contains the gene c-kit. The porcine c-kit homologue has been identified as the causative gene for the dominant white phenotype in the pig, and the murine c-kit homologue has long been studied as the gene responsible for dominant white and some white spotting patterns in the mouse. In this study, genomic DNA from solid colored, dominant white, "and white", and "van" patterned cats was sequenced and analyzed for single nucleotide polymorphisms (SNPs) in the c-kit gene; additionally, c-kit cDNA from one solid colored cat and one dominant white cat was sequenced and analyzed for mutations. Several mutations were identified in both the genomic and cDNA sequence, and have been analyzed for potential causative effect and for segregation through families of white spotted cats.

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Feline Inherited Facial Defect Linkage Analysis

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Linkage analysis is a fundamental research tool for identifying candidate regions of chromosomes for disease traits. The value of linkage analysis is base on the power of the pedigree. Power is a statistical estimate based on structure and samples size of a population. For the feline inherited head defect, power was not obtained for linkage analysis until 50 DNA donations, 72% affected kittens, were received in the first quarter of 2005. These cats were related to 131 samples received over the previous 10 years. The project sample database has over 515 DNA samples, but the 215 DNA samples (181 of which were used in the linkage analysis) that form 3 pedigrees have been one major key to the successful identification of a region containing the feline head defect.

Linkage analysis of the Burmese, and Burmese/Bombay/American Shorthair pedigrees has limited the feline head defect to a 15Mb region of a feline chromosome. Within the region there are over 250 genes, of which, at least 6 have known function in developmental pathways. Research is continuing to narrow the region of interest, which will hopefully identify the causative gene. The continuing donation of affect DNAs and their parent's DNA will continue to increase the power of the analysis and should lead to identification of the causative gene. The gene will be analyzed for mutations affecting gene function. Once a mutation completely linked to the disease, is found, a DNA test will be developed suitable for commercial production.

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Do Mild, Infrequent Seizures Constitute Inherited Idiopathic Epilepsy in Dogs?

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Findings by our group suggest that when examining the mode of inheritance for "idiopathic" epilepsy, dogs with very infrequent and/or mild seizures should be included along with the more severe cases. Because mild, infrequent seizures rarely warrant treatment, the importance of these cases often is discounted. However, we have found that even very mild cases may provide crucial information on the inheritance of idiopathic epilepsy. (Note that if seizures of any severity are known to be due to another disease/disorder, such as liver disease, head trauma, an infectious disease such as distemper, etc., then the seizures would not be considered "idiopathic" epilepsy.)

Let us consider a case of an otherwise healthy dog that had two mild seizures several months apart. The owner reported that the dog staggered around as if drunk (but did not fall), drooled, and acted disoriented for 2-3 minutes. Understandably, many veterinarians would not diagnose this as epilepsy because it would not require treatment and because it is not possible to know with 100% certainly if these mild episodes are seizures. Further, many experienced dog owners and breeders may not even realize that these episodes are most probably seizures. In fact, these mild episodes often are never reported to the breeder, even when the owner has frequent contact with the breeder. Breeders find out about such episodes only when they (or a researcher) directly ask the owner if their dog ever had seizures or episodes of unusual behavior. Thus, when breeders try to evaluate if their bloodlines show any evidence of inherited epilepsy, they often do not even know about these mild cases. Importantly, as illustrated by the information presented below, mild cases often occur in the same families as the more severe, clear-cut cases of epilepsy; and if only the more severe cases are considered, breeders and researchers are likely to miss seeing a clear inheritance pattern. Thus, what may, in fact, be a simple recessive disorder may appear to be due to a more complex mode of inheritance, or worse, no inheritance at all.

In a large pedigree of Standard Poodles that we analyzed, we considered any Poodle to be "affected with idiopathic epilepsy" if all of the following three conditions held:

1) The Poodle had one or more episodes for which the owner's description was judged by our research staff to be a seizure. We included very clear seizures that would be considered "grand mal" (technically called "generalized tonic-clonic" seizures), as well as much milder episodes that we judged to be "focal" or "partial" seizures. (These are called "focal" or "partial" seizures because the seizure involves only a limited area of the brain.) These milder episodes included the example given above as well as other episodes, such as trembling and hiding (for no reason), uncontrolled repetitive lifting of leg(s) in a manner similar to a marionette, etc. In most of these episodes, the dog's consciousness was impaired, but it was never completely lost (as assessed by asking owners to rate dog's responsiveness to them during the worst part of the episode). We judged these milder episodes to be seizures because they strongly resemble episodes seen in humans that have been verified with electroencephalograms (EEGs) to be "focal" seizures.
2) The Poodle's first observed seizure occurred younger than 8 years of age. In this family, the average (mean) age when seizures began was 3.7 years, with the youngest being 6 months and the oldest being 7 years.
3) There was no evidence of any illness or injury that was likely to have caused the seizures.

Based on these criteria, there were **27** Poodles that were considered to be affected with idiopathic epilepsy. There were 57 that were not affected; that is, the owner was certain that they never observed

any potential seizure activity. There were 38 Poodles for whom we either had no information (couldn't contact owner) or, in a few cases, the information was too unclear to classify the dog as affected or unaffected. What was compelling about this family is that the data are consistent with a simple recessive mode of inheritance. Statistical tests using formal segregation analysis showed that the best model was simple recessive (with complete or almost complete penetrance and a gene frequency of 0.39). A visual inspection of the pedigree (included in poster) also revealed a simple recessive mode of inheritance. For example, when an unaffected sire that was suspected of being a "carrier" was bred to an affected bitch, an average of 45% of their offspring were affected. (The expected frequency based on simple recessive inheritance would be 50%.) Similarly, when two probable "carriers" were bred together, 33% of their offspring were affected. (Expected frequency for offspring of two carriers is 25%.)

In contrast, many fewer dogs were classified as being affected with idiopathic epilepsy when we considered only "grand mal" seizures, which involved uncontrolled shaking and/or rigidity of the entire body and complete loss of consciousness. Specifically, if we only consider as affected dogs with two or more grand mal seizures, **10** Poodles would be classified as affected. This constitutes only 37% (10/27) of the number we considered to be affected when we also included the milder cases. Thus, if we only considered Poodles with grand mal seizures to be affected, we would not have found as clear a pattern of inheritance. In fact, one argument that some breeders and owners have presented to us for why they believe that idiopathic epilepsy is not inherited in a clear fashion, or not inherited at all, is that there are too few cases in some supposedly affected bloodlines for it to be inherited as a simple recessive trait. Based on our data, we offer another potential explanation for why epilepsy often does not appear to be inherited with a clear mode of inheritance. We suggest that it is because many cases are either not brought to the attention of the breeder (or researcher) and/or their seizures are dismissed as too mild or infrequent to be indicative of idiopathic epilepsy. Of course, until we or other researchers discover the gene (or genes) for epilepsy in Poodles and other breeds, it will not be possible to know the mode of inheritance with certainty. However, we argue that the kind of mild cases described here should be considered "affected" not only because they strongly resemble focal seizures seen in humans, but because it was only when these mild cases were analyzed along with the more severe cases that a clear mode of inheritance emerged. Perhaps, both severe and mild cases are found in the same families because in addition to the gene responsible for whether the dog has epilepsy, there may also be a "modifier" gene that affects the severity of seizures. Last, but importantly, we wish to thank the breeder who provided us with complete litter records. Without complete litter records, we would not have been able to collect enough data on this bloodline to determine a clear mode of inheritance.

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Poster Abstract

CONTROLLING PROGRESSIVE RETINAL ATROPHY IN A LABRADOR RETRIEVER BREEDING COLONY

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In 1992, a four-year-old working dog guide and his full-sister, a breeder, were diagnosed with progressive retinal atrophy (PRA). To halt further spread of this inherited autosomal recessive disease, The Seeing Eye ultimately employed three tools. First, traditional test matings were done whereby young males of unknown genetic composition were mated with known carriers or with PRA affected mates. The progeny were monitored to see if any PRA affected offspring were produced. Second, a computer program was written to calculate the probability that a young breeding candidate of unknown genetic composition was a heterozygous carrier, based on all information known about related animals in the breeding colony. These calculated probabilities helped guide breeder selection decisions in the absence of a DNA marker-based test that would otherwise have separated heterozygous carriers from homozygous normal dogs. Third, a major research initiative was funded to develop a DNA test that uses either markers or identifies the actual gene. Using tools one and two, the disease was controlled by 1997, when the last of 38 confirmed cases was born. This date coincided, approximately, with release of the first version of a DNA marker test that could be used to identify heterozygous carriers. In early 2005, the marker test became a gene based test. The Seeing Eye invested almost $800,000 in the research project that eventually led to this DNA marker test, but this was less than half of total funding required to complete the project.

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Poster Abstract

A Second-Generation Genome-Wide Screen for Linkage to Canine Hip Dysplasia

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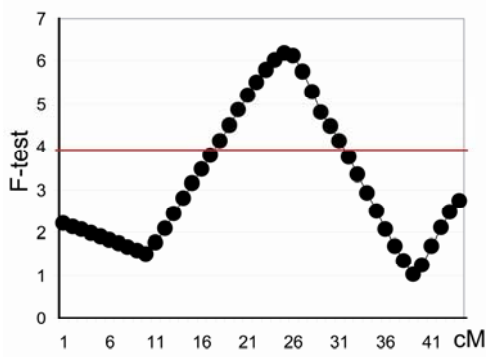
Canine hip dysplasia (CHD) is a common developmental complex trait characterized by hip joint laxity and subluxation and secondary hip osteoarthritis. To dissect the underlying genetics of this common heritable disease in dogs, an experimental pedigree was established by crossing unaffected Greyhounds and dysplastic Labrador Retrievers. Hip phenotypes measured at 8 months and analyzed included the distraction index, the dorsolateral subluxation score and the Norberg angle. A first genome-wide screen on 152 dogs from this 3 generation pedigree was completed with 250 microsatellites at the NHLBI Mammalian Genotyping Service, Marshfield WI. We reported (Todhunter et al, 2005 Mammalian Genome) identification of putative QTL for canine hip dysplasia ($p<0.05$, chromosome-wide) on CFA04, 09, 10, 11 ($p<0.01$), 16, 20, 22, 25, 29 ($p<0.01$, LOD score 2.8), 30, 35, and 37.

To improve the marker coverage and to provide a better resolution in defining the QTL location, a second genome-wide screen was undertaken with 323 additional markers from the Minimal Screen Set 2 (MSS2). Twenty-five chromosomes were genotyped and integrated with the previous screen, resulting in 460 unique marker genotypes. Genotyping is continuing on the remaining 14 chromosomes. Interval mapping analysis was performed using the combined backcross/F_2 design module from QTL Express, a web-based software which regresses identical-by-descent marker probabilities on the phenotypes. Estimates were obtained for the additive and dominance effect of the putative QTL at each location across the chromosome. The location giving the highest F ratio statistic was considered to be the best estimate for the position of the QTL. Chromosome-wide significance thresholds for each trait were determined empirically by permuting the marker data; the threshold was obtained from 500 permutations. Putative QTL were identified on 11 out of the 25 chromosomes tested with the full set of markers. The QTL detected on chromosomes 11 and 29 (Figure 1) remain with the highest LOD scores and are the focus of initial fine mapping.

Figure 1. Profile plot of F-test statistics (vertical axis) of the QTL underlying the NA on chromosome 29. The peak represents position of the QTL in cM on the X axis across the chromosome. The threshold at a chromosome-wide significance level of 0.05 is drawn as a solid red line across the plot.



To further validate these results, 130 purebred Labrador Retrievers were genotyped using a revised Mammalian Genotyping Service set of 249 markers at Marshfield Medical Research Foundation. Genotyping on this Labrador Retriever population with the MSS2 markers is completed on 25 chromosomes. QTL mapping is performed using a new module of QTL Express designed to analyze data from purebred populations with complex pedigrees using a variance-based approach. Fine mapping of the validated QTL will be undertaken using SNP haplotypes on the crossbred pedigree, purebred pedigree and also other breeds afflicted with the disease.

Identifying QTL which either confer protection or increases the risk of CHD should provide dog breeders with the tools needed to implement an effective selection program to reduce the frequency of CHD and veterinarians and dog owners with effective methods to identify dogs at risk of developing CHD early in life, when preventive measures can be implemented. Identifying the mutation(s) contributing to CHD will increases our understanding of CHD and should open up new and more effective therapies for this orthopedic disease of dogs.

Support: Morris Animal Foundation; Collaborative Grant Program, College of Veterinary Medicine and Biotechnology Center for Advanced Technology, Cornell University; American Border Collie Association; Van Sloun Foundation; Masterfoods Inc; Mammalian Genotyping Service

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SCREENING INACCURACY IMPEDES GENOMIC RESEARCH AND GENETIC CONTROL OF CANINE HIP DYSPLASIA

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Introduction: The aim of genomics is to decipher how genes function and provide an understanding of the link between genotype and phenotype. Therefore accurate characterization of phenotype is integral to genomic advancements. The diagnosis of canine hip dysplasia (CHD) has for decades been based on an empirically accepted phenotype derived from the ventrodorsal radiograph of the canine hips and pelvis. This radiograph is scored subjectively for the presence of subluxation of the coxofemoral joint, or secondary osteoarthritis (OA). It has been generally accepted that the subjective scoring of hip phenotype at 2 years of age accurately reflects the true hip phenotype of the dog for life. This investigation compared OFA-type scores and PennHIP scores at 2 years of age, with end-of-life histopathologic hip phenotypes.
Methods: 48, 8-week old Labrador Retrievers from 7 litters were followed for life. Hips were radiographed in the hip-extended position at 30, 42, and 54 weeks of age, then yearly until end of life. Hip radiographs were evaluated by a board certified radiologist for the presence and severity of OA. All radiographs were also scored using the criteria of the OFA. Distraction radiography was performed at 2 years of age according to the PennHIP method. Gross and microscopic histopathology of the hips was performed on 45/48 dogs after succumbing to natural causes.
Results: Radiographic prevalence of OA increased linearly from 15% at 2 yrs of age to 67% at end-of-life. Of 29 dogs receiving OFA-type 'normal' scores from the hip-extended radiograph, 16 (55%) were radiographically dysplastic by end-of-life and 92% had histopathologic evidence of OA. At 2 years of age PennHIP scores predicted all 48 dogs to be susceptible to OA (DI range 0.36 – 0.92): 43 of 45 dogs (96%) showed Histopathologic OA by end of life. Median disease free interval was 3 years for dogs with DI>0.6 and 12 years for dogs with DI< 0.4.
Discussion: The linear increase in OA over life refutes the commonly held belief that hip OA occurs either by two years of age or much later in the geriatric years. Therefore "idiopathic" or "old-age" OA of the hip are likely misnomers. The high rate of 'false negative' diagnoses associated with subjective scoring of the hip-extended radiograph impedes genetic progress via selective breeding and also confounds advancements in genomic research. Other scoring systems based on hip-extended radiography used worldwide would likely fare similarly. The PennHIP DI predicted with 96% accuracy the dogs that would go on to develop the OA of CHD later in life. Results of this study (and those previously published) indicate that rapid genetic improvement in hip phenotype will occur using the distraction index as a selection criterion. Similarly, more rapid progress in genomic research can be expected by keying on this newer hip phenotype.
Acknowledgment: Nestlé Purina Pet Care Co, St Louis, MO

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Poster Abstract

A Practical Veterinary Cancer Registry

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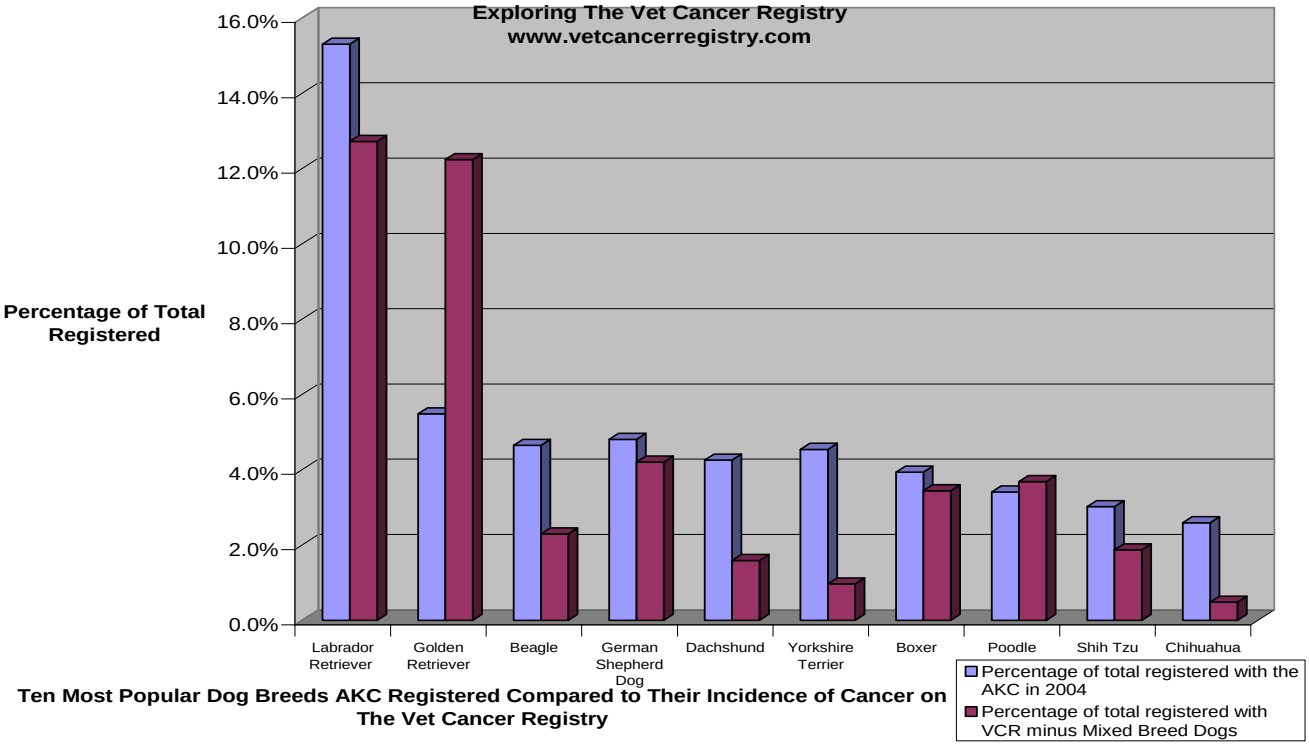
The Vet Cancer Registry is a web based (www.vetcancerregistry.com) data collection point for confirmed veterinary cancer cases. Cases have been registered from all over the world and only those cases with confirmed diagnoses are accepted into the database. This service is totally free and has been developed to be user friendly. There are currently more than 8600 cases on the website and cases are being added at a rate of about 12 –18 per week. There are 6,456 dogs and 2,192 cats registered at this time in the registry.

Although the data are of a general nature, by looking at thousands of cases at one time one can notice trends that would not be evident in evaluating only a handful of cases. The search format is extremely interactive and allows for creative pursuits that are left to the imagination of the investigator. There is also a mechanism to query case submitters and to encourage collaboration among researchers.

This effort grew out of the obvious need to collate data about dog and cat brain tumors in order to obtain any meaningful data. The International Brain Tumor Registry evolved to include all types of tumors and the Vet Cancer Registry was "born".

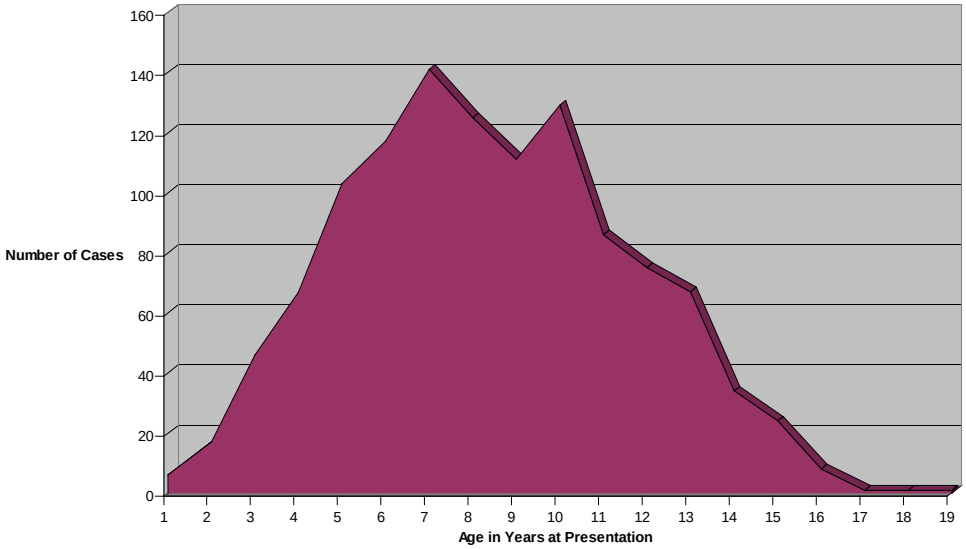
We encourage all veterinarians to contribute data and hope that all persons exploring the large number of cases will appreciate a sense of real value among this growing collection of cases from around the world. All of these results are free to all persons with access to the internet.

The following four charts demonstrate the kinds of information readily available from www.vetcancerregistry.com.

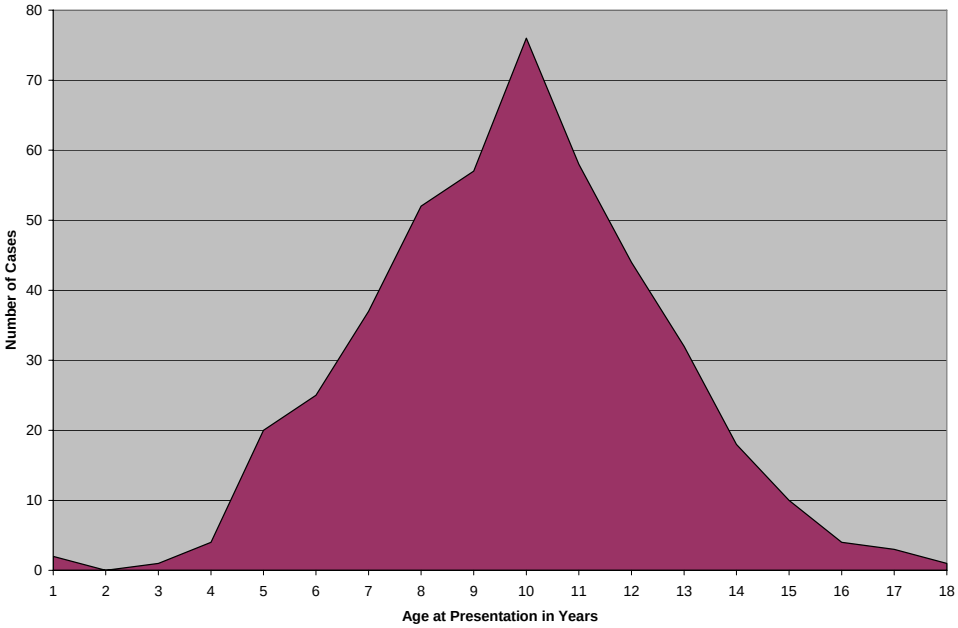


Ten Most Popular Dog Breeds AKC Registered Compared to Their Incidence of Cancer on The Vet Cancer Registry

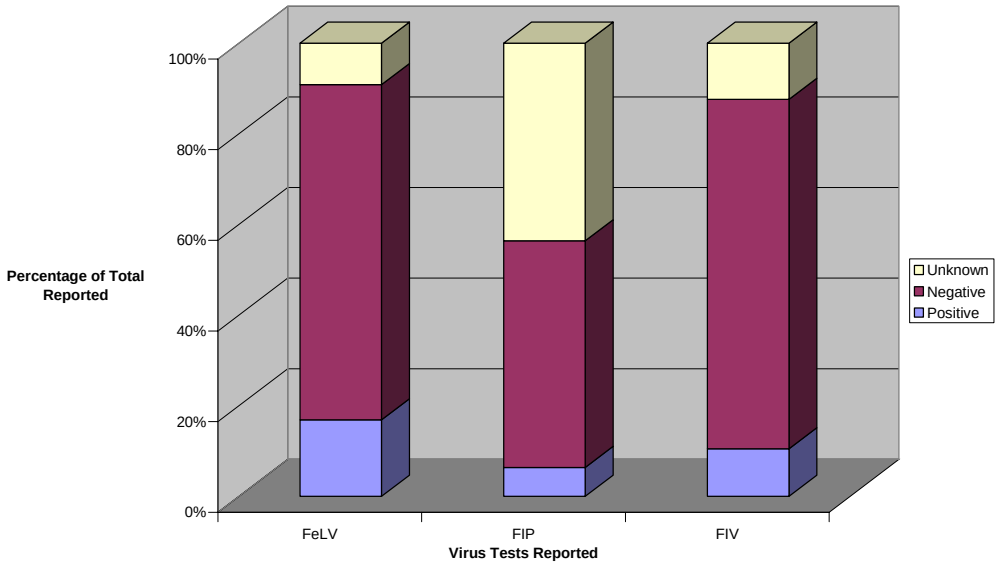
Lymphosarcoma in Dogs by Age
(1159 total cases)
www.vetcancerregistry.com



Hemangiosarcomas in Dogs by Age
(444 total cases)
www.vetcancerregistry.com



FeLV, FIP and FIV reported cases in cats with confirmed cancers at The Vet Cancer Registry
www.vetcancerregistry.com



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Poster Abstract

Comparative Gene Expression Analysis of Canine and Human Osteosarcoma

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Introduction: Canine osteosarcoma is a valuable model of pediatric osteosarcoma, validated at both a histological and biological level. The goal of this study was to characterize this model at the genomic level through a comparison of gene expression signatures of canine and pediatric osteosarcoma.
Methods: RNA was extracted from 15 canine and 15 pediatric primary osteosarcomas and selected normal canine and human tissues. Affymetrix oligonucleotide arrays representing dog (Dog Genome chip v1.0) and human (U133A Human chip) were used. Hierarchical clustering was performed on both species separately. Shared orthologues, represented on the arrays were normalized across species and then clustered. A preliminary dog gene list of interest was identified by subtraction of dog osteosarcoma genes from those expressed in normal tissues.
Results: Normalized cluster analysis of dog and human array data sets revealed a clear distinction between osteosarcoma and normal tissues. Mapping of overlapping genes between dog and human present on both array sets followed by pooled normalization yielded 7000-9000 genes for clustering of dog and human data sets. Interestingly two osteosarcoma clusters including both dog and human osteosarcoma samples were found. Highly expressed genes in canine osteosarcoma compared to canine normal tissues included genes associated with connective tissue, cell adhesion and extra-cellular matrix proteins.
Conclusions: This data demonstrates the strong similarity between canine and human osteosarcoma based on global gene expression. Ongoing evaluation will validate the biological similarity in gene expression between dog and human osteosarcoma and will define specific genes or pathways common and distinct in these species.

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Do Peripheral Lymphocytic Chromosomal Aberrations in Dogs With Lymphoma Change During and After Treatment?

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Objectives:

Cancer is the leading cause of death in dogs. Canine lymphoma is particularly devastating because it is frequently diagnosed and almost always fatal. Chromosomal aberrations from tumor biopsy samples have been studied, and certain repetitive anomalies were shown to correlate with prognosis. A relationship between cytogenetic changes in peripheral blood lymphocytes in human lymphoma and leukemia patients was recently established. Not only was predictive information obtained, but response to therapy could also be monitored. Evaluating peripheral lymphocytes instead of tumor is advantageous because: 1) More patients can be evaluated since it the rate of successful "spreads" is higher 2) No biopsy is required, so there is less expense and patient discomfort, and 3) peripheral lymphocytes are available even after the tumor has gone into remission creating a previously unexploited window for evaluation. If meaningful cytogenetic changes from lymphocytes are identified, this can become a powerful clinical tool in the treatment of canine lymphoma. Developing methods for early screening and/or predicting response to therapy would have a major clinical impact in this important disease.

Supporting data:

Previous work using flow cytometry has demonstrated that canine lymphoma patients are marginally hypoploid. We anticipate that the patients will have slightly abnormal chromosome numbers at the time of diagnosis. Their abnormal chromosome counts will worsen during the course of treatment and then return to near normal counts after treatment ceases. Once the disease recurs, chromosome counts will return to or exceed their most abnormal count. We have identified numerical aberrations in 5 lymphoma patients, with 2 of the patients having multiple time points. Peripheral changes in chromosomes could provide a unique window for determining what is happening to the patient. Chromosome counts can identify numerical changes, such as chromosome deletions or duplications.

Conclusions:

We have determined that chromosomal aberrations can be identified in the peripheral blood of canine lymphoma patients. Additionally, the frequency of aberrations changes during the course of treatment.

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THE CANINE RAGE - HMGB1 COMPLEX, A HAND ON METASTASIS?

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Objectives: Primary objective was to characterise the canine receptor for advanced glycation end products *(RAGE)* gene. The RAGE receptor is a multiligand member of the immunoglobulin superfamily of cell surface molecules known to be causally involved in a variety of pathophysiological processes, e.g. immune/inflammatory disorders, Alzheimer disease, and tumorigenesis. Recently, the RAGE receptor was described to bind a number of ligands with diverse structural features. One of these ligands is amphoterin, synonymously called HMGB1. The RAGE – HMGB1 complex has been shown to have significant influence on inflammation and metastasis by taking significant effect at invasiveness, growth and motility of tumour cells. *In vitro* and *in vivo* approaches showed that blocking of this complex resulted in drastic suppression of tumour cell growth. Recently we characterised the canine *HMGB1* gene and protein completely.

Results: Here we present the complete characterisation of the canine *RAGE* gene including its 1384 bp mRNA, the 1215 bp protein coding sequence (cds), the 2835 bp genomic structure, the chromosomal localisation on CFA 12, the gene expression pattern in healthy tissues, and its 404 amino acid protein with a weight of 43113.19 Daltons. Further on we compared the cds of six different canine breeds for SNPs.

Conclusions: The complete characterisation of the canine RAGE-HMGB1 protein complex could serve as base for future clinical studies aimed at the development of blocking strategies to inhibit metastatic behaviour of canine and human tumours.

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Poster Abstract

HMGA2 IS OVEREXPRESSED IN CANINE PROSTATE CARCINOMA

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The number of dogs developing tumours of the prostate is currently increasing. Furthermore the dog is - beside humans- the only known mammalian species that spontaneously develops this locally invasive disease with a high metastatic potential. Both species show striking similarities in the development and progress of the disease: Prostate carcinoma most commonly appear in older patients, the tumours are likely to metastasise to distant regions by blood or lymphatic system, and the tumours vary with respect to their clinical behaviour. Molecular indicators allowing for a valid prognosis of these cancers are of considerable interest, because based on the histology of the lesions alone it is often not possible to recognize sufficiently the malignant potential of the tumour in terms of local invasiveness and metastatic spread.

HMGA proteins are members of the High-Mobility-Group family of proteins which normally are expressed during embryonic development and are down-regulated in adult tissues. Currently there are three well known members of the HMGA family: *HMGA1a* and *HMGA1b*, deriving from alternatively spliced mRNAs of the same gene and *HMGA2*, which is encoded by a separate gene. Due to their ability to mediate the binding of other transcription factors without transcriptional activity per se, HMGA-proteins are called architectural transcriptions factors. In human carcinomas the overexpression or aberrant expression often correlates with the degree of neoplastic cell transformation and metastatic tumour progression. In tissue specific cell-lines, the application of an adenovirus carrying the *HMGA1* gene in antisense orientation abrogated the effects mediated by over expression of *HMGA1*. Taken together HMGA expression is considered as a possible molecular marker in prostate cancer diagnosis.

HMGA proteins are highly conserved during evolution. In previous investigations we were able to demonstrate the existence of *HMGA* genes in the canine genome as well as the close similarity between canine and human genes and proteins. In the present study we have determined the *HMGA2* expression patterns by real-time quantitative RT-PCR in prostatic tissues from 16 dogs with diverse histological findings. The study includes 4 samples of non-neoplastic tissues, 3 hyperplasias, 3 cysts, and 6 carcinomas. The results show that expression of *HMGA2* is highest in carcinomas, less intense in benign neoplasms with intermediate values for cysts and hyperplasias and is markedly low in non-neoplastic tissues. Actually, there is a 19-fold divergence in expression between the highest transcript level of non-neoplastic tissues and the lowest transcript level observed in carcinomas. In our study all malignant neoplasias showed expression levels beyond 50,000 transcripts per 250 ng total RNA, whereas none of the non-malignant tissues showed expression levels above this value. These results indicate that *HMGA2* indeed may serve as a molecular marker in prostate cancer diagnosis.

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The Ins and Outs of Pedigree Analysis, Genetic Diversity, and Genetic Disease Control

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As breeders, you engage in genetic "experiments" each time you plan a mating. The type of mating selected should coincide with your goals. Outbreeding brings together two animals less related than the average for the breed. This promotes more heterozygosity, and usually more variation in a litter. A reason to outbreed would be to bring in new genes or traits that your breeding stock does not possess. Outbreeding can also mask the expression of recessive genes, and allow their propagation in the carrier state.

Linebreeding attempts to concentrate the genes of a specific ancestor or ancestors through their appearance multiple times in a pedigree. The ancestor should appear behind more than one offspring in the sire and dam's pedigree. Otherwise you are only linebreeding on the single offspring. A linebreeding may produce an offspring with magnificent qualities. However, if those qualities are not present in any of the ancestors that have been linebred on, the individual may have a wonderful show career, but it may not breed true. Careful selection of mates is important, but careful selection of offspring from the resultant litter is also important to fulfill your genetic goals. Without this, you are reducing your chances of concentrating the genes of the linebred ancestor.

Inbreeding significantly increases homozygosity, and therefore uniformity in litters. Inbreeding can cause the expression of both beneficial and detrimental recessive genes through pairing up. Inbreeding cannot change, or create undesirable genes. It only exposes them through homozygosity. Inbreeding can also exacerbate a tendency toward disorders controlled by multiple genes, such as hip dysplasia and congenital heart anomalies. Unless you have prior knowledge of what milder linebreeding on the common ancestors has produced, inbreeding may expose the offspring (and buyers) to extraordinary risk of genetic defects. Research has shown that inbreeding depression, or diminished health and viability through inbreeding is directly related to the amount of detrimental recessive genes present. Some lines can thrive with inbreeding, and some cannot. Increased homozygosity through inbreeding can also decrease the diversity of major histocompatability (MHC) genes that affect the immune system, and play a role in auto-immune disorders.

The inbreeding coefficient is an estimate of the percentage of all the variable gene pairs that are homozygous due to inheritance from common ancestors. It is also the average chance that any single gene pair is homozygous due to inheritance from a common ancestor. In order to determine whether a particular mating is an outbreeding or inbreeding relative to your breed, you must determine the breed's average inbreeding coefficient. The average inbreeding coefficient of a breed will vary depending on the breed's popularity or the age of its breeding population.

For the calculated inbreeding coefficient of a pedigree to be accurate, it must be based on several generations. Inbreeding in the fifth and later generations (background inbreeding) often has a profound effect on the genetic makeup of the offspring represented by the pedigree. In pedigree studies, the difference in inbreeding coefficients based on four versus eight-generation pedigrees varied immensely. A four-generation pedigree containing 28 unique ancestors for 30 positions in the pedigree could generate a low inbreeding coefficient, while eight generations of the same pedigree, which contained 212 unique ancestors out of 510 possible positions, had a considerably higher inbreeding coefficient. What seemed like an outbred mix of genes in a couple of generations appeared as a linebred concentration of genes from influential ancestors in extended generations.

Many breeders plan matings solely on the appearance of an animal and not on its pedigree or the relatedness of the prospective parents. This is called assortative mating. Breeders use positive assortative matings (like-to-like) to solidify traits, and negative assortative matings (like-to-unlike) when they wish to correct traits. Some individuals may share desirable characteristics, but they inherit them

differently. This is especially true of polygenic traits, such as ear set, bite or length of forearm. Breeding two phenotypically similar but genotypically unrelated individuals together would not necessarily reproduce these traits. Conversely, each individual with the same pedigree will not necessarily look or breed alike. Therefore, matings should be based on a combination of appearance and ancestry.

Some breeds and breeders have concerns about genetic diversity. Molecular genetic research in many of these breeds shows that there is more diversity (heterozygosity) present than breeders realize. Some breed clubs advocate codes of ethics that discourage linebreeding or inbreeding, as an attempt to increase breed diversity. The types of matings utilized do not cause the loss of genes from a breed gene pool. It occurs through selection; the use and non-use of offspring. Regardless of the popularity of the breed, if everyone is 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. The frequency of his genes will increase, possibly fixing breed related genetic disease through the founder's effect. If some breeders linebreed to certain individuals that they favor, and others linebreed to other individuals that they favor, then breed-wide genetic diversity is maintained. Animals who are poor examples of the breed should not be bred simply to maintain diversity. Related individuals with desirable qualities will maintain diversity, and improve the breed.

If you linebreed and are not happy with what you have produced, breeding to a less related line immediately creates an outbred line and brings in new traits. Repeated outbreeding to attempt to dilute detrimental recessive genes is not a desirable method of control. Recessive genes cannot be diluted; they are either present or not. If an individual is a known carrier or a high carrier risk through pedigree analysis, it can be retired from breeding, and replaced with one or two quality offspring. Those offspring can be bred, and replaced with quality offspring of their own, with the hope of losing the defective gene.

Trying to develop your breeding program scientifically can be an arduous, but rewarding, endeavor. By taking the time to understand the types of breeding schemes available, you can concentrate on your goals towards producing a healthy and worthy representative of your breed.