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## 27th Annual Canine Symposium

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# 27th Annual Canine Symposium

The 27th Annual Canine Symposium took place on January 25 at VHUP. The event, held in honor of Dr. M. Josephine Deubler, who organized the first Canine Symposium in 1970, focused on canine genetic diseases.



## Genetic Disease

Dr. Donald F. Patterson, professor of medical genetics at the School, opened the seminar by displaying the cover of a recent issue of *Time* magazine that investigated dog breeding practices. He called the article — which asserted that purebred dogs are riddled with disease because of irresponsible breeding methods — an unjustified indictment of the dog breeding community. He said the problems can be more accurately assessed only by understanding the complex mechanisms of genetic diseases.

Genetic diseases are caused by mutations — or alterations in the structure and function of genes, rather than by the environment. Genes are comprised of DNA sequences. These sequences serve as templates for messenger RNA, which directs protein synthesis. Hence, genetic defects ultimately result in the manufacture of abnormal proteins — perhaps enzymes, biological receptors or structural proteins comprising cell membranes and connective tissues. Genes, which direct growth, development and function of the body's organs throughout life, number some 50,000 in every mammal. The genome of dogs differs from that of humans by only about 10-20 percent of the nucleotides. Thus, explained Dr. Patterson, "You can expect that all mammals will generally have the same number and kinds of genetic diseases."

Yet while some 5,000 genetic disorders have been mapped out in humans, a mere 350-or-so have been documented in the dog. Owing to a series of major scientific breakthroughs, this represents a logarithmic increase from the number of canine inherited diseases described in the 1930's, when just a handful of such

anomalies was recognized. "Because of the tremendous progress in the development of vaccines, antibiotics and nutritious diets," said Dr. Patterson, "we've gotten rid of a lot of the non-genetic problems and been able to concentrate our efforts on genetic diseases."

Our expanded understanding of genetic defects, which, in recent years, has led to the identification of about five to ten canine genetic diseases annually, can also be attributed to the development of modern diagnostic methods. A large percentage of genetic maladies documented in the dog thus far are orthopedic, ocular or neurological, perhaps because of the more obvious clinical signs they produce. But researchers are now striking major advancements in those genetic abnormalities that cause more subtle signs and challenge diagnostic methods.

One such case is vitamin B12 (cobalamin) deficiency, which was first described by a veterinary medical geneticist at the School. The first known affected dogs presented at a few months of age. They showed general signs of failure to thrive, including anorexia, anemia and weakness, and were small in relation to their litter mates. Using paper chromatography, researchers identified methylmalonic acid in the urine. The presence of this abnormal metabolite signals a defect in either an enzyme involved in the metabolism of organic acids or an enzyme cofactor. The problem in this case was found to be a lack of cofactor (vitamin B12) receptors in the ileum, where they are inserted following genetic translation. Unlike many genetic diseases, cobalamin defects have an effective therapy — parenteral administration of vitamin B12.

Vitamin B12 deficiency is an autosomal recessive disease, as are most understood genetic disorders of purebred dogs. In his retort to the *Time* magazine story, Dr. Patterson said that inbreeding — the mating of individuals that share common ancestry, does not cause genetic disease. Rather, he said, inbreeding can increase the chance of a disease-causing recessive mutant gene being transmitted in double dose to the offspring.

In order to eliminate recessive

diseases, Dr. Patterson explained, we must be able to recognize affected individuals and refrain from breeding them. But, he added, "That's not enough, because most of the genes that cause these recessive disorders are in the population in carriers. We don't know that an animal is a carrier unless it's bred to another carrier and produces affected individuals."

If, for example, the frequency of a defect were four percent and the affected individuals were removed from the breeding population, it would take over ten generations to reduce the frequency of disease to one percent. But if both the carriers and those affected were extracted from the breeding population, the disease would be eliminated in one generation. So the key to abolishing canine genetic diseases, Dr. Patterson said, is to identify carrier dogs.

To this end, the School has undertaken four major initiatives: research in biochemical and molecular abnormalities involved in specific genetic diseases; development of carrier tests based on detection of either abnormal gene products or structural changes in the DNA of mutant genes; operation of a genetics/pediatrics clinic at VHUP, where breeders and owners of animals with known or suspected genetic diseases can seek diagnostic services and genetic counseling; and development of a compendium of genetic diseases, to be published in book and software forms within the next year.



## Screening for Inherited Diseases

Dr. Urs Giger, professor of medicine and medical genetics, discussed screening for inherited disease. In contrast to the common dominant inheritance of diseases in humans, most hereditary diseases in dogs are recessively inherited. In autosomal recessive inheritance both asymptomatic parents may contribute an abnormal (mutant) gene to an offspring which will then have two mutant genes (homozygous) and is, therefore, affected. Most metabolic and eye disorders are inherited

## Molecular Genetic Tests

by an autosomal recessive trait. In x-linked recessive disorders, the asymptomatic dam will pass on one of her x-chromosomes to the male and female offspring, however, if the male inherits the x-chromosome containing a mutant gene, the males will be affected with e.g., hemophilia or muscular dystrophy. Finally, with complex traits such as hip dysplasia and certain cancers, environmental factors also play a role in the expression of the disease. Thus, in order to control hereditary diseases in dogs, it will be important not only to identify affected dogs, but also carriers which carry one copy of a mutant gene, but have no clinical signs.

Veterinarians and breeders have several tools to identify diseased animals. Since many genetic diseases are breed-specific, the signalment of an animal may suggest a disorder known to occur in that breed. Most diseases are associated with characteristic clinical signs which occur at a typical age. Neonates and juvenile dogs are more likely to suffer from a genetic disorder (this is known as the fading puppy syndrome), although a few genetic diseases may only cause signs in adulthood. Failure to thrive, growth retardation, and malformations are commonly seen, and neurologic and ophthalmologic signs may also be evident. In contrast to malnutrition, infections, and intoxications, the clinical manifestations are usually chronic progressive, however, genetic predisposition to infection and bleeding may be associated with intermittent signs. An easy way to recognize an unthrifty puppy early, is to regularly weigh puppies and compare with littermates. Routine blood and urine tests as well as imaging studies such as radiographs, ultrasound and ophthalmoscopy rule out many acquired diseases and may further suggest an inherited disorder.

The Veterinary School's Section of Medical Genetics has developed and established screening tests for many metabolic or hematologic hereditary disorders (see *Josephine Deubler Genetic Disease Testing Laboratory*). Since inborn errors lead to dysfunction of a biological pathway they can be identified by product deficiency and accumulation of substrates or alternative substances. A small urine

sample is often more helpful for these screening tests than serum or other specimens. For instance, specific sugars are found in urine of puppies with certain skeletal and neurological abnormalities known as mucopolysaccharidoses. Cystine, a poorly soluble building block of proteins, accumulates in dogs with cystinuria and can lead to kidney and bladder calculi, resulting in inability to urinate, an emergency situation.

We are aiming to not only identify the failing biological pathway in affected animals, but to determine the responsible protein that is defective. In fact, various gene defects may result in the failure of the same biologic function, thus, identification at the protein or DNA level is important to reach a specific diagnosis. The best examples are deficiencies of enzyme activities, inability of a receptor to bind, or a lack of transporters to move substances across a membrane. Such tests are now available for several dozens of genetic defects in dogs.

These specific functional and immunologic tests are not only useful to confirm a specific defect in an affected dog, but also to identify carriers. Carriers or heterozygotes, who carry one normal and one mutant gene, are asymptomatic, but can pass on the mutant gene to their offspring. Thus, in order to produce healthy dogs it is pivotal to recognize carriers and eliminate them from breeding. Although parents and offspring of affected dogs are obligate carriers, for others laboratory tests are needed to know whether they are carriers or normal. Instead of having the normal 100% specific enzyme function, carriers have intermediate activity or amount of approximately 40-60% compared to controls. Although very helpful, these carrier tests are time consuming and technically demanding, and require special specimen submission. Because of these difficulties, these carrier tests are not completely accurate. Therefore, it is exciting that for some of these inherited disorders, the molecular genetic defect has been identified which will simplify testing in the near future for many hereditary disorders in dogs.

Biochemical tests are useful in diagnosing specific inherited diseases. However, said Dr. Paula Henthorn, associate professor of medical genetics at the School, they have limitations. For example, they occasionally fail to distinguish carriers from normal dogs.

"Sometimes what we detect in the lab doesn't really represent what's going on in the animal," Dr. Henthorn explained.

The future generation of genetic tests will be based on DNA analysis. DNA, or deoxyribonucleic acid, is a double strand comprised of billions of the four nucleotide bases. "If you thought of each base pair as a bead on a string, that string would stretch from here to San Francisco," said Dr. Henthorn.

Of even greater consequence, a substitution, addition or deletion in just one of the six trillion base pairs in the genome can dramatically alter the protein a gene encodes. Such minute modifications can have deleterious consequences. For example, phosphofructokinase (PFK) deficiency, which leads to hemolytic anemia and exercise intolerance in the English springer spaniel, results from a single base substitution. X-linked severe combined immunodeficiency (XSCID), which is lethal in young dogs, is caused by a deletion of only four nucleotides.

Molecular genetic testing can be performed in puppies shortly after birth. In testing for a particular disorder, one must know what sequence mutation to look for. DNA can be isolated very non-invasively, perhaps in a drop of blood, hair follicle, frozen semen, or cheek cells. The polymerase chain reaction (PCR) is used to amplify selected DNA segments up to billions-fold so they can be examined. Restriction enzymes are then employed to cut the fragments of interest. These fragments are then run on gel electrophoresis, which separates them according to size and thereby distinguishes normal from abnormal fragments.

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Both PFK deficiency and XSCID, which are caused by mutations that create new restriction sites, can be diagnosed in this manner. But detecting the genetic aberration in a single dog is only half of the battle, Dr. Henthorn said.

“Once we’ve looked at an individual animal, we have to analyze the results in the larger context of what we know about the breed or the pedigree.”

Over 244 dogs were screened for PFK deficiency between 1993 and 1995. These mass diagnostic efforts shed light on the occurrence of the mutation throughout the springer spaniel breed. Likewise, the recognition of a single case of XSCID led to broad testing of the affected dog’s pedigree. Researchers at the School traced the defect back to the dog’s grandmother, flagged female carriers and advised owners on modifying their breeding strategies.

“These tests involve genetic counseling to understand the frequency of the marker you’re looking at and the inheritance in the pedigree,” Dr. Henthorn explained.

These sophisticated diagnostics are presently only available for heritable diseases caused by single-gene defects. But in this rapidly burgeoning field, Dr. Henthorn added, tests for multiple-gene disorders, such as hip dysplasia, will be developed in the years to come.



## Hereditary Diseases Affecting Immune System

Primary immune deficiencies are genetic defects that compromise the immune response and thereby render affected individuals highly susceptible to infection. Dr. Peter J. Felsburg, professor of immunology at the School and chairman of the department of clinical studies at VHUP, explained the etiologies of several inherited immune disorders and discussed diagnostic methods.

White blood cells, or leukocytes, are the work horses of the immune system.

Their constituents, including B and T lymphocytes, monocytes and neutrophils, course through the peripheral circulation. Leukocytes develop from pluripotent stem cells and are renewed throughout life.

The major function of B lymphocytes is to produce antibodies (humoral immunity) that confer protection against bacterial infection. T lymphocytes, which direct cell-mediated immunity, fend off intracellular invaders, such as fungi, protozoa and viruses; a subset of T lymphocytes, helper T cells, regulate and enhance humoral immunity. Monocytes and neutrophils, the “garbage collectors” of the immune system, phagocytize foreign invaders. Complement - enzymatic proteins in the serum, combines with the antigen-antibody complex to stimulate lysis of potentially pathogenic antigen. A flaw in any of these immune components can cause an immunodeficiency syndrome, Dr. Felsburg explained.

“Where the genetic defect is in the development of the immune system determines how severe the immune deficiency is.”

Some 50 heritable immune deficiencies have been identified in humans. In the dog, only four or five have been documented. “These same 50 disorders should also be seen in the dog, and in higher incidence because of inbreeding,” said Dr. Felsburg.

The clinical features of immune incompetence in the dog include recurrent, chronic infections that are refractory to treatment; chronic diarrhea; growth retardation; and adverse reactions to modified live vaccines.

Several simple tests are used to diagnose canine immune deficiencies. They include quantification of antibodies or complement, measurement of B- and T-lymphocyte maturity through analysis of cell surface markers, and challenge assays to assess the competence of both the T lymphocytes (lymphocyte transformation test [LTT]) and the phagocytic system.

Among the significant primary immune deficiencies that have been reported in the dog are XSCID, selective IgA deficiency and leukocyte adhesion deficiency (LAD). In XSCID, both humoral and cell-mediated immunity are compromised. Af-

flicted dogs experience chronic infections that typically begin at a few months of age. These infections, usually bacterial, are generally unresponsive to antibiotic therapy. Affected dogs are smaller than their litter mates and exhibit marked failure to thrive.

Interleukin-2, or IL-2, stimulates T lymphocytes to proliferate in normal dogs. Dogs with XSCID lack IL-2 cell surface receptors. Likewise, they do not respond to IL-2 therapy. Two tests are used to diagnose XSCID. With the first, T cells are stimulated with antigen and IL-2 is added. T cell response is absent in affected dogs. The second test uses antibodies against IL-2 receptors to determine whether these receptors are present. Because XSCID is a recessive disease, carriers are not clinically affected. In hopes of purging the breeding population of this deadly gene, researchers at the School have developed carrier tests for XSCID.

Selective IgA deficiency, which leads to less severe recurrent infections, occurs quite commonly in dogs. It can be diagnosed with LTT or by measurement of the serum IgA concentration, which is profoundly depressed in affected dogs.

LAD is caused by defective integrins, proteins that enable phagocytes to bind to microorganisms. Signs of LAD, which occur at a few weeks of age, include recurrent pyogenic infections and poor wound healing. LAD is often diagnosed using flow cytometry to discern the presence of normal adhesion molecules.

Once an immunodeficiency has been identified using one of myriad screening tests, Dr. Felsburg said, more sophisticated assays can be employed to locate where the defect is. Researchers are working to pinpoint the genes responsible for these problems, and then develop carrier tests.



## Heritable Renal Diseases

A variety of serious kidney disorders are encoded by genes. Likewise, they have breed predispositions, said Dr.

Meryl Littman, associate professor of Medicine at VHUP.

The kidney plays several homeostatic roles. It filters the blood through its glomeruli — small, convoluted tufts of capillaries, and excretes toxic waste products. The renal tubules resorb substances from the glomerular filtrate — such as amino acids, sugars, water and electrolytes, in amounts necessary to maintain hydration, acid-base balance, blood pressure and other normal parameters. Also, the kidney manufactures important vitamins and hormones.

As a homeostatic organ, the kidney is so efficient that it can serve all of the body's functions with just a portion of its nephrons, or functional renal units. The dog, for example, can lose one of its two kidneys and remain healthy. With a 67 percent loss of renal mass, it might exhibit polyuria/polydipsia (pu/pd), but serum measures of renal function, such as creatinine and blood urea nitrogen (BUN), are not affected. In fact, renal failure does not typically occur until about three-quarters of the renal parenchyma is destroyed.

Clinical signs of renal failure include pu/pd, dehydration, anorexia, vomiting, weight loss, anemia, hypertension and ascites. Laboratory tests typically show elevated BUN, creatinine and phosphorus.

Several inherited renal defects — renal dysplasia, glomerular diseases, tubular diseases and structural abnormalities — can lead to renal failure. Yet when they occur, these problems should not be assumed to be genetic; infectious and metabolic mechanisms, for example, may be the cause. In assessing their etiology, it is important to survey the affected dog's pedigree and consider whether a breed predisposition exists for the disorder.

Renal dysplasia is a congenital or neonatal disorder that results in maldevelopment of the kidneys. The renal reserve can be severely compromised by renal dysmaturity, predisposing the dog to progressive renal failure. Renal dysplasia is thought to be familial in the Lhasa apso, Shih Tzu, miniature schnauzer, Doberman pinscher, chow chow, soft coated wheaten terrier, golden retriever, and standard poodle breeds.

Clinical signs of renal dysplasia include poor growth, pu/pd, cachexia and “rubber jaw.” Blood work reveals elevated BUN and creatinine; urinalysis shows low urine specific gravity and possibly signs of urinary tract infection; and on radiographs, the kidneys appear small. A diagnosis can also be rendered by kidney biopsy, which may reveal maldeveloped fetal glomeruli and mesenchyme, and renal atrophy. Renal dysplasia is thought to be an autosomal recessive trait, but no carrier tests have yet been developed.

Unlike renal dysplasia, which usually presents in young dogs, glomerulopathies may emerge clinically at any age. They are characterized by protein-losing nephropathy, which leads to edema/ascites, hypertension and thromboembolism. Affected dogs have elevated BUN and serum creatinine, low serum albumin, and proteinuria. Glomerulonephritis and amyloidosis may be evident on biopsy. Like renal dysplasia, glomerulopathies seem to gravitate toward certain breeds of dogs, including beagles, Basenjis, Samoyeds, Labrador and golden retrievers, soft coated wheaten terriers, Bernese mountain dogs, Dobermans, bullmastiffs, bull terriers, English cocker spaniels, Rottweilers, and Sharpeis. Likewise, Dr. Littman cautioned, the pathogenesis of these disorders may vary from breed to breed.

“They may look the same on paper but there may be different genetic reasons for the problem,” she said.

Certain dog breeds are also predisposed to tubular diseases and structural anomalies. Telangiectasia occurs in some Pembroke Welsh corgi dogs. The Siberian husky appears prone to ectopic ureter and subsequent hydronephrosis.

“I think you can see how complex these renal disorders are and the variety of diseases that might present,” said Dr. Littman.

The genetic defects inherent in most inherited kidney disorders have not yet been identified, she added. But once these modes of inheritance are worked out, special breeding strategies may be devised to reduce the occurrence of these often-debilitating maladies.

## Genetic Basis of Hip Dysplasia

Sound imaging techniques for diagnosing the presence of hip dysplasia are currently available. By incorporating the results of these tests into breeding programs, said Dr. Gail K. Smith, professor of surgery at the School and chief of the section of surgery at VHUP, we can select for better hips in future generations of dogs.

Hip dysplasia is the most common heritable orthopedic disease of the dog. Due to a misshapen femoral head (ball) or shallow acetabulum (socket), the ball-and-socket hip joint does not form a tight union in dysplastic dogs. Consequently, these individuals have lax, or loose-fitting, hips. Joint laxity may, depending upon the severity, over time lead to degenerative joint disease (DJD), or arthritis.

Hip dysplasia in the dog was first described in the 1930s. After decades of research on the disorder, why are there still so many dogs walking around on bad hips? For one thing, little is known about the genetic scheme and molecular patterns of hip dysplasia. What is understood, however, is that hip dysplasia arises from a complex form of inheritance. Its expression is polygenic and multifactorial; in other words, many genes, as well as environmental factors, influence its development.

The other reason hip dysplasia is ubiquitous is that dysplastic dogs have not been kept out of the breeding pool because, until recently, their disorder was not accurately diagnosed. In 1983, Dr. Smith developed the PennHIP® program, which utilizes a highly reliable method for diagnosing hip dysplasia. By the PennHIP® method, radiographs are taken when the dog's hips are in the neutral (standing) position, which allows maximum laxity. (The traditional position used for evaluating hip status was legs fully extended.) The femoral heads are then manually pushed laterally out of the acetabula as far as they will easily go. A

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distraction index (DI), which measures the distance the heads can be pushed out of the joint, is then calculated. The DI is numbered 0 (no laxity) through 1 (complete laxity). A DI of .75, for example, indicates that the hips can be translated 75 percent from the congruent position.

But, said Dr. Smith, “The fact that we can measure laxity is meaningless unless we can link it to future development of osteoarthritis.”

Ongoing research indicates that hip laxity is the most important factor in predicting DJD. Tighter hips are better hips, Dr. Smith said. But, he added, arthritis susceptibility is also highly breed dependent. In other words, different breeds of dogs have different degrees of tolerance of laxity in their hip joints; for example, German shepherd dogs are more likely to develop arthritis, given the same amount of laxity, than are Rottweilers. Environmental factors can also dramatically influence the expression of arthritis in an arthritis-prone dog.

Studies show that an individual’s DI is consistent over time. Dogs have essentially the same amount of laxity as puppies as they do years later. Thus, hip laxity can be accurately determined as early as four months of age, well before a dog is ready to be bred. This is important because hip dysplasia is a highly heritable disorder.

Heritability is a measure of the total variability in phenotypic traits attributable to genetic differences among individuals. Hip dysplasia has a high degree of heritability, according to the results of an extensive, soon-to-be-published study Dr. Smith conducted that showed dysplastic dogs parent dysplastic puppies. Breeders can place heritability in their corner by applying selection pressure to their breeding populations, Dr. Smith explained. The more selection pressure they apply, the greater the change in the trait from one generation to the next. Key to this strategy is accurate identification of healthy individuals.

“We must rely on diagnostic tests to help us cleanse this gene pool of the bad genes,” Dr. Smith said.

Breeding the extremes for a particular trait will attenuate of the expression of

that trait rather rapidly. In a study conducted in the mid ‘80s, test matings of an isolated population were performed. After evaluating the hips of 71 German Shepherd dogs, a male and a female were identified with DIs similar to those of racing greyhounds, 0.21 and 0.19 respectively. These two dogs were mated and the resultant litter had a mean DI of .27, which represented a 60 percent shift from the original breeding population. Successive breedings were monitored for several years thereafter. By 1991, two generations later, 80 percent of the kennel’s output had DIs below .3.

Such success can be replicated in other populations of dogs. By utilizing PennHIP’s services to obtain accurate ratings of hip laxity, breeders might then design breeding protocols that select from the tight half of the breed in each generation interval. Thus, they can effect a moderate rate of genetic change with each consecutive generation without losing desirable traits or increasing the likelihood of introducing new undesirable characteristics.



### Hereditary Eye Diseases

Canine inherited retinal degeneration causes characteristic optic changes that are consistent among various breeds. But what appears to be a single disorder, said Dr. Gregory M. Acland, adjunct associate professor of ophthalmology in medical genetics at the School, is not.

“There are lots of clues that the disease may be different intrinsically from breed to breed,” he said.

Progressive retinal atrophy (PRA) is a gradual, blinding disorder. Affected dogs develop degenerative changes in the retina with age. Early in the disease, they become night blind. As more of their photoreceptors degrade over time, these dogs lose their day vision as well. The pupils of their eyes become increasingly dilated and prone to developing cataracts.

Upon indirect ophthalmoscopic examination, several pathologic changes are evident: hyperreflectivity of the tapetal

fundus; reduced diameter and branching pattern of retinal blood vessels; and atrophy of the optic nerve head. Diagnosis can be confirmed by either electroretinography or retinal morphological examination.

The major indicator that PRA represents several different pathogenetic mechanisms is the breed-related difference in the age of onset and rate of progression of the disease. Certain breeds, such as the collie, Irish setter, Norwegian elkhound and miniature schnauzer, have early-onset forms of retinal degeneration. In these breeds, the disease results from abnormal or arrested development of the photoreceptors and affects dogs early in life. In other breeds, such as the miniature poodle, Labrador retriever and cocker spaniel, PRA has much later onset.

Studies were conducted to determine whether all of the early-onset forms — though they occur in several dog breeds, represent the same molecular disease pattern. In one trial, the test mating of an affected Irish setter to an affected elkhound produced a litter of unaffected puppies. The phenotypically-normal puppies (carriers of both disorders) were then bred to affected collies. This second litter consisted of all normal puppies. Given that PRA is an autosomal recessive trait in all breeds but the Siberian husky — in which it is x-linked recessive, it is apparent that different genetic forms of PRA occur in the three breeds.

Likewise, test matings were conducted between dogs of several breeds that develop late-onset PRA. The results of these matings pointed toward a single gene at work. Thus, the late-onset forms of PRA result from mutations in the same gene.

Mutations in a variety of phototransduction genes have been implicated in retinitis pigmentosa, the equivalent disease in people to PRA in dogs, and in retinal degenerations in mice. These include for example, mutations in the genes for rhodopsin — the photosensitive pigment in rods, and for the phototransduction enzyme phosphodiesterase (PDE). Defects in the PDE-beta gene, for example, have been found to cause PRA in dogs and humans; PDE-alpha mutations cause retinitis pigmentosa

in humans; and PDE-gamma gene knock-outs produce retinal degeneration in mice.

But confounding these data is the fact that anomalies in a single gene such as RDS/peripheral can continue to lead to a variety of disorders with different patterns of inheritance. In fact, individuals from the same pedigree with the same mutation may exhibit different disorders phenotypically.

“The pathogenesis of these diseases is not quite as clear as we had hoped,” said Dr. Acland. Considerable effort has been expended in evaluating these genes as candidates for the mutant locus in several forms of PRA, and a PDE-beta mutation has been identified as the cause of PRA in Irish setters. Despite this success, however, the end result of all other studies to date has been to prove that PRA is not caused by mutations in these phototransduction genes.

In order to delineate these disease mechanisms, researchers are working to pinpoint the genes responsible for PRA in specific breeds. A reliable DNA test for the presence or absence of rod-cone dysplasia 1 (*rcd1*), which causes early-onset PRA in Irish setters, already exists. But the molecular bases of the other early-onset forms, as well as late-onset progressive rod-cone degeneration (*prcd*), are still being mapped out.

Research on the molecular foundation of retinal degeneration is now turning toward mapping the genes and loci responsible for disease, as well as developing marker tests to spot defective alleles and trace their patterns of inheritance. These investigations have broad implications, Dr. Acland said.

“Progressive retinal atrophy is really a paradigm for all the hereditary eye diseases.”

...and perhaps for other genetic diseases as well.



## Treatment of Genetic Diseases

Even the best detection systems to diagnose and prevent genetic diseases don't alleviate the need for therapy to treat disorders produced by new mutations, said

Dr. Mark E. Haskins, professor of pathology and medical genetics at the School.

“It's possible to treat these diseases by getting around the genetic problems that exist,” he said.

Several therapeutic approaches exist. The simplest of these is direct, phenotypic treatment. These include reconstructive surgery for structural malformations, such as polydactylism, hip dysplasia, and patent ductus arteriosus.

“But this type of treatment is dissatisfying in some respects,” Dr. Haskins said.

The reason, he explained, is that it doesn't really get to the core of the problem. Using NIH funding, Dr. Haskins is testing a variety of treatment modalities for genetic defects.

For metabolic problems, there are therapies that work at the biochemical level. They might limit the availability of a compound in the diet that can become toxic due to genetic deficiencies in processing, remove the toxic product made by the body, or provide a missing non-protein product, such as vitamin B<sub>12</sub>.

For disease caused by dysfunctional or deficient proteins, it's possible to either enhance the activity of the defective enzyme or provide the normal gene product, such as insulin or enzymes. For example, a dog with exocrine pancreatic insufficiency has inadequate secretion of digestive enzymes. These enzymes can be therapeutically replaced in the food, thereby eradicating the clinical manifestations of maldigestion and malabsorption. For a dog with von Willebrand's disease, which is characterized by the deficiency of clotting factors that leads to prolonged bleeding, the missing coagulation proteins can be restored by plasma infusion.

Enzyme replacement can also be used for lysosomal storage disorders. These diseases encompass a variety of rare, genetic (mostly autosomal recessive) defects described in several breeds, including the beagle, German shorthaired pointer and wirehaired dachshund. Lysosomes - small intracellular organelles that contain hydrolytic enzymes — are the “little garbage disposals of cells,” Dr. Haskins said. Deficiency of any of these enzymes can lead to accumulation of metabolites inside the lysosome. Dr. Haskins

likened the lysosomes that become grossly enlarged in these storage disorders to ping pong balls that grow to the size of basketballs.


“We're trying to understand why taking a cell and filling it with basketballs as opposed to ping pong balls makes these animals look the way they do, and then treat them,” Dr. Haskins explained.

Animals with one group of these disorders, mucopolysaccharidosis, exhibit stunted growth, facial dysmorphism, corneal clouding, organomegaly, and neurological and skeletal abnormalities. Human children with mucopolysaccharidosis are mentally retarded and typically die by the age of ten.

Lysosomal storage diseases are caused by defects in the genes coding for lysosomal enzymes. Replacement enzymes can be produced in vitro by genetically-engineered cells and then infused into affected individuals. This has been shown to be successful in children, but may not work well in the dog because of an immune response to the new enzymes and also must be given IV weekly for the life of the dog.

Heterologous transfer through bone marrow transplants, given intravenously from normal to affected siblings, has been effective in the dog. In this method — known as “cross correction” — Dr. Haskins said, “We give normal cells as enzyme factories. They make the enzymes, which are then delivered to the rest of the body. The hope is that these enzymes travel to the bone, cornea, heart valves and all the other affected organs.”

Gene transfer can also be a permanent fix to the problems caused by mutant genes. It involves cloning normal copies of a defective gene and then delivering them, via retrovirus vectors, to the patient's own abnormal cells. Still in the experimental stages, this method, which also utilizes bone marrow transplantation, has several hurdles to overcome before it can be used clinically.

These innovative therapies incorporate different approaches ultimately designed to alleviate disease in affected dogs. Yet with years of experimentation ahead, they are still at the bottom of the refinement curve. 

—Joan Capuzzi, V'98